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***Image-Pro® Plus***

**Version 7.0 for Windows™**

**Reference Guide**

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Delft University of Technology  
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for his help in developing the margination measurement algorithms used in this product.

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## Image-Pro Plus Tool and Command Reference

### Image-Pro Tools

The following tools are located on the toolbar immediately below the menu bar. If no toolbar is being displayed below the menu bar, you may show it by invoking the *Hide/Show Tools* command on the *Window* menu or pressing <F5>. If the menu bar and title bar are not being displayed, you may invoke them by pressing <F4>.



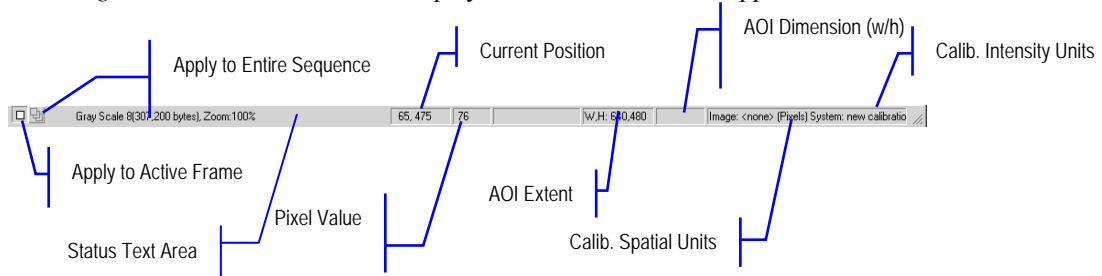
This toolbar is a “dockable” toolbar; that is, the toolbar can be moved to different positions on your screen or resized, as well as hidden completely.

When you position the mouse cursor over one of the icons, a brief description of the command appears. In order for these tool tips to be visible, the main application window must be active.



### The Status Bar

*Image-Pro Plus* has a status bar displayed at the bottom of the application area.



## Image-Pro Tools

The *Status Bar* provides general-purpose information about the open image.

- **Status Text Area** may provide information about a particular menu item, act as a progress indicator for lengthy operations, or describe the attributes of the currently active image.
- **Current Position** gives the location of the cursor in pixels.
- **Pixel Value** displays the intensity value of the pixel underneath the cursor.
- **AOI Extent** displays the location of the AOI in terms of four pixel locations: left, top, right, and bottom.
- **AOI Dimension** gives the width and height of the AOI in pixels.
- **Intensity Units** indicates the intensity calibration units.
- **Spatial Units** indicates the spatial calibration units.

When an operation is in progress, the message bar will display a tracking bar, and the sequence buttons will be replaced with a *Cancel* button. To cancel the operation, click the *Cancel* button or press <Esc>.



The *Apply to Active Frame* and *Apply to Sequence* buttons appear at the extreme left end of the status bar. These buttons can be used to apply a specific operation to the frame currently in the application area, or to the active portion of a sequence of images. (Please refer to the description of the *Sequence Toolbar* later in this manual.)

*Image-Pro Plus* allows you to enable or disable status bar fields other than the status text area by right clicking on the status bar. You will see a pop-up menu of the indicator fields on the status bar (listed above).

Click the cursor next to the name of the field you wish to enable. Enabled fields are checked.

To resize any of the fields in the status bar, place your cursor over one of the dividers between the fields. The cursor will change to a double-headed arrow. Click and drag the cursor left to make the field smaller, or to the right to enlarge the field.



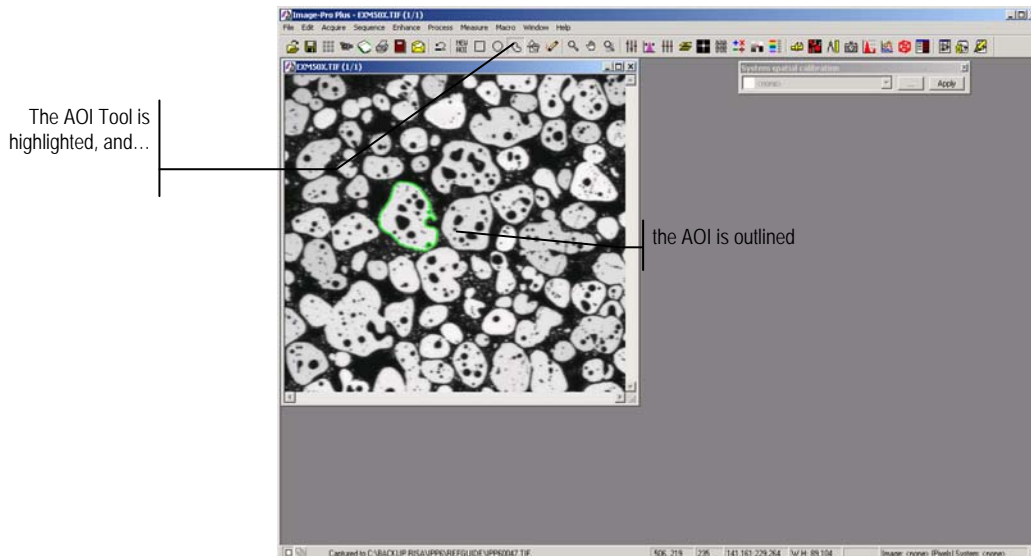
**AOI Tools:** The Area-of-Interest (AOI) tools are used to isolate an area from the rest of an image. When an AOI is active in an image, many of *Image-Pro's* commands will operate only upon the pixels within the AOI. The filter command, for example, filters only the pixels within an AOI if one is active when it is used.

*Note - Not all of Image-Pro's commands are constrained by an AOI. Some, such as **Save** and **Color Segmentation** operate upon the entire image regardless of whether an AOI is active or not. This behavior is generally noted in the command descriptions contained in this reference.*

An *Area-of-Interest* can be defined in any shape (it will always be a closed figure, however). It is created by:

- 1) **Selecting the tool for the AOI** shape you need. This is done by clicking the appropriate AOI tool button (Rectangular, Elliptical or Freeform -- see AOI button descriptions below). When an AOI tool is selected, the button will darken to indicate that it is enabled.
- 2) **Drawing the shape** in the image with your mouse (see the drawing techniques used for each AOI type in the button descriptions below).

Once an AOI has been created in an image it can be toggled off and on by clicking its AOI tool button. When an AOI is active, the outline of the AOI appears in the image, and its AOI tool button is highlighted.



## Image-Pro Tools

You may toggle off an active AOI by clicking its AOI Tool button. When toggled off, the AOI disappears from the image and its AOI tool button returns to normal.

*Image-Pro* allows you to have multiple AOIs active and visible when you are in Multiple AOI mode (see *Multiple AOI Tool* below). When not in Multiple AOI mode, only one AOI can be active (and visible) at any given time, although you may keep one of each type (that is, up to three AOIs) defined in an image. For example, you can define a rectangular AOI, toggle it off, then define an elliptical AOI, then toggle it off, and then define a freeform AOI. When you return to the rectangular tool, *Image-Pro* will reactivate the rectangular AOI you defined. Likewise, when you select the elliptical or freeform tool, the last elliptical or freeform AOI is activated.

The AOI Manager allows you to preserve individual AOIs for future use. See the *AOI* command on the *Edit* menu for more information.

The individual AOI tool buttons, used to create and activate AOIs, are described further below.



**New AOI Tool:** Use the AOI reset tool when you want to eliminate the currently defined AOI and create a new one of the same type.

For example, if you already had a freeform AOI active in your image, but you wanted to create a new one, you would click the **New AOI** button. This would clear the old AOI from the image and give you the freeform drawing cursor so you could define a new one.

See below for the steps used to define an AOI.

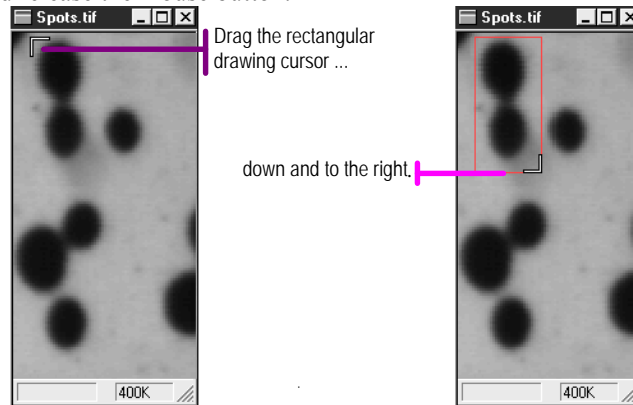


**Rectangular AOI Tool:** Use this tool to create and/or activate a square or rectangular AOI in your image. To enable the tool, click it. The button will be highlighted when it is enabled. Holding down the <Shift> key while creating or editing a rectangular AOI will create a square AOI (height = width).

The behavior of this tool will vary depending upon whether a rectangular AOI has already been defined in the image.

**If you have already defined a rectangular AOI** in the image, enabling this tool will activate it (the AOI outline will appear in the image). If you want to create a new rectangular AOI, click the **New AOI** button. This will eliminate the current AOI and give you the drawing tools to define a new one.

**If you have not already defined a rectangular AOI** in the image, the rectangular drawing cursor will be given to you. Move this cursor to the upper-left corner of the AOI, then drag it to the lower-right corner and release the mouse button.



The rectangle you have just defined will appear as an AOI in your image.

You may adjust the boundaries and position of a rectangular AOI while it is active. To adjust a boundary, place the cursor along the edge or corner of the AOI and drag it when the 2-way cursor appears. To move the AOI, place your cursor in the middle of the rectangle and drag it when the 4-way cursor appears.



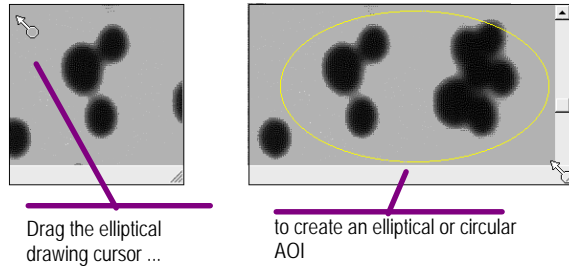
**Elliptical AOI Tool:** Use this tool to create and/or activate a circular or elliptical AOI in your image. To enable the tool, click it. The button will be highlighted when it is enabled. Holding down the <Shift> key while creating or editing an elliptical AOI will create a circular AOI.

The behavior of this tool will vary depending upon whether an elliptical AOI has already been defined in the image.

**If you have already defined an elliptical AOI** in the image, enabling this tool will activate it (the AOI outline will appear in the image). If you want to create a new elliptical AOI, click the **New AOI** button. This will eliminate the current AOI and give you the drawing tool to define a new one.

**If you have not already defined an elliptical AOI** in the image, the elliptical drawing cursor will be given to you. Drag the cursor until the circular or elliptical shape you need is produced, then release the mouse button.

## Image-Pro Tools



The ellipse you have just defined will appear as an AOI in your image.

You may adjust the boundaries and position of an elliptical AOI while it is active. To adjust a boundary, place the cursor along the edge of the AOI and drag it when the 2-way cursor appears. To move the AOI, place your cursor in the middle of the ellipse and drag it when the 4-way cursor appears.



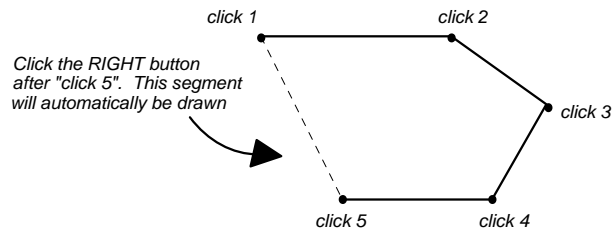
**Freeform AOI Tool:** Use this tool to create and/or activate a freeform AOI in your image. A freeform AOI is made up of any polygonal shape you care to define. To enable the tool, click it. The button will be highlighted when it is enabled. The behavior of this tool will vary depending upon whether or not a freeform AOI has already been defined in the image.

**If you have already defined a freeform AOI** in the image, enabling this tool will activate it (the AOI outline will appear in the image). If you want to create a new freeform AOI, click the **New AOI** button. This will eliminate the current AOI and give you the drawing tool to define a new one.

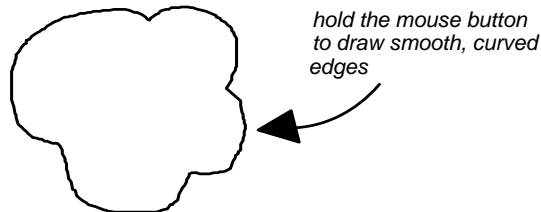
**If you have not already defined a freeform AOI** in the image, the freeform drawing cursor will be given to you. You can define a freeform AOI using one or more of the following techniques:



**Defining polyline vertices:** use this technique to create polygons that are made up of straight line segments. To create such polygons, simply click the left mouse button at each vertex (including the beginning point) in the polygon, then click the right mouse button to close the figure. The example below shows the 5 mouse clicks needed to create this 5-sided figure.



**Drawing freehand:** use this technique to create smooth edges. Smooth edges are created by simply holding the left mouse button down while you draw with the cursor. This method was used to create the curved edges in the figure below.



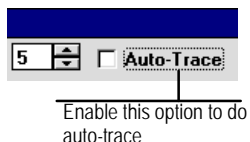
To close the figure, double-click the left mouse button, or click the right mouse button once. *Image-Pro* will automatically connect the first and last points in your line.

**Auto-tracing an object:** when you select the **Freeform AOI Tool**, the **Edge Tracking** toolbar appears in the upper-left corner of the *Image-Pro* window.

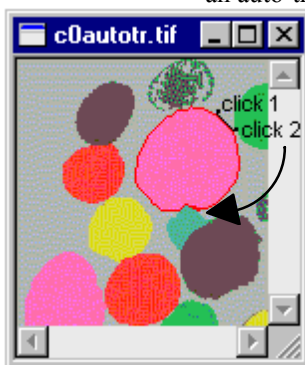


The **Auto-Trace** option lets you create an AOI by automatically tracing an object in your image.

## Image-Pro Tools



Once the **Auto-Trace** option has been selected, you must define the first two points along the edge you want to trace. The first point defines the beginning of the outline, and the second point defines the direction in which you want the outline to proceed. The example below shows how you would begin an auto-trace for an object.



After the first two points, *Image-Pro* will automatically trace the rest of the object.

When the beginning point is reached, *Image-Pro* will stop the trace. Click the right mouse button once to close the figure.

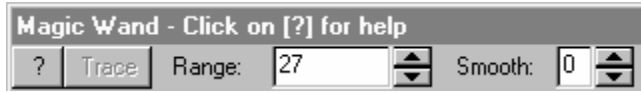
The trace will also stop if *Image-Pro* loses the edge it is tracing, or encounters the edge of the image window. When this occurs, you may either click the right mouse button to end the trace, disable the **Auto-Trace** option and complete the trace manually, or lead *Image-Pro* to the next point along the edge (just click a point where you want the trace to continue).

*Image-Pro* will not pick up the trace at all if it does not discern an edge.

**Magic Wand:** The magic wand tool makes it easier to outline an irregular AOI. Simply place the wand cursor inside the area that you want to trace, and click the left mouse button once. The magic wand will automatically trace the outline of the object(s) based on the color similarities or difference of intensity ranges between the pixel under your cursor, plus or minus a specified tolerance interval.

To outline an different region, place the cursor on that region and click with the left mouse button. To outline more than one region, hold the <Ctrl> key down while clicking on the region you want to outline. Click the right mouse button to validate the AOI and outline it in green.

The magic wand also has its own toolbar:



**? or Help:** Clicking this button displays the magic wand help.

**Trace:** Switch to the **Edge Tracking** toolbar (see following section).

**Range:** A percentage (0 - 99) of the maximum intensity for the image class. The following table displays the minimum and maximum gray levels for each class:

Image class:	Range min value	Range max value
8	0	255
12	0	4095
16	0	65535
24 RGB	0	255
36 RGB	0	4095
48 RGB	0	65535
32 floating point	0%	100%

**Smooth:** Degree of smoothing in freeform AOI (see following section).

The options in the Edge Tracking toolbar are used to fine tune *Image-Pro's* edge detection mechanism. Adjust these values if *Image-Pro* is having difficulty following an edge, or is tracing elements you do not want to included in your AOI.

**Threshold:** Set this value to describe the level of contrast between the feature you are tracing and surrounding elements. The threshold scale extends from 1 to 10. A low value represents low contrast; a high value, high contrast.

**Smooth:** This value (0-9) specifies the amount of post-filtering of the outline you want. 0 = no smoothing, 9 = high degree of smoothing.

**Speed:** Set this value to control the speed at which the trace is performed. A value from 1 to 5 can be specified. The lower the value, the slower the trace is drawn. You might slow down a trace to make it easier for you to track its progress.

**Noise:** Set this value to specify the number of pixels (1 to 6) *Image-Pro* should use between nodes along the edge it outlines. A large value results in a smoother outline (*Image-Pro* won't trace every nook and

## Image-Pro Tools

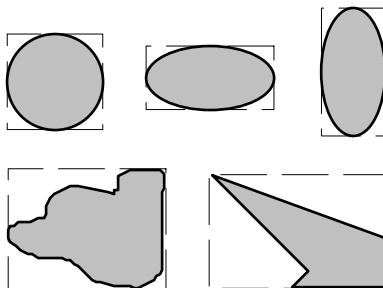
cranny), but it may cause *Image-Pro* to lose the edge more frequently. Also, specifying values over 3 or 4 can cause *Image-Pro* to overshoot curves along the edge.

*Note - You can interrupt an auto-trace at any point by pressing the space bar. You may back up the trace by pressing Backspace. To continue the trace again, simply click on the next point.*

Regardless of which method you have used to create your freeform AOI, your freeform shape will appear in your image when the right mouse button is clicked to close the figure.

The boundaries and position of a freeform AOI cannot be modified once it has been defined. If you need to change the position or edge of a freeform AOI, you must redraw it. To do this, click the **New AOI** button to eliminate the existing freeform AOI from the image, then draw the AOI again.

**Important** - some operations that read or write to elliptical and freeform AOI's require rectangular dimensions. An FFT, for example, can only operate upon a rectangular set of pixels. The same is true of the **Save As** and **Paste** commands. In these instances, the rectangular requirements are satisfied by using the pixels encompassed within a nonrectangular AOI's "bounding-box" to perform the operation. The bounding-box is defined by the smallest rectangle that completely encompasses the nonrectangular AOI. The drawing below depicts the bounding boxes that would be used for a few example elliptical and freeform AOIs.

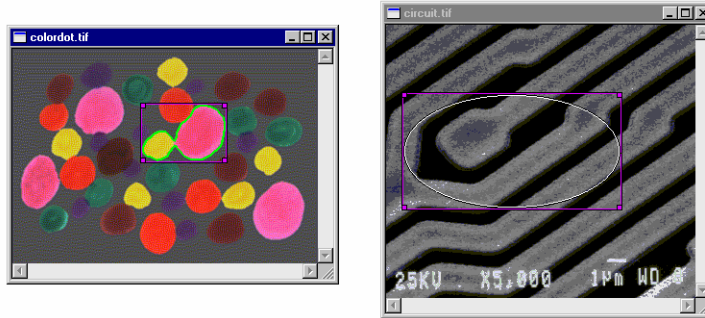


Both the AOIs themselves and the point of origin for the bounding box are important considerations in understanding how an individual command operates upon the multiple AOI. When *Image-Pro* can perform a command on the AOI itself, it does so. Otherwise, it uses the bounding box for the AOI.

Commands that operate upon two images, such as the *Operations* command, add a certain complexity. Consider, for example, an

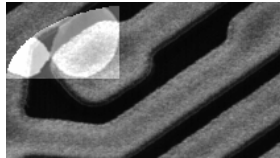
## Image-Pro Tools

*Operations : Add* command performed on the following two images, each having an active AOI (the bounding box for each AOI is shown).



In both instances, *Image-Pro* performs the following steps:

- Align the two images at the point of origin (0,0) for each AOI.
- Perform the *Add* operation only where the **Active image**'s AOI is active and the **Other image**'s bounding box coincides (that is, if the two bounding boxes are of different size, the *Operations* command will be performed only for that portion that they both intersect).
- Present the result in the **Active image**'s bounding box.



If “circuit.tif” is active, and “colordot.tif” is added to it, the resulting image shows that the bounding box of “colordot.tif” was not as wide as that of “circuit.tif.”



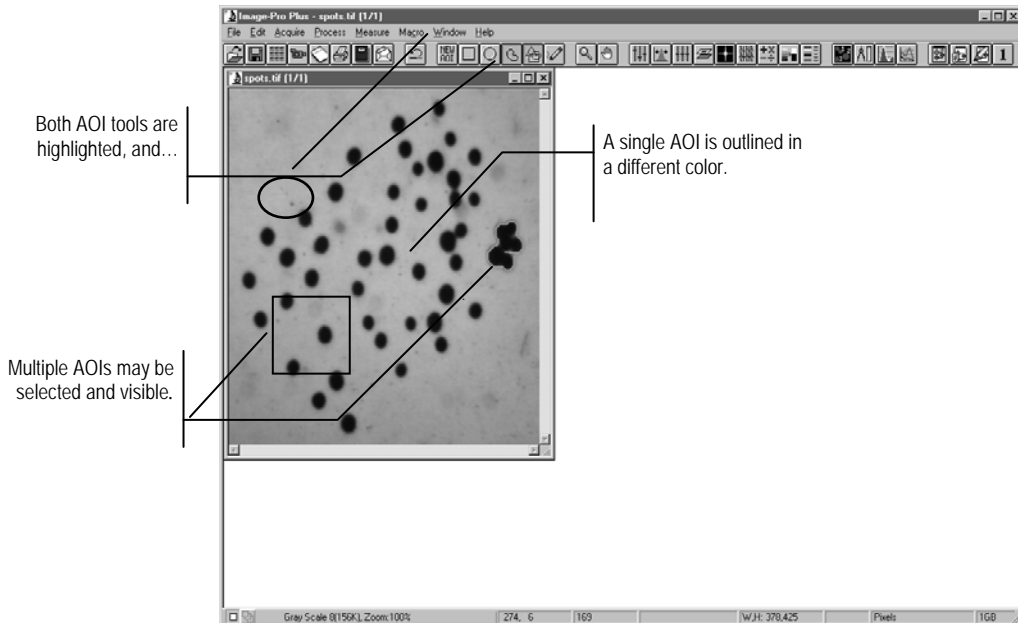
If “colordot.tif” is active, and “circuit.tif” is added to it, the resulting image shows that the bounding box of “colordot.tif” was not as deep as that of “circuit.tif.”

## Image-Pro Tools



**Multiple AOI Tool:** The **Multiple AOI Tool** is used to associate more than one area of interest with an image for simultaneous processing. A **Multiple AOI**'s outlines appear in a different color to distinguish them from that of a single AOI.

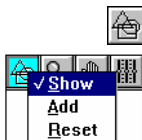
In the process of creating Multiple AOIs, the **Multiple AOI Tool** and one of the **Single AOI Tools** will both be highlighted.



To create a Multiple AOI,



**1) Create an AOI:** Click the AOI tool you want to use and place the AOI where you want it (the **Multiple AOI tool** need not be on at this point). The rectangular, elliptical, and irregular tools work as described above.



**2) Add it to the Multiple AOI:** Click the **Multiple AOI** button. A pop-up menu appears. Click **Add**. An AOI outline in a distinctive color is superimposed over the single AOI. The single AOI remains active, and you may move it and change its shape, or you may leave it in place.

Repeat steps 1 and 2 for as many AOIs as you want (the maximum is limited by the available memory on your computer).

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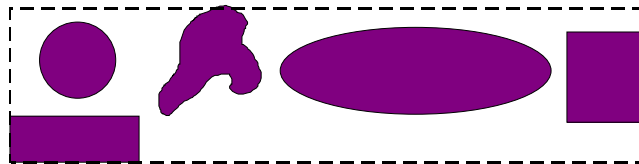
**Note** *Image-Pro* commands that operate upon an AOI will use both the **Multiple AOI** and the active **Single AOI**. If you do not want one of them to affect your application, clear its AOI tool.

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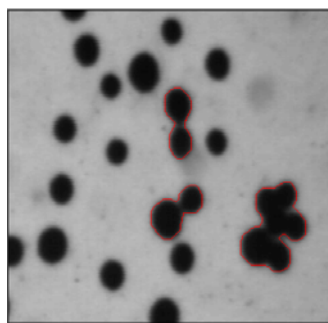
To make the Multiple AOI inactive, click the **Multiple AOI** button, then click **Show** to turn it off.

To reset the multiple AOI, click the **Multiple AOI** button, then click **Reset**. All the AOIs in the **Multiple AOI** will be reset.

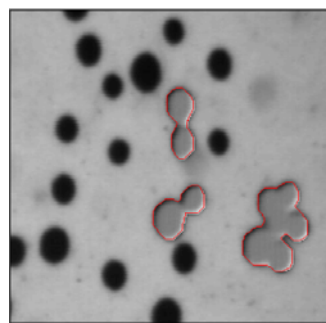
The point of origin for a multiple AOI is determined by its bounding box. A multiple AOI's bounding box is defined by the smallest rectangle that completely encompasses all individual AOIs. The drawing below depicts the bounding box for the multiple AOI.



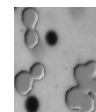
Both the AOIs themselves and the point of origin for the bounding box are important considerations in understanding how an individual command operates upon the multiple AOI. Suppose, for example you want to apply a “sculpt” filter to a multiple AOI in an image, then duplicate that image. The filter is applied only to the portion of the image contained within each individual AOI, while the *Duplicate* command uses the multiple AOI's bounding box for its borders.



*Original image,  
with Multiple AOI*



*Result of “sculpt” filter*



*Result of  
Duplicate command*

## Image-Pro Tools



**Zoom Tool:** The **Zoom Tool** is used to magnify or reduce the appearance of the image in the window. The Zoom Tool does not change the actual size of your image in terms of spatial resolution; it simply enlarges or reduces your view of the image by the specified percentage.



To use the **Zoom** tool, click the **Zoom** button (the button will be highlighted indicating that it is enabled), and move the cursor into the image window. The cursor will change into a magnifying glass.

**If you want to magnify an area.** Place the magnifying glass over the area that you want to magnify, then click the left mouse button to “Zoom-in” on that area. *Image-Pro* will enlarge the view by a factor of two. Clicking again will enlarge the original image by a factor of 4, clicking again by 8 and so forth up to 16.

To “undo” the magnification (i.e., “zoom-out” back out), click the left mouse button while holding down the **Shift** key. Each click of the **Shift-left** mouse button will “undo” the magnification, a level at a time.

**If you want to reduce the image view.** Place the magnifying glass anywhere in the image and click the left mouse button *while holding down the Shift key* to “Zoom-out” on the image. *Image-Pro* will reduce the screen dimensions of the image by half. You can continue to click until the image is reduced to 10% of its original screen size.

To enlarge the image again, click the left mouse button.

*Note - You can also magnify and reduce an image using the **Zoom** command in the pop-up menu that you create by clicking the right mouse button. This command allows you to select **Zoom In**, **Zoom Out**, or **Zoom** to a specific magnification. See **The Image Window** below.*



**Pan Tool:** The Pan Tool is used to position an image that does not fit entirely within the image window. It is an alternative to using the arrows on the scroll bars for positioning the image within the window.

To use the **Pan** tool, click the **Pan** button (the button will be highlighted indicating that it is enabled), and move the cursor into the image window. The cursor will change into the panning hand. Place the hand over the area of the image you want to move, then drag it to its new position.

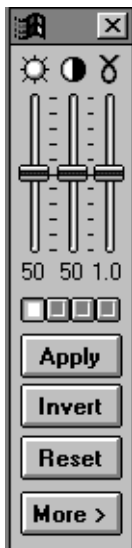


**Sync Pan, Scroll, and Zoom.** This tool is used to synchronize the **Pan**, **Scroll**, and **Zoom** movements in several open workspaces.








**Contrast Enhancement Button:** Click this button to display the BCG and other Contrast Controls. To remove, or hide, the BCG Controls, click this button again.



**BCG Controls:** The Brightness, Contrast and Gamma (BCG) controls are used to adjust the visual qualities of your image. There is an independent slider control for each characteristic:

 **Brightness:** Move this slider up to increase, or down to decrease, the image brightness. A value of 50 represents no change to the image values.

 **Contrast:** Move this slider up to increase, or down to decrease, the image contrast. A value of 50 represents no change to the image values.

 **Gamma:** Move this slider up to increase the Gamma value (increase dark area contrast) or down to decrease the Gamma value (increase bright area contrast). A value of 1 represents no change to the image values.

***Important** - these controls change the appearance of your image as it is interpreted through the Lookup Table. They do not actually change the pixel values in your image bitmap. If you want to apply the changes to your bitmap you must write the values to the bitmap by using the **Apply Contrast** command or by clicking the **Apply** button.*

**Command Tools** The **Command** Tools give you a quick way to access *Image-Pro's* major functions. This button can be used as an alternative to selecting the command from the *Image-Pro* menus.

The commands associated with each button are listed below. For a full explanation of the commands that these buttons invoke, refer to the menu command descriptions contained in this reference.

*Note - If a command is not available to you, the command tool will not respond when you click on it. In addition, the tool tip will not be given. (A tool tip is a brief description of the command, which appears when the mouse pointer pauses over a button.)*

## Image-Pro Tools



**Open Document from Disk Command Button:** Use this tool to activate *Image-Pro's Open* command.



**Save Document to Disk Command Button:** Use this tool to activate *Image-Pro's Save* command.



**View Image Database Command Button:** Use this tool to activate *Image-Pro's Image Database* command.



**Video/Digital Capture Command Button:** Use this tool to activate *Image-Pro's Acquire* command.



**Scan to New Document Command Button:** Use this tool to activate *Image-Pro's Scan* command.



**Print Current Document Command Button:** Use this tool to activate *Image-Pro's Print* command.



**Report Generator Command:** Use this button to launch *Image-Pro's Report Generator* feature.:



**Undo Previous Command Button:** Use this tool to Undo or Redo the last operation.



**AOI Management Command Button:** Use this tool to activate *Image-Pro's AOI Manager* command.



**Annotate Image Command Button:** Use this tool to activate *Image-Pro's Annotate* command.



**Close Open Documents Command button:** Use this tool to close any open images in the *Image-Pro* workspace.



**Close Open Tool Command button:** Use this tool to close any open tool dialogs in the *Image-Pro* workspace.



**Acquire Image Sets Command button:** Use this tool to activate *Image-Pro's AFA* command.



**Deconvolve Image Sets Command button:** Use this tool to activate *Image-Pro's SharpStack* command.



**Visualize 3D Image Sets Command button:** Use this tool to activate *Image-Pro's 3D Constructor* command.



**Manage Image Sets Command button:** Use this tool to activate *Image-Pro's Set Manager* command.



**Contrast Enhancement Button:** Use this tool to activate the *BCG and other Contrast Controls*.



**Automatic LUT Equalization Command Button:** Use this tool to activate *Image-Pro's Best-Fit Equalization* command.



**Reset Contrast Table Command Button:** Use this tool to activate *Image-Pro's Reset Contrast Table* command.



**Segment Command Button:** Use this tool to activate *Image-Pro's Segmentation* command.



**Background Operations Command Button:** Use this tool to activate *Image-Pro's Background Correction* command.



**FFT Command Button:** Use this tool to activate *Image-Pro's FFT* (Fast Fourier Transform) command.



**Spatial Filter Command Button:** Use this tool to activate *Image-Pro's spatial and morphological Filtering* command.



**Arithmetic and Logical Operations Command Button:** Use this tool to activate *Image-Pro's Operations* command.



**Colorize Image Command Button:** Use this tool to activate *Image-Pro's Pseudo-Color* command.



**Count and Measure Command Button:** Use this tool to activate *Image-Pro's Count/Size* command.



**Spatial Calibration Command Button:** Use this tool to activate *Image-Pro's Spatial Calibration* command.



**Manual Measurements Command Button:** Use this tool to activate *Image-Pro's Measurements* command.



**Snap Command Button:** Use this tool to activate *Image-Pro's Snap* command.



**Histogram Command Button:** Use this tool to activate *Image-Pro's Histogram* command.



**Line Profile Command Button:** Use this tool to activate *Image-Pro's Line Profile* command.



**Macro Management Command Button:** Use this tool to activate *Image-Pro's Macro* command.

## *Image-Pro Tools*



**Record Macro Command Button:** Use this tool to activate *Image-Pro's Record Macro* command.



**Edit Macro Command Button:** Use this tool to activate *Image-Pro's Macro Editor* command



**Open 3D Viewer Command Button:** Use this tool to open *Image-Pro's 3D Viewer* window.



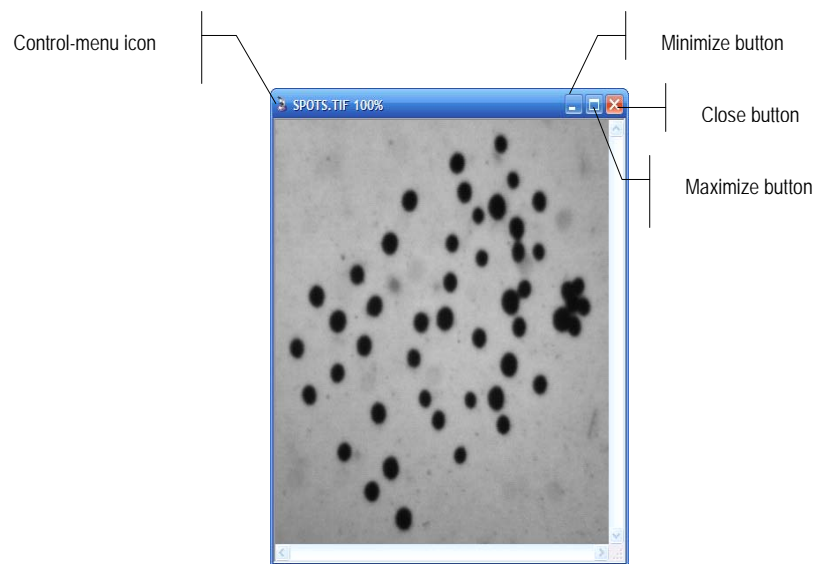
**Dark Mode Command Button:** Use this tool to switch to *Image-Pro's* dark mode for low-light situations.

## The Image Window

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Open images are placed into image windows within the imaging area of the *Image-Pro* application window. You may have more than one image open simultaneously. The active image will be the frontmost image window, and its title bar will be highlighted.

Each image window has its own set of **Control-menu**, **Minimize**, **Maximize**, and **Close** buttons.



Like any window, an image window can be moved and resized. To move an image window, drag its title bar. To resize an image window that is not maximized, drag its borders to the desired size.

The **Minimize** and **Maximize** buttons can also be used to affect the disposition of your image window. The **Maximize** button will enlarge the image window to its maximum possible size in the imaging area. The **Minimize** button will reduce the image to an icon (you might want to minimize an open image to reduce screen clutter). Minimized images are placed along the bottom of *Image-Pro's* imaging area. You can reactivate a minimized image by double-clicking its icon.

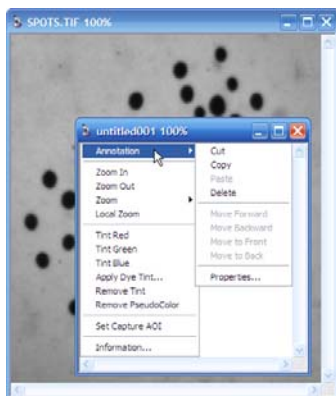
The **Close** button offers a quick way to close the image. Click the **Close** button once to close the image immediately.

## Image-Pro Tools

*Note: By default, Image-Pro will issue a warning message, reminding you to save this image, before closing a modified image. To turn off this warning, visit the Edit:Preferences menu and disable this option.*



The *Image-Pro* icon on an image workspace window is used to access the image window's system command menu. This menu contains the standard Windows system menu commands.



In addition, a few commands that are unique to *Image-Pro* may be obtained by placing the mouse cursor over the image and clicking the right button.

The **Context Menu** displays commands that are used to enlarge/reduce the active image, color it, and obtain information about the image. Additional commands may be added to this menu by the program feature or tool, such as the Annotation commands, shown at the left.

**Zoom In:** Use the **Zoom In** command to double the view of the image in the window. To double the view a second time, repeat the process.

**Zoom Out:** Use the **Zoom Out** command to reduce by half the view of the image in the window. To halve the view a second time, repeat the process.

**Zoom:** Use the **Zoom** command to enlarge or reduce the view of the image in the window. This command can be used as an alternative to the **Zoom** tool located on the ribbon along the left edge of the screen. When you select the **Zoom** command, a pop-up menu is presented. In this menu select the size at which you want to view the image. Choices range from 10% to 1600%. Selecting 100% returns an image window to its actual size.

**Local Zoom:** Use this command to enlarge the area of the active image directly under your cursor.

**Tint Red, Tint Blue, or Tint Green:** These options can be used to tint the image one of the primary colors. The image may be added to a color composite if the *Generate color composite automatically on image open* is selected.

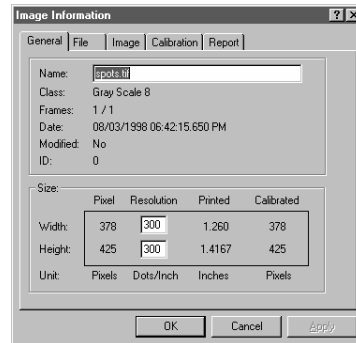
**Apply Dye Tint :** This command will open the **Edit Dye List** dialog, where you can select a dye in the **Name** List. When you click OK, that dye will be applied to the image. This will tint the image the selected color and also set the channel and wavelength properties.

**Remove Tint/Remove Pseudocolor:** These commands are available when the active image has a tint or pseudocolor, and will remove any tinting or pseudocoloring (respectively) from the image.

**Display with Tint/Pseudocolor:** This command let you apply color to an AOI or an entire image. Tint/PseudoColor will be disabled if the image is color or does not have either a tint or a pseudoColor, and enabled if a grayscale image has either. It will be checked by default, meaning that if there is a tint or pseudocolor, the image will display with the coloring, but you can click the item to turn the display off without disturbing the tint or pseudocolor itself.

**Set Capture AOI:** This command will be available when the active image has an AOI applied. When invoked, the acquisition area will be set to the bounding box of the AOI.

**Information :** Use the **Information** command to obtain information about the image.



*Note - When you have an Image-Pro operations dialog open, commands specific to that operation may be added to the pop-out menu. This is the case with the annotation command shown in the pop-out menu on the previous page.*

## Managing Open Image Windows

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When you have several images open, perhaps with accompanying dialog boxes, you may use several *Image-Pro* tools to manage them. We recommend that you close windows when you have finished using them. When you need to have several open, you may place them as you will, using the *Tile Images*, *Cascade Images*, or *Overlap Images* command, or by dragging each one by its title bar. The active image has its title bar highlighted and will be on top of any other images that are stacked (although the **Palette** dialog box, if open, may overlay it).

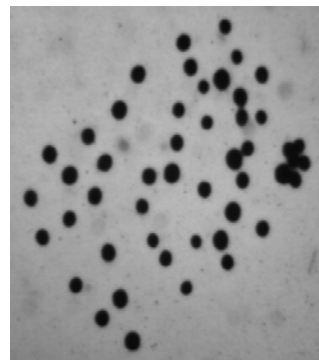
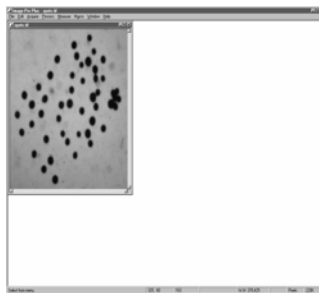
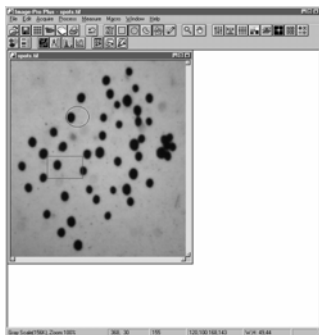
To select a different image, you may do any of the following:

- ◆ click anywhere on it with the left mouse button;
- ◆ choose it by name from the list at the bottom of the *Window* menu;
- ◆ choose the *Next Image* (<F2>) or *Prev. Image* (<F3>) command on the *Window* menu;
- ◆ use the <Ctrl>+<Tab> key combination to toggle between the current active image and the most recently active image.

To create a larger imaging area (in order to view more of an oversize image, for example), you may do either of the following:

- ◆ select the <F4> function key (*Hide/Show Borders*) to remove the *Image-Pro* window border (including title bar, command bar, toolbar, and status bar);
- ◆ select the *Hide/Show Tools* command (<F5> function key) to remove the *Image-Pro* toolbar.

The image on the left below is the normal view of an image in the *Image-Pro* application window; the image in the center shows *Image-Pro* with its toolbar removed; the image on the right shows *Image-Pro* with its borders removed.





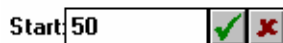
*Image-Pro* commands can be accessed while the borders are hidden by using the keyboard instead of the mouse. To access a pull-down menu, press Alt plus the menu's access key (e.g., <Alt>+<F> for the *File* menu, <Alt>+<E> for the *Edit* menu). Then select the command within the menu using the arrow keys and the enter key or the command's own access key. Some commands, such as *Undo*, also have a shortcut key combination (<Alt+Bksp> for *Undo*), which allows you to access the command directly.

To restore the toolbar or borders, repeat the process that removed them (e.g., <F4> function key to show borders again).

## Image-Pro Dialog Boxes

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Most of *Image-Pro's* commands utilize dialog boxes within which you specify options and parameters pertaining to the task at hand. A variety of standard Windows user interface techniques are utilized within the dialog boxes -- list boxes, group boxes, buttons, edit boxes and so forth, which you are already probably familiar with. In addition to these you may see the following buttons when typing a numeric value into an edit box while using *Image-Pro*:



These symbols can be used to enter or reset the value you have just typed. Clicking the check mark enters the value, and performs a limit test upon it if appropriate. As an alternative to clicking the check mark, you can press Enter. If the value is not within the allowable range the check mark and x-symbol will not be cleared. Clicking the x-symbol resets the contents of the field to its original value.

## Common Dialog Boxes

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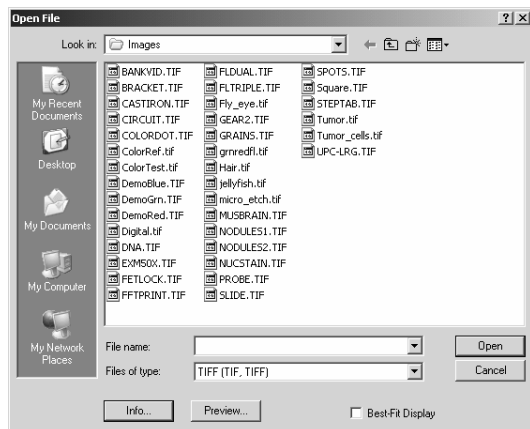
*Image-Pro* uses common dialog boxes for the commands that open, load, and save files. We describe here the features that these dialog boxes hold in common. Individual dialog boxes will then be described briefly as they appear in their proper order.

### Open and Load Dialog Boxes

*Image-Pro* uses several commands that open or load specific types of files (such as *Image : Calibration : Open*, *Edit : AOI : Load*, and *Windows : Show Gallery : File : Open*). In addition, the *File : Open* command is based on the same common dialog box, but with additional features; those features that it holds in common operate in exactly the same way.

## Image-Pro Tools

The **Open** dialog box commonly appears as follows:



**Look in:** This list box shows the available folders (sometimes called “directories”) and files. To see how the current folder fits in the hierarchy on your computer, click the down arrow. To see what is inside a folder, click it. The folder's contents will then be listed in the large list box (see **File list**, below). If you close *Image-Pro* and reopen it later, the program will remember the location of the last file you opened, and will return you to the same location.



**Up One Level:** Click this button to open the folder one level higher than the one selected in **Look in**.



**New Folder:** Click this button to create a new folder when saving.



Click the **List** button (on the left) to display the files in columns in **File list**.

Click the **Details** button (on the right) to display the files in **File list** one per row, with size, type, and date information included.

**File list:** This list box shows the folders and files in the location selected in **Look in**. To see what is inside a folder, double-click it. To open a file immediately, double-click it.

**File name:** This text box provides a space for you to type the name of the file you want. You can use \* as a wildcard. For example, you can type \*.\* to see a list of all files. You can also type the full path of a file. For example, you can type c:\myimages\image1.tif. Or, if you have used a long filename, you might type c:\myimages\view from the top.tif.

Alternatively, you can click any file in the **File list**, and the **File name** is entered for you.

If you are opening a specialized file, such as a calibration file, a filename for that file type (e.g., .CAL) will be offered for your consideration.

## *Common Dialog Boxes*

**Files of type:** This list box shows the types of files to display. This is useful for narrowing the list of files displayed to only those files with the filename extension you want..

If you are opening a specialized file, such as a calibration file, only files of that type (e.g., .CAL) can be opened.

**Open:** Click this button to close this dialog box and open the file with the name, file type, and location you specified.

**Cancel:** Click this button to close this dialog box without saving any changes you have made.

## Image-Pro Tools

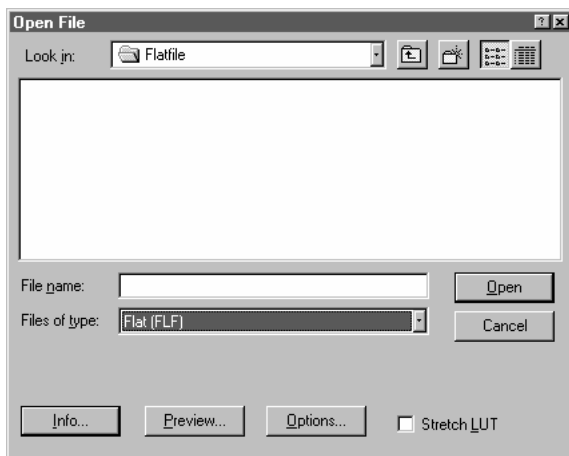
### Opening and Saving Flat Files

If you have images stored in FLAT file format, you will want to be able to open and save them using *Image-Pro Plus*. To do so, you will need to define the image type in the **Open** and **Save** dialogs.

#### To Open a Flat File:

For example, if you want to define and open a new image type, called MYTYPE. These files will have the file extension “.MYT”. Follow the steps below to open a flat file:

1. Click on the **Open** command from the *File* menu.
2. Select the **FLAT** file type, as shown below:



3. Click on the **Options** button to display the following dialog box:



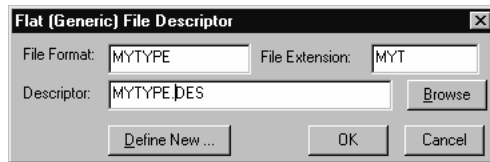
4. Change the **File Format** to MYTYPE and the **File Extension** to MYT.
5. Use the **Browse** button to select the descriptor file or type in the name i.e. `mytype.des`
6. Click **OK** to define the new image type.
7. Cancel the **Open** command.

8. Reselect the **Open** command.
9. The MYTYPE file type will be there, just as TIF and PCX files are.
10. Select your MYT flat file image and it will be displayed on the screen.

You can perform the same operations using the **Save** command to save a flat file image.

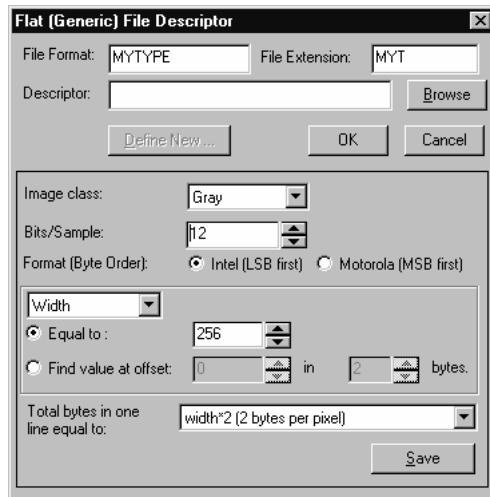
### Defining a Flat File Descriptor

You can use the Flat File dialog (below) to generate descriptions of your files.



1. Select the **File Format** and **File Extension** for the files you are going to create.
2. Click on the **Define New** button from the Flat File Descriptor dialog.

You will see the following dialog box:



3. Select the image class.
4. Select the bits/sample (8,12, 16)
5. Select the byte order
6. Define the Width, Height, Header Size and line offset.

## Image-Pro Tools

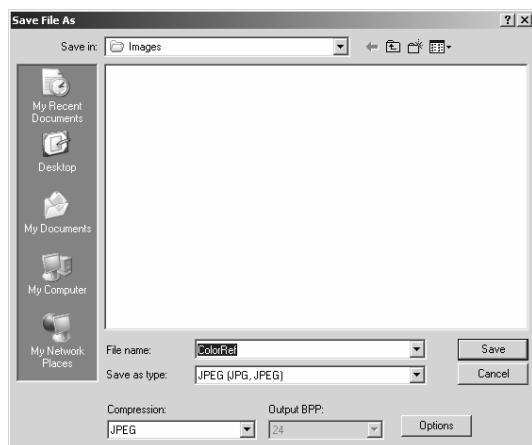
7. Click **Save** to save your new description in a file.
8. Click **OK** to finish.

You should have very specific information about the image before attempting to save it into a flat file. Please refer to *Appendix B, Flat File Format Specifications*, for more information.

## Save and Save As Dialog Boxes

*Image-Pro* uses several commands that save files (such as *Image : Calibration : Save Active* and *Measure : Count/Size : File : Data to File*). In addition, the *File : Save As* command is based on a nearly identical common dialog box, but with additional features; those features that it holds in common operate in exactly the same way.

The **Save** dialog box commonly appears as follows:



**Save in:** This list box shows the available folders and files. To see how the current folder fits in the hierarchy on your computer, click the down arrow. To see what is inside a folder, click it. If you close *Image-Pro*, and reopen it later, the program will return you to the location of the last file that you saved.



**Up One Level:** Click this button to open the folder one level higher than the one selected in **Save in**.



**New Folder:** Click this button to create a new folder..



Click the **List** button (on the left) to display the files in columns in **File list**..

Click the **Details** button (on the right) to display the files in **File list** one per row, with size, type, and date information included..

**File list:** This list box shows the folders and files in the location selected in **Look in**. To see what is inside a folder, double-click it. To save to an existing file, double-click its name..

**File name:** This text box provides a space for you to type the name of the file you want. You can use \* as a wildcard. For example, you can type \*.\* to see a list of all files. You can also type the full path of a file. For example, you can type `c:\myimages\image1.tif`. Or, if you use a long filename, you might type `c:\myimages\view from the top.tif`

Alternatively, if you want to overwrite an existing file, you can click its listing in the **File list**, and the **File name** is entered for you.

If you are saving a specialized file, such as a histogram file, a filename for that file type (e.g., .HST) will be offered for your consideration. You may overwrite or modify this suggested name as you choose.

**Save as type:** This list box shows the type of files to save. This is useful for narrowing the list of files displayed to only those files with the filename extension you want

If you are saving a specialized file, such as a histogram file, you will only be able to save to that type.

**Save:** Click this button to close this dialog box and save the file with the name, file type, and location you specified.

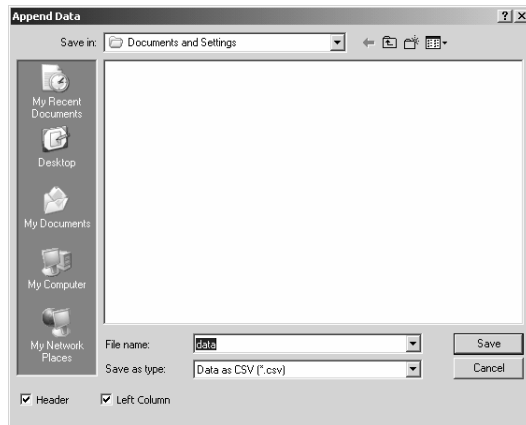
**Cancel:** Click this button to close this dialog box without saving any changes you have made.

## Append Data Dialog Box

*Image-Pro* uses several commands (such as *Measure : Count/Size : File : Append Data to File* ) that attach files to the end of other files. In addition, the *File : Save As* command is based on a nearly identical common dialog box, but with additional features; those features that it holds in common operate in exactly the same way.

## Image-Pro Tools

The **Append** dialog box commonly appears as follows:



**File name:** This text box provides a space for you to type the name of the file where you want to append the information.

**Folders:** You can type the full path of a file. To attach to an existing file, you can click its listing in the **File list**, and the **File name** is entered for you.

If you are saving a specialized file, such as a histogram file, a filename for that file type (e.g., .HST) will be offered for your consideration. You may overwrite or modify this suggested name as you choose.

**Save as type:** This list box shows the type of files to save. This is useful for narrowing the list of files displayed to only those files with the filename extension you want..

If you are saving a specialized file, such as a histogram file, you will only be able to save to that type.

**Header:** Check this box to create a header in the appended file. When appending, if you want to append this data directly after the preceding data, you will need to turn off the header check box.

**Left Column:** Check this box to align the appended file with the left column of the existing file. When appending, you will want to use the same settings as you did for the previous file.

**OK:** Click this button to close this dialog box and append the file with the name, file type, and location you specified.

**Cancel:** Click this button to close this dialog box without saving any changes you have made.



## Data Exchange

---

*Image-Pro* offers several ways for you to transfer data between *Image-Pro* and other Windows applications. These include clipboard transfers and dynamic data exchange.

### Clipboard Transfer

In Windows, the Clipboard is a temporary storage location used to transfer data (including images) between documents and between applications. *Image-Pro's Edit* menu has one command for placing image data on the Clipboard (*Copy*) and one for moving it from the Clipboard to the destination location in the active image (*Paste*). When you *Copy* an item from your document (such as copying an AOI from an image), that item is placed on the Clipboard. It is not removed from the original location, nor is it copied to any other location than the Clipboard. To place the item in the destination image, use the *Paste* command; this puts whatever image data are currently on the Clipboard into the new location. (Note that if the Clipboard contains any data other than image data, the contents cannot be pasted into your image.) You may then use the 4-way mouse cursor to move the item to be pasted within its destination document; set the item by clicking the right mouse button. Pasting an item does not remove it from the Clipboard; placing something else on the Clipboard overwrites it. If nothing is on the Clipboard, the *Paste* command will be dim.

Only one item may be placed on the Clipboard at a time. The next time you place information on the Clipboard, even if it is from a different application, the new information overwrites what you had placed on the Clipboard.

### Dynamic Data Exchange

Dynamic Data Exchange (DDE) is another mechanism to exchange data between applications. *Image-Pro* allows you to insert reports directly into Microsoft's Excel spreadsheet application. The *Window : Show Output Window* command has a *File* menu containing the *Data Export* command. When you select *Data Export*, the transfer takes place right away (unless *Image-Pro* is unable to open Excel).

You may prefer to set some options before you perform the data exchange. Do so with the *Export Options* command, which lets you set the name of the sheet in Excel that will receive the data, as well as the row and column number with the sheet and whether or not the row and column are to be incremented before sending data the next time (thus allowing a time sequence of information to be preserved). The Export Options dialog is shown here:

## Image-Pro Tools

DDE is a language-dependent protocol. We recommend that you use it with an English version of Excel only. If you want to work with another language, modify the English commands found in `IPWIN32.INI` in section `[DDEEXCEL]`. The "Comma" parameter is not an Excel command; it tells *Image-Pro* to use the comma (",") instead of the period (".") for a decimal point.

DDE commands are language specific and IPP uses English commands by default. For non-English Excel (Microsoft office) the translated version of commands can be specified in `ipwin.ini` in `DDEEXCEL` section. By default this section includes only a couple of items, such as:

```
[DDEEXCEL]
Sheet=
Append=1
ProgramPath=C:\Program Files\Microsoft
Office\Office10\EXCEL.EXE
```

Language-specific DDE commands can be added below. Here are the possible entries (in English):

```
Activate=ACTIVATE
Select=SELECT
Workbook=WORKBOOK
Cell=R%C%
Paste=PASTE
System=SYSTEM
Topics=topics
Comma=0
```

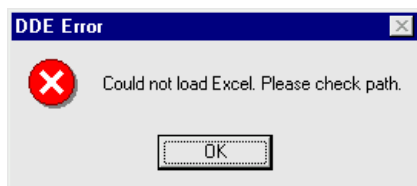
The cell addressing is controlled by the "Cell=R%C%" line.

For German it has to be : Cell=Z%S%  
For French and Spanish: Cell=L%C%

Numeric values will by default be formatted using the operating system's regional setting for the decimal separator (e.g. the '.' decimal point character in the United States). A line can be added to the `[DDEEXCEL]` section to override the default character that is used for numeric formatting. The "Comma" setting is not an Excel command; it tells *Image-Pro* whether to use the comma (",") instead of the period (".") for a decimal point: Comma = 1 set to 1 to use comma separator, 0 to use '.'

## Using Data Export

Select the **Data Export** command to transfer data to Microsoft's Excel spreadsheet. Before selecting **Data Export**, you should use **Export Options** to determine the correct placement (within Excel) for the data. When you select **Data Export**, the transfer occurs with little visible evidence that it has happened. By switching to the Excel application, you can confirm that the transfer has occurred. If you select **Data Export** before opening your Excel application, *Image-Pro* attempts to start it. If it cannot, the following message appears:

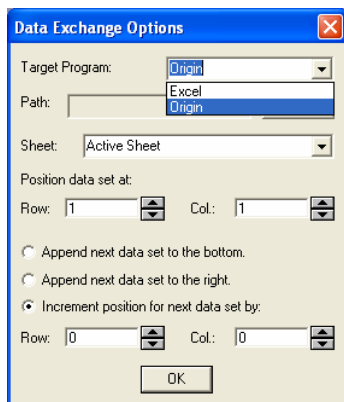


If you receive this message, check to be sure that your computer's Search Path includes the directory containing Excel and that you have sufficient memory to run both simultaneously.

If you have Origin installed on your computer, *Image-Pro Plus* can export data to that program as well.

**Export Options** : Select the **Export Options** command to tell *Image-Pro* how and where to export the current data via DDE.

When you select the **Export Options** command, the **Dynamic Data Exchange Options** dialog box is displayed:



## Image-Pro Tools

**Target Program:** Select *Excel* or *Origin* as the target program.

**Sheet:** Select the sheet within *Excel* to which you want the data transferred. If *Origin* is selected as the target program the *sheet* combo-box is populated with the worksheet names of the *Origin* program. “*Active sheet*” is the default choice.

---

**Note:** The **Path** and **Browse** buttons are disabled in *Origin*, because *Origin* is located by registry entries and does not require user input. When *Origin* is selected as the target program all export operations called from multiple data windows via the “*Export to Excel*” menu item will be redirected to *Origin*.

---

**Position data set at:** Row and Column: Use the spin buttons to select the starting row and column within Excel to receive the transferred data. This setting tells Image-Pro where to place the data the first time you execute the Data Export command after setting this option. If you want successive calls to be placed below or to the right of the current data, choose one of the option buttons below.

**Append next data set to the bottom:** Choose this option button if you want successive Data Export commands to array your data below the previous data. *Image-Pro* will augment the row by the amount needed.

**Append next data set to the right:** Choose this option button if you want successive Data Export commands to array your data to the right of the previous data. *Image-Pro* will augment the column by the amount needed.

**Increment position for next data set by :** Choose this option button if you want successive Data Export commands to array your data a fixed number of rows and columns to the right and down. The default increment setting of 0,0 will overlay successive data.

## **The File Menu**

---

*File* menu commands are used to capture, open, close, print, store, and retrieve images. You may also use the commands on this menu to convert an image or group of images from one format to another. The *File* menu also contains the *Image-Pro Internet* functions that allow you to transfer text and images from one *Image-Pro* user to another.

File:New

## New

---

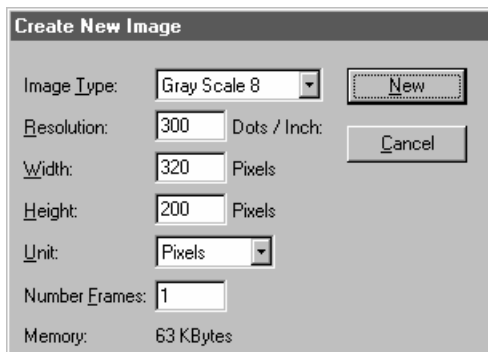
Use the *New* command to create an empty image window of the specified dimensions and class. After creating an empty image window, image data can be copied into it using the *Paste* command.

---

**Note:** To create an image window from the contents of the clipboard, use the *Paste New* command.

---

When an empty window is created, all pixels are initialized with the highest intensity value for its type (e.g., 255, or white, for an 8-bit *Gray Scale* image). If a *Palette* window is created, its palette will be initialized with 256 increasing levels of gray.



**Image Type:** From this list box select the kind of image you want to create. **Image Type** defines the type and depth in *bits per pixel* (BPP) of your new image. The following image types can be selected:

IMAGE TYPE	BITS PER PIXEL
<i>Gray Scale 8</i>	8 BPP
<i>Palette</i>	8 BPP (256 colors)
<i>Gray Scale 12</i>	12 BPP
<i>Gray Scale 16</i>	16 BPP
<i>Floating Point</i>	32 BPP
<i>True Color / RGB 24</i>	24 BPP (8 bits per color channel)
<i>RGB 36</i>	36 BPP (12 bits per color channel)
<i>RGB 48</i>	48 BPP (16 bits per color channel)

**Resolution:** Within this box you can specify a logical image resolution in terms of dots-per-inch (DPI). If the **Units** selection is set to **Inches**, this value will be used to calculate the spatial resolution (i.e., dimensions in pixels) of your new image. For example, if you set **Resolution** = 100, and **Width** and **Height** = 2" x 2", your image dimensions will be established at 200 pixels x 200 pixels. If the **Units** selection is set to **Pixels**, you may assign a logical resolution to the image, but it will not be used to determine the image's spatial resolution.

After the image is created, **Resolution** can be viewed and changed using the *Info*. command. *Image-Pro* stores the resolution value with most file types (TIFF, for example). *Image-Pro* will use this information only to determine the image size for printing; however, many external programs including the *Report Generator*, use this resolution to scale an image when it is imported; (see the *Info*. command for more about this).

**Width:** Enter image width in inches if the **Unit** field is set to **Inches**, or in **Pixels** if the **Unit** field is set to **Pixels**. The width value establishes the number of pixels the new image window will contain in the horizontal direction.

**Height:** Enter image height in inches if the **Unit** field is set to **Inches**, or in **Pixels** if the **Unit** field is set to **Pixels**. The height value

*File:New*

establishes the number of pixels the new image window will contain in the vertical direction.

**Unit:** From this list box, select the terms (**Inches** or **Pixels**) in which you want to express the **Width** and **Height** values. When **Inches** is selected, the image's spatial resolution is derived by multiplying the **Resolution** value by the **Width** and **Height** values (e.g., if **Resolution** is set to 100, and **Width** and **Height** to 2" x 2", image dimensions will be established at 200 pixels x 200 pixels). When **Pixels** is selected, image dimensions are established directly from the **Width** and **Height** values that you enter.

**Number of Frames:** If you are creating a sequence of images, enter the number of frames in the new sequence. Otherwise, for single images, enter 1.

**Memory:** This item displays the amount of memory the image requires. Memory requirements are determined by:

$$\text{Height} * \text{Width} * \text{BPP} * 1/8$$

where **Height** and **Width** are measured in pixels.

---

**Note:** Gray Scale 12 uses 16, not 12, as BPP in the above memory requirement calculation. Although a Gray Scale 12 image uses only 12 bits per pixel, the 12 bits occupy 2 bytes (16 bits) of memory. Similarly, RGB 36 uses 48 BPP in the calculations.

---

**New:** Click this button to create a new, *empty* window of the specified size and class.



## Open

---

Use the *Open* command to open an existing image file, or to create a new image from just a portion of an existing image. *Open* can also be used to preview a miniature of the image, or to view its statistics and information without actually having to open the image itself. These capabilities can be used to locate a particular image quickly.

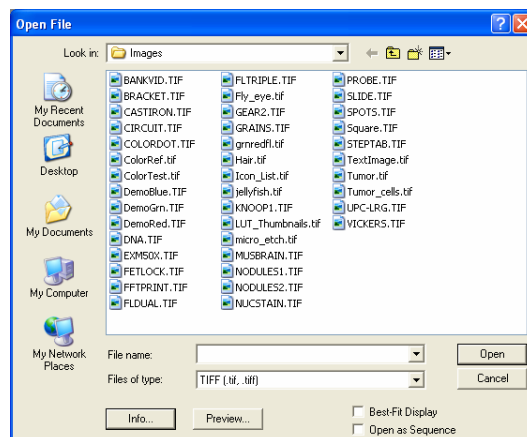
*Image-Pro* supports, and will open, many image format types. These are identified in the Files of type list box. You may also open an image sequence (\* .seq), *Image-Pro* workspace (\* .ipw) or audio/visual (\* .avi) file. *Image-Pro Plus* allows you to open many multi-dimensional set formats, including Image-Pro set (\* .ips), and InVivo (\* .exp, \* .qrs, and \* .qcl) file formats.

When you open an image, *Image-Pro* places it into a new image window. It becomes the active image. More than one image can be open within *Image-Pro* simultaneously.

---

**Note:** *Image-Pro* maintains, at the bottom of the *File* menu, a list of the last eight files you have opened. You can access any of these files by simply clicking on its file name. If no files are listed (beneath Exit), then you must use the *Open* command to open the file.

---



## File:Open

**File Name:** From this list box, select the name(s) of the file(s) you want to open. You can either type the name of the file (with its entire path, if it is not in the current folder), or use the **Look in** and **Files of type** selections to obtain a list of file names from which you can select.

Double-clicking on a file name in the large list box (where both folder and file names are listed) will automatically open it.

---

**Note:** If you simply type in the file name, be sure that the **Files of type** field correctly identifies the format of the file you want to open. Otherwise you will receive an error message when *Image-Pro* tries to open the file.

---

**Files of type:** In this list box, select the image format of the file you want to open. If you select **All Formats**, *Image-Pro* uses the file's extension to identify its format (e.g., TIF for TIFF, PCX for ZSoft files, BMP for bitmap files, and so forth). If your image file does not use standard format-identifying extensions, you must type the name of your file in the **File Name** field, *then* select its format from the **File Type** list box. Otherwise, *Image-Pro* will select a format based on the file name extension.

---

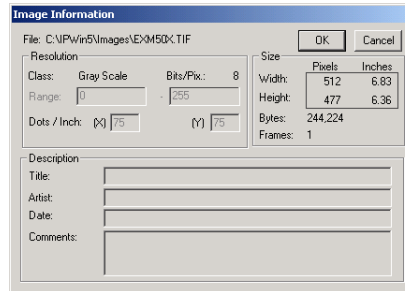
**Note:** *Image-Pro* will read only video data from a multimedia AVI file. It does not support sound, even if a sound card is present. *Image-Pro* will read only the bitmap (raster, not vector) data from a PICT file. Multi-dimensional set files, such as *Image-Pro Set* and *InVivo* file formats may open multiple image workspaces.

---

**Look in:** In this list box, select the folder from which you want to select your file

**Info:** Click this button to display the following statistics and information about your image. See *Info* command for a description of the fields in this box.

File:Open



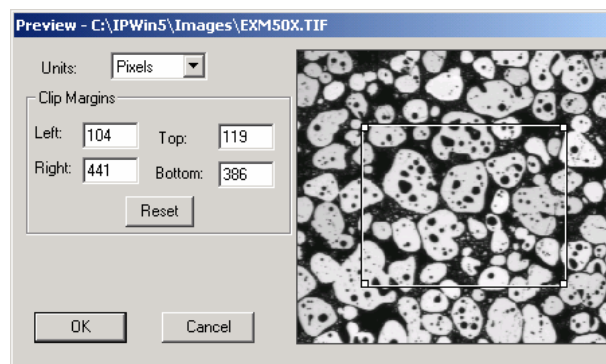
---

**Note:** Description information (e.g., **Title**, **Artist**) will be displayed only if such information had been stored with the image. Otherwise these fields will be blank.

---

When you have finished viewing the image information, click **OK** or **Cancel** to return to the **Open** dialog box.

**Preview:** Click this button to preview your image in miniature size. In preview mode, you can use the **Clip Margins** options to select a portion of your image to open, rather than opening the whole thing.



---

**Note:** When you preview a file that supports multiple image resolutions, you will be asked to select at what resolution you would like to open the image. If you are previewing a file simply to see if you have selected the correct one, select the lowest resolution available for the Preview, and then cancel out of the Preview mode before loading the image.

If you are previewing the file to select a sub-area to load, make sure that you are selecting the resolution that you actually want to load and

## File:Open

use. Once you have selected a resolution to preview, and click **OK**, the file will be loaded at that resolution.

---

**Units:** From this list box, select the unit of measure you want the **Clip Margins** values expressed in.

**Clip Margins:** Within the margin boxes, enter the corner coordinates of the image area you want to open. The **Clip Margins** values determine whether all or part of the image is opened. If these values are untouched, the entire image will be opened; if the values are changed, *Image-Pro* will open the part of the image specified by these fields.

To open just a portion of your image, enter the coordinates of the upper-left and lower-right corners of the region you want opened. These coordinates can be specified by typing the appropriate coordinate value in each of the margin boxes, or by adjusting the frame around your image using your mouse (this way is easier -- see tip below).

If you modify the corners of your image, then change your mind, you can quickly reset the corner coordinates to their original positions by clicking the **Reset** button.

Once you have defined the portion of the image you want to open, press the **OK** button to return to the **Open** dialog box, and proceed to open the image as usual.

---

### *TIP*

A frame, initially encompassing the entire image, is located around the miniature in the preview box. Using the handles located at each corner of this frame, you can position it to define just the portion of the image you want to open.

- ◆ To reduce or increase the dimensions of the frame, place your cursor on one of the corner handles and drag it vertically, horizontally or diagonally, to stretch or shrink the frame to the size you need.
  - ◆ To reposition the frame, place your cursor anywhere within the frame and drag it into position.
- 

**Note:** When you open just a portion of your image, the portion you select is loaded into a new, untitled image window. This is done to protect against overwriting the original, full-sized file with a file containing only the selected portion of the image data.

---

*File:Open*

**Frames:** If you are opening an image sequence ( \* . seq ) file, multimedia ( \* . avi ) file, or *Image-Pro* workspace file ( \* . ipw ) you may preview one or more of the frames.

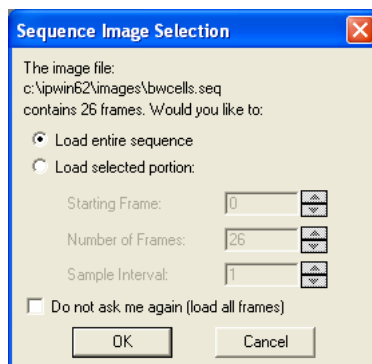


Move the slider bar from side to side to view one or more frames. The frame number is displayed over the slider bar. (The number in the slider bar only applies to the frame in the preview.)

Click **OK** to open the image (or sequence), **Cancel** to return to the application area.

**Best Fit Display:** This option takes effect only for *Gray Scale 12*, *Gray Scale 16*, *RGB 36*, and *RGB 48* images. Select this check box if you want *Image-Pro* to modify the display range to compensate for the narrow dynamic range of an image. Please see the **Display Range** command on the *Enhance* menu for a more complete description.

**Open As Sequence:** Check this box to open multiple files as an image sequence. When opening an image sequence, multimedia, or *Image-Pro* workspace file, you may choose to open only a portion of the file, as shown here.



*File:Open*

Click the appropriate radio button to load the entire sequence, or a portion of the sequence. Use the spin buttons to specify which frames to load.

**Do not ask me again:** To always open whole sequences, check this box.

Click **OK** to load the image(s).

## **Image Database**

---

Use the *Image Database* command to access Media Cybernetics' *IQbase 2.6*. Follow the instructions on your *Image-Pro Plus 7.0* installation CD to use your complementary 6-month trial version of the *IQbase Image Database*. Once you have installed *IQbase 2.6*, you will be able to archive images from *Image-Pro Plus* to *IQbase* quickly and easily. For more information about *IQbase 2.6*, please refer to the *IQbase Users Guide* PDF and online help provided as part of your *Image-Pro Plus* package.

## **Reload**

---

Use the *Reload* command to fill the active window with a new copy of the file it is linked to (the file listed on the window's title bar).

You might want to use this command if, after considerable editing, you wanted to restart with an untouched copy of the image. *Reload* is also very useful in automated procedures where a routine needs an image free from any effects of a preceding routine.

---

*Note:* *Image-Pro* loads the active window from the current copy of the file associated with it. If you have made changes to the stored file (whether from this window or another) after the image was opened, the changes will be reflected in the reloaded image.

---

Performing a *Reload* on an untitled image window has no effect.

Performing a *Reload* has no effect on the Lookup Table. If you have modified the Brightness, Contrast, Gamma settings, they will remain as you set them. If you want to return the LUT to the original settings, use the **Reset** button on the BCG toolbar.



## **Close**

---

Use the *Close* command to close an active image and remove its window from the screen. When an image is closed, its associated Histogram and Line Profile windows are closed along with it.

---

**Note:** If you have modified an image before attempting to close it, *Image-Pro* can issue a warning asking if you want to save it first. To do so, set *Edit : Preferences* to issue a warning. Otherwise, set the preference not to warn you, and *Image-Pro* will close the file immediately with no changes and no warning.

---

## Save

---

Use the *Save* command to immediately store the contents of the current window to its file (the file listed on the window's title bar) while leaving the image active in its window. If the image is in an untitled window, *Image-Pro* will display the *Save File As* dialog box.

The *Save* command can be used to save your most recent changes to disk. It is often performed as a precautionary measure during lengthy or involved processes to reduce the amount of reprocessing that might be required in the event of a system failure or operational error. When you close an image and choose not to save its changes, *Image-Pro* discards all changes made since the last *Save* operation.

---

**Note:** The **Save** command always saves the contents of the entire window, even if there is an AOI defined within it, or only a portion of the sequence is open. If you need to store just the contents of an AOI, or the active part of a sequence, use the **Save As** command.

---

## Save As

---

Use the *Save As* command to store the contents of the current window, the current AOI, or the active part of a sequence, to a file that you specify, in the format that you specify. At the end of a *Save As* operation, your image window will be associated with the new file and the new format (i.e., its title bar will display the new file name).

The *Save As* command serves several important uses beyond simply storing an image to a new file name. It is also used to:

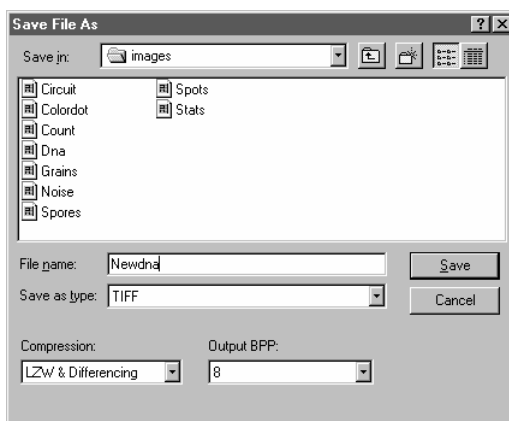
- ◆ convert a single image from one format to another (see the *Convert* command for converting multiple files). For example, if you needed a TIFF file converted to JPEG format, you would simply open the TIFF image, then use *Save As* with the JPEG format option to save it to a new file.
- ◆ save an image with different compression or quality options from those it was originally created with. For example, if your original TIFF file was uncompressed, you can use *Save As* with the LZW compression option to store it in compressed form.
- ◆ save an image to another disk or folder
- ◆ save the active portion of a sequence of images.

---

**Note:** Files containing multiple frames, or sequences, are supported only in the IPS, TIF, SEQ, IPW, and AVI formats. All other formats will save only the active frame.

---

*Save As* will allow you to store the active image back to its original file name.



## File:Save As

**Save in:** Find the folder into which you want to save the file. You may create a new folder using the **New Folder** button.

**File name:** Enter the name of the file you want the image saved to. To specify the file's location, you can either enter its entire path (disk and folder), or specify its location using the **Save in** list box.

**Save as type:** In this list box, select the format in which you want the image saved.

**Save in:** In this list box, select the drive and folder to which you want your image file saved.

**Compression:** In this list box, select the form of compression you would like the file stored in. The available compression methods will vary from one file format to another. For example, LZW (Lempel-Zif encoding) compression is an option with TIFF files. Moreover, some formats have no compression options (**None** is the only choice). For JPEG files, a compression factor of 25 is used.

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**Note:** If you plan to use the image with another program, be sure it is compatible with the selected compression method. Not all programs support compressed and uncompressed format variants.

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**Output BPP:** In this list box select the bit depth (bits-per-pixel) to store the image in. The choices in this list box will vary from one file format to another. For example, TIFF supports several depths ranging from 2 BPP to 32 BPP.

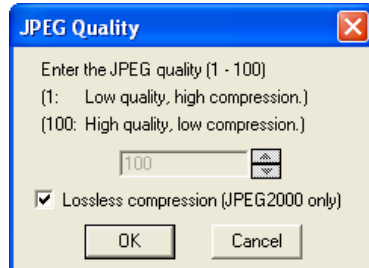
Storing an image at a lower bit depth will reduce its size (in terms of disk and memory space, not in terms of spatial dimensions), but will result in loss of information. To reduce its depth, the least significant bits in each pixel are eliminated. A *Gray Scale* image written to 4 BPP would be reduced from 256 gray levels to 16. Once an image is stored without this information, it can never be recovered. **Output BPP** should be changed only when image quality is less important than file size.

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**Note:** **Output BPP** is not available with JPEG format.

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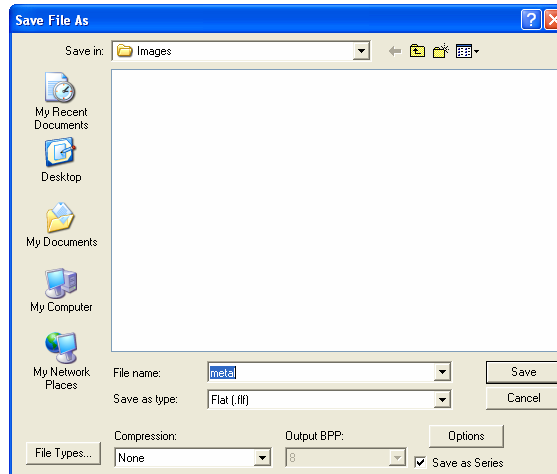
If you are saving files in the JPEG or JPEG 2000 format, you will see the **JPEG Quality** options dialog:



This dialog allows you to select the level of quality and compression that will be used to store your image. Lossless compression is available only in the JPEG 2000 format.

Click **OK** to save your JPEG file, or **Cancel** to return to the **Save As** dialog.

**Save As Series:** This option is enabled when the active image is a multi-frame sequence. When the option is selected, the frames from the active image will be saved as a series of separate single-frame image files, with sequentially numbering added as a suffix to the selected file name.

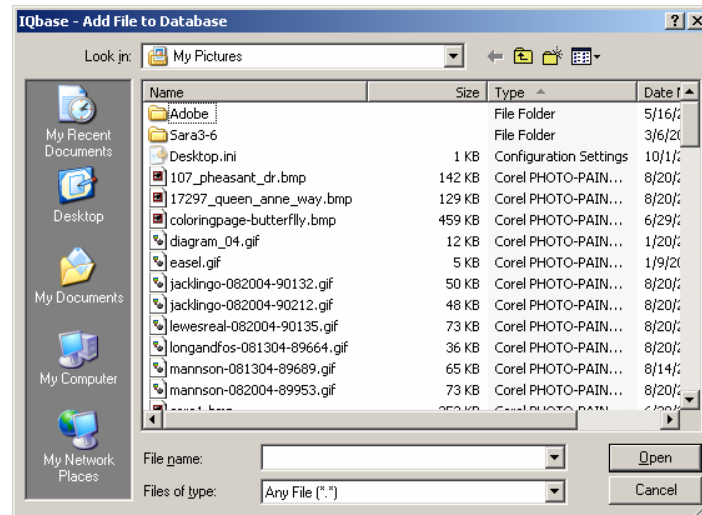


Note that when this option is selected or deselected, the list of file types that are available will change, as not all file formats are capable of saving multi-frame images.

## Archive

---

Use the *Archive* command to save an image to a folder in *IQbase 2.6*. The *Add File to Database* dialog box appears.



1. Navigate to the image file you want to add and click on it to select it.
2. Click **Open**.
3. The new file appears in the **Folders** area of the *Databases* dialog box.

A complementary 6-month trial version of the *IQbase Image Database* is included on the *Image-Pro Plus 7.0 CD*. Once you have installed *IQbase 2.6*, you will be able to archive images from *Image-Pro Plus* to *IQbase* quickly and easily. For more information about *IQbase 2.6*, please refer to the *IQbase Users Guide PDF* and online help provided as part of your *Image-Pro Plus* package.

## Convert /Batch Conversion

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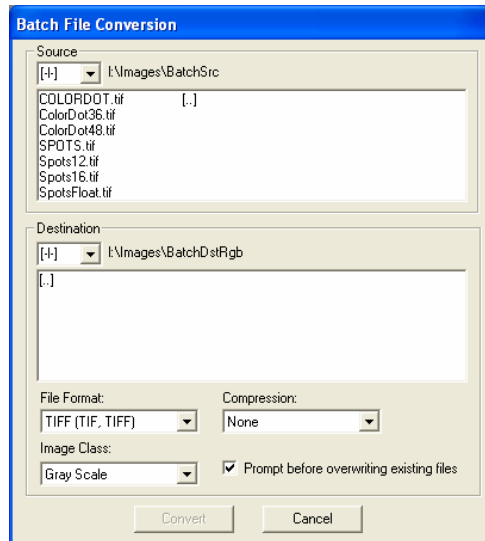
Use the *Convert* command to convert multiple files from their existing file format to a new file format. It can be used to convert a group of files of varying formats (e.g., *True Color* PCX, *Grayscale* TIFF, etc.) to a single format in one operation. It can also be used as an alternative to the *Save As* command for converting a single file from one format to another.

*Convert* writes the converted files to a folder that you specify. Converted files are given the same 8 character file names as the files from which they are converted, and are assigned an extension denoting the format they have been converted to (default extensions are noted in parenthesis after format name in the **File Format** list box).

If a file with the same name is found in the destination folder, *Image-Pro* will present a warning, asking whether the file should be overwritten.

*Convert* does not delete the source files.

When you select the *Convert* command, you will see the following screen:



**Source:** From this list box, select the image files you want to convert to the new format. Clicking on a drive letter will select that device; clicking on a folder name will display the image files within that folder.

### *File:Convert /Batch Conversion*

To select a file, click on its file name. To deselect a file you have inadvertently selected, click on its file name again. To select multiple files, press the <Ctrl> button when clicking an additional file. If you have selected a file folder that you do not have permissions to view, you may see nothing in the **Source** list other than the [...] item that will allow you to return to the previous folder.

**Destination:** From this list box, select the folder in which you want the converted files stored. The destination folder cannot be the same as the source folder. Double-clicking once on a drive letter will select that drive; double-clicking once on a folder name will select that folder.

**File Format:** In this list box, select the format you want the selected source images converted to.

**Image Class:** In this list box, select the image class you want the source images converted to. The classes available in the **Convert To** list box will vary depending upon the format you have selected in **File Format**. In JPEG format, for example, only the *True Color* and *Gray Scale* classes are available. If you are converting to a Palette image, the M/Color method will be used to create the palette.

**Prompt Before Overwriting Existing File:** Check this box to turn on the overwrite warning that appears for every single file conversion if the conversion is done more than once in the same folder.

**Compression:** In this list box, select the form of compression you would like the files stored in. This option should be selected *after* you have made your **File Format** and **Image Class** selection, because the available compression methods vary from one file format to another, and from one class to another. For example, LZW (Lempel-Zif encoding) compression is an option with TIFF files, but it is not available in PCX. Some formats have no compression options (**None** is the only choice).

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**Note:** If you plan to use the image with another program, be sure it is compatible with the selected compression method. Not all programs support compressed and uncompressed format variants.

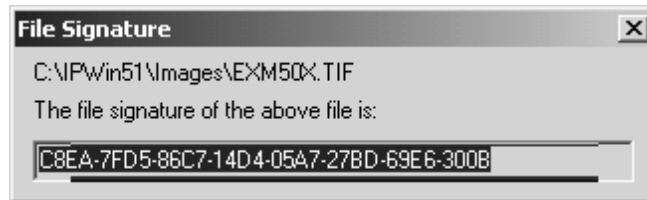
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## File Signature

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The **File Signature** feature calculates and displays the digital signature of a selected file. The signature enables you to determine if a file has changed from a previously known point. When activated, you will first see the standard **File Open** dialog. Use the **File Open** dialog to select the desired file. When you click the **Open** button, you will see the **File Signature** dialog:



You may copy this value to a string on the Clipboard, but you cannot edit it. The digital file signature will be inserted whenever an image workspace is opened or saved to or from a file.

The **File Signature** is different from the **Image Signature** function in that headers and other file information, such as image type, are included with the **File Signature**. The **Image Signature** appears on the *Process* menu.

When the **Audit Trail** feature is active (see *Edit:Preferences: Application* tab) the digital file signature of each new file will be automatically entered whenever you open or save a workspace. Whenever you select **File Signature** from the *File* menu, a copy of the digital file signature will be added to the **Audit Trail**.

## Image Signature

---

The **Image Signature** feature calculates and displays the digital signature of the active image. The signature enables you to determine if an image has changed from a previously known point. When activated, you will see this dialog:



You may copy this value to a string on the Clipboard, but you cannot edit it. If you activate a different image, the **Update** button is also activated. Click the **Update** button to see the signature of the new image. The digital image signature will be inserted whenever a new workspace is opened.

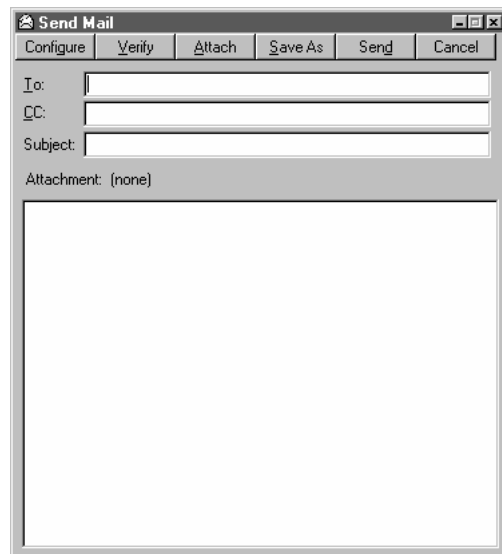
This feature differs from the **File Signature** in that it only considers visible image parameters to generate the string. **File Signature** is located on the *File* menu.

The Image Signature feature is also linked to the **Audit Trail** (*Edit: Preferences: Application* tab). The digital image signature is automatically added to the Audit Trail whenever a new image workspace is created. Whenever you select **Image Signature** from the *Process* menu, a copy of the image file signature will be added to the **Audit Trail**.

## Send Mail

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Use the *Send Mail* command to send text and images to one or more people using Internet or Microsoft e-mail. You can compose a message and include one of the active images as a file attachment. When you click on the *Send Mail* command, you will see the following dialog:



Enter the e-mail address(es) of the person or people who will receive this mail in the **To:** field. The **CC:** field allows you to send other people copies of this message. The **Subject:** field provides a header for your message. You must fill in at least one recipient in the **To:** field.

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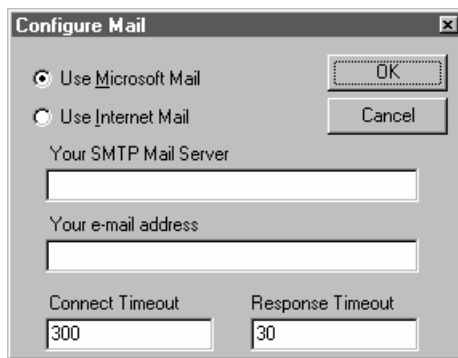
**Note:** The format of the e-mail address specified in the **To:** and **CC:** fields will depend on your e-mail system configuration. Enter the addresses in the same format that you would use in your main e-mail program.

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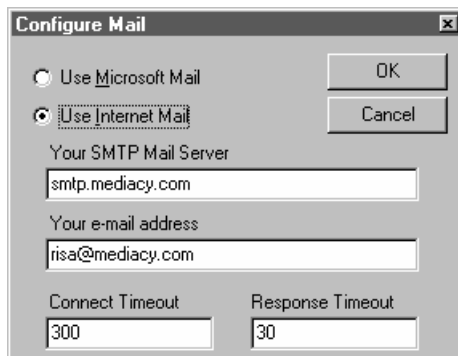
The **Send Mail** window can be resized by clicking on the control buttons, or dragging the window handles. You may have more than one **Send Mail** window open at the same time.

**Configure:** Click on this button to set up your e-mail addresses. The *Send Mail* command supports both Microsoft Mail system protocol,

and the Internet e-mail SMTP protocol. Clicking on this button displays the following dialog:



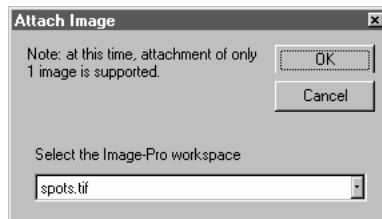
Select either **Microsoft Mail** or **Internet Mail**. *Microsoft Mail* does not have any user-defined settings. If you are using *Internet Mail*, you must enter the SMTP server and e-mail address information accurately for the *Send Mail* function to work. A sample configuration is shown below:



SMTP mail server information can be entered using either a domain name, as shown above, or an IP address, such as 172.17.0.1. The timeout parameters should not be adjusted, unless you are specifically directed to do so by *Image-Pro Plus* technical support.

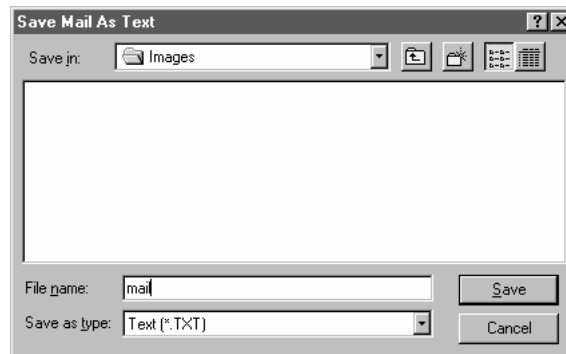
**Verify:** Click on this button to validate the addresses in the **To:** and **CC:** fields. This is only useful when using Microsoft Mail, as it is not possible to verify Internet mail addresses beforehand.

**Attach:** The **Attach** button permits you to attach an image file to your e-mail message. Any open Image-Pro workspace can be selected from the workspace list and attached. When you click on the **Attach** button, and have an image open, you will see the following message:



The current contents of the application area will be attached to the e-mail as a file. *Microsoft Mail* only supports attaching files, so the image must be stored on disk. If the image has not been saved to a file, or has been modified, you will be prompted to save the image prior to sending the message. At present, it is possible to attach only one image at a time. Attaching a new image replaces any previous attachment.

**Save As:** Clicking on this button allows you to save your e-mail message in text file format. You will see the following dialog box:



You can select the drive and folder where you want your mail stored. E-mail messages may only be stored in text (.TXT) format. Images that are sent as attachments are stored in their original form. For more information about *Save* and *Save As* commands, please refer to the previous chapter of this manual.

**Send:** Clicking on this button delivers your e-mail message to the intended recipients.

**Cancel:** Clicking this button returns you to the *Image-Pro* application area without sending your e-mail message.

## Remote File Transfer

---

Use the *Remote File Transfer* command to open files stored on any remote FTP server, or save files to a remote server. This feature makes *Image-Pro* an FTP client for the Internet File Transfer Protocol (FTP). Both standard anonymous FTP and account/password log-in FTP are supported. Clicking on this command displays the following options:

- ◆ Open remote file
- ◆ Save remote file
- ◆ Personal file server.

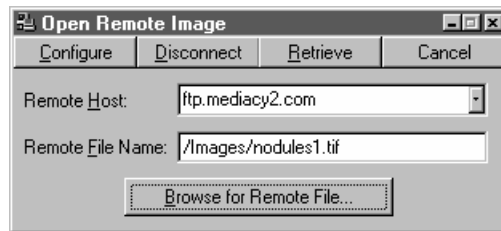
*Open* allows you to browse the remote file system for the desired file, select a file, and transfer it to your local system. Any transferred files that are recognized as image files will automatically be opened in a new image workspace. You will be prompted to save *unrecognized* files in a location of your choice in your local file system.

*Save* allows you to save your selected image or file to a location on the remote server. Images will be saved in the original image format if possible, or in TIFF format (the default). You may have multiple **Open** and **Save** dialogs open at the same time.

*Personal File Server* allows you to configure your *Image-Pro* system as a personal FTP server. You can choose to make open images and/or files available to remote clients. You control whether to allow anonymous log-in, or to require a specific account and password, as well as all the aspects of what to make available to those clients.

## Open Remote File

Clicking on this option displays the Open Remote Image dialog:

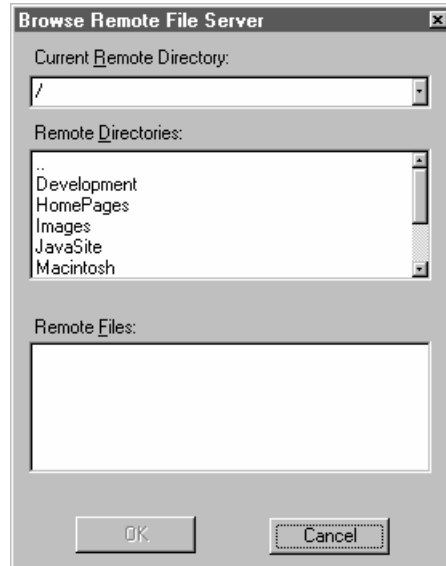


**Remote Host** is the name and/or Internet address of the remote server (or Host) where your file is located. The drop-down box contains the names of the last four Hosts that you used, or you may enter a new Host name. You may specify the Host using either an IP address, such as 172.17.0.8 or by using a domain name, such as ftp.mediacy.com.

**Remote File Name** indicates the directory location and name of the file to look for. Clicking on **Browse for Remote File** allows you to search the server, and will insert the path into the Remote File Name field.

**Browse for Remote File** retrieves a directory of the remote file system, allowing you to search for the file you want. You must specify the remote Host and connect to it before you can browse for a file. Clicking on this button displays the following dialog:

*File:Remote File Transfer*



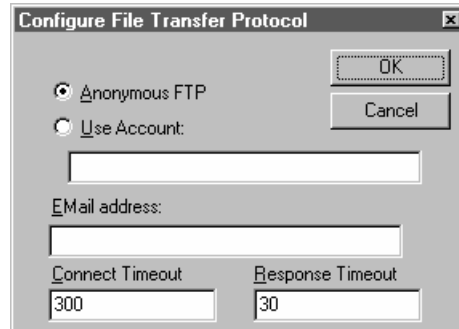
**Current Remote Directory** displays the directory where you are currently searching. The drop-down box displays any other directories that you have recently searched on this Host.

**Remote Directories** displays the list of directories on the Personal file server. File sub-directories and UNIX links appear in this list. When you double-click on a directory name in this list, the remote directory is changed to the current directory, and the contents of the other windows is refreshed. Each path that you have browsed is added to the **Current Remote Directory** combo box.

**Remote Files:** This combo box displays a list of all the files in the current server directory. When you double-click on a name in this list, or click the **OK** button, this becomes the current **Remote File Name**.

**Configure:** Clicking on this button allows you to set up the transfer protocols. Clicking on **Configure** displays the following dialog:





The **Anonymous FTP** and **Use Account** buttons are used to select the log-in method when connecting to the FTP server. The **E-mail address** field contains your e-mail address, which is customarily used as the password with anonymous log-ins. When the **Use Account** button is selected, this field will change to **Account Password**, and the password will be echoed with asterisks (\*).

Under most circumstances, using anonymous FTP should be sufficient. In this case, all you need to enter is your e-mail address.

The timeout parameters should not be adjusted, unless you are specifically directed to do so by *Image-Pro Plus* technical support.

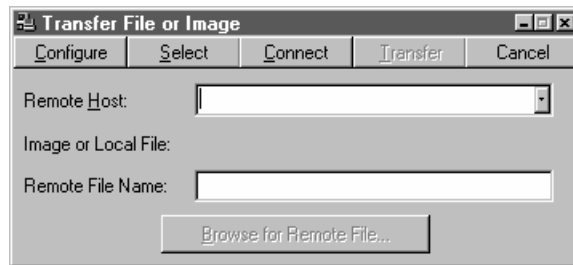
**Connect:** Clicking on the **Connect** button connects you to the remote server. After the connection is made, **Connect** becomes **Disconnect**.

**Retrieve:** Once you have identified the file you want, clicking on the **Retrieve** button will get the file for you.

**Cancel:** Clicking on the **Cancel** button closes your remote FTP connection without transferring any files.

## Save Remote File

Clicking on this option displays the following dialog:

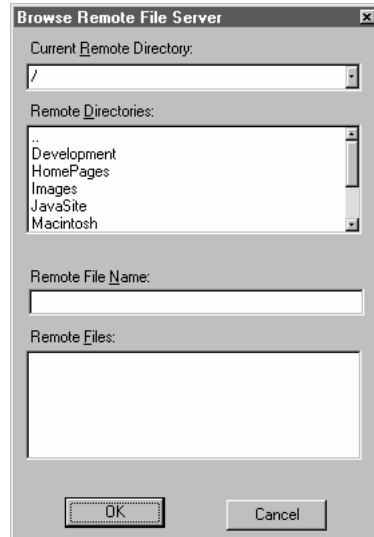


**Remote Host** is the name and/or Internet address of the remote server where your file is located. The drop-down box contains the names of the last four Hosts that you used, or you may enter a new Host name. You may specify the Host using either an IP address, such as 172.17.0.8 or by using a domain name, such as ftp.mediacy.com.

**Image or Local File:** If you have a file open in the *Image-Pro* application area, its name will appear here.

**Remote File Name** indicates the directory location and name of the file to look for. Clicking on **Browse for Remote File** allows you to search the server, and will insert the path into the Remote File Name field. The drop-down box displays any other directories that you have recently searched on this Host.

**Browse for Remote File** retrieves a directory of the remote file system, allowing you to search for the file you want. You must specify the remote Host and connect to it before you can browse for a file. Clicking on this button displays the following dialog:



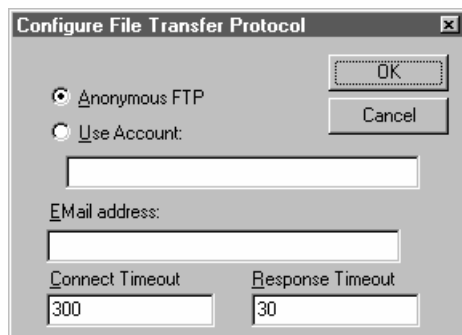
**Current Remote Directory** displays the directory where you are currently searching.

**Remote Directories** displays the list of directories on the Personal file server. File sub-directories and UNIX links appear in this list. When you double-click on a directory name in this list, the remote directory is changed to the current directory, and the contents of the other windows is refreshed. Each path that you have browsed is added to the **Current Remote Directory** combo box.

**Remote File Name:** This box displays the name and path of the remote file that you have currently selected.

**Remote Files:** This combo box displays a list of all the files you have browsed. When you double-click on a name in this list, or click the **OK** button, this becomes the current **Remote File Name**.

**Configure:** Clicking on this button allows you to set up the transfer protocols. Clicking on this button displays the following dialog:

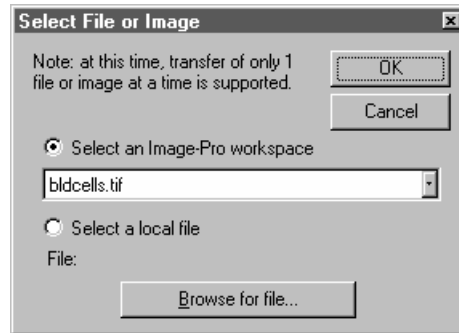


The **Anonymous FTP** and **Use Account** buttons are used to select the log-in method when connecting to the FTP server. The **E-mail address** field contains your e-mail address, which is customarily used as the password with anonymous log-ins. When the **Use Account** button is selected, this field will change to **Account Password**, and the password will be echoed with asterisks (\*).

Under most circumstances, using anonymous FTP should be sufficient. In this case, all you need to enter is your e-mail address.

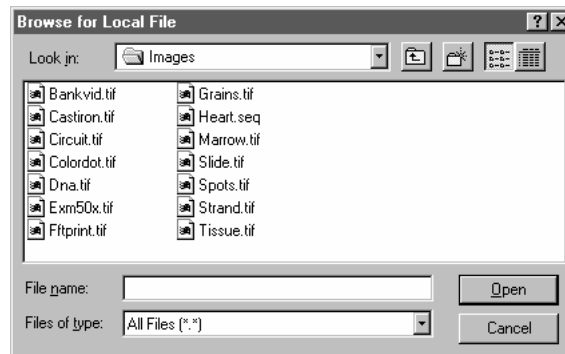
The timeout parameters should not be adjusted, unless you are specifically directed to do so by *Image-Pro Plus* technical support

**Select:** Clicking this button enables you to select the file for transfer. You will see the following dialog:



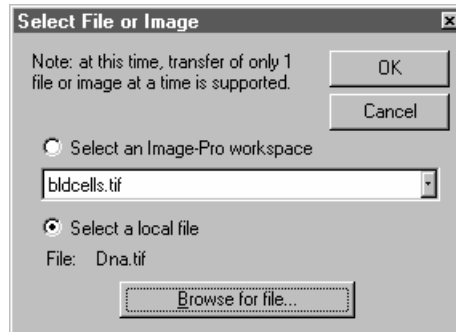
Clicking on **Select an Image-Pro** workspace will transfer the image file currently open in *Image-Pro Plus*.

To select a local file from a diskette or hard drive, choose **Select a local file**. Then, click on **Browse for file**. You can selected the desired file from the following dialog:



*File:Remote File Transfer*

Double-click on the file name, or select it and click the **Open** button. The name of your selected local file will appear on the **Select File** dialog, as shown below:



Click **OK** when you have the image you want to transfer, or **Cancel** to change your mind.

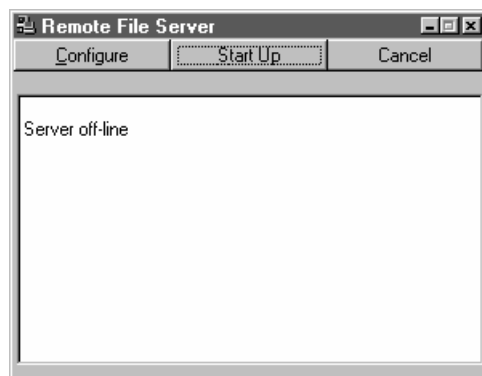
**Connect:** Clicking on the **Connect** button connects you to the remote server.

**Transfer:** Once you have identified the file you want, clicking on the **Transfer** button will send the file for you.

**Cancel:** Clicking on the **Cancel** tab closes your remote FTP connection without transferring any files.

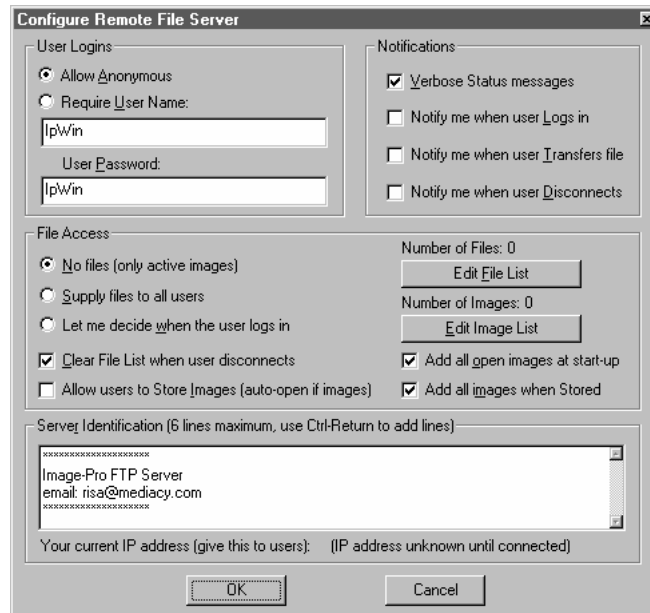
## Personal File Server

The **Personal File Server** options allow you to set up and customize *Image-Pro Plus* as the remote server. This allows other users to have access to the files you have made available, using their copies of *Image-Pro* or any other FTP client software. Your *Image-Pro* system controls the degree of access each user has. Clicking on this option displays the following dialog:



Any messages about the status of the server will appear in this window.

**Configure:** This button displays the user log-in, notification, and file access controls used by the system administrator for security purposes. Clicking on this button displays the following dialog:



**User Log-In:** This group contains the controls that permit anonymous log-ins, or require the user name and password.

**Notifications:** This group controls over the verbosity level, and specifies when you will see status messages. **Verbose status** provides information about the connection process. It is useful for diagnosing failed connection attempts. The three additional checkboxes control whether an audible alert and a status message will indicate when user logs in, transfers a file, and/or disconnects from the remote server.

**File Access:** This area contains controls that determine which files the client will see in the server's virtual file system. By default, the server will not list any files from the user's local file system, but this can be changed by checking **Supply files to all users** or **Let me decide when the user logs in**.

**Clear File List when user disconnects** assures that the file list is emptied when the user leaves, preventing sensitive file lists from being revealed to subsequent users.

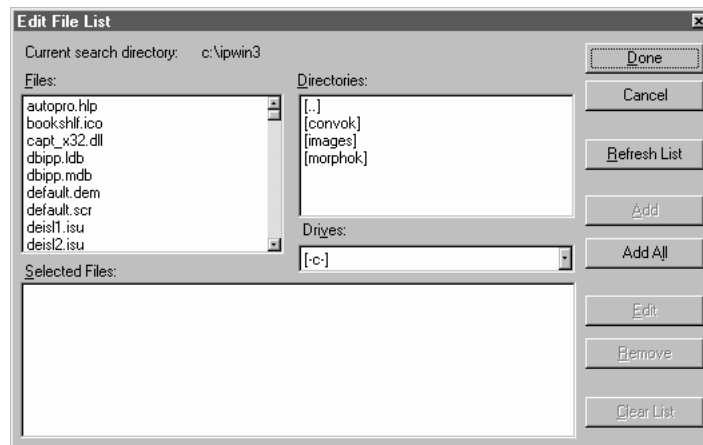
**Allow Users to Store Images (auto-open if images)** determines if your file server will allow users to store files. Only the virtual *images* subdirectory will permit users to save files, and only if this option is checked. If a remote user does transfer a file to the images directory, it will automatically be opened if *Image-Pro* recognizes it as an image file.



**Add all open images at start-up** will automatically add all open images to the *Image List* when the server is started.

**Add all images when stored** will automatically add any images stored by remote users in the virtual images directory to the *Image List*.

The **Edit File List** button controls which files the user will see in the virtual “misc” directory when he or she connects. Clicking on this button displays the following dialog box:



The **files, drives, and directories** list boxes are used to locate the files of interest. Whenever a file is highlighted, the **Add** button is enabled.

**Selected Files:** This list box contains the presentation name and path of the requested file(s). The presentation name is usually the actual file name, except in the case of duplicates or long file names that contain spaces. In the case of duplicates, a number is appended. In the case of long file names, spaces are replaced with periods.

**Add:** Clicking this button includes the highlighted file name in the **Selected Files** list.

**Add All:** Clicking this button includes all the files from the files list in the **Selected Files** list.

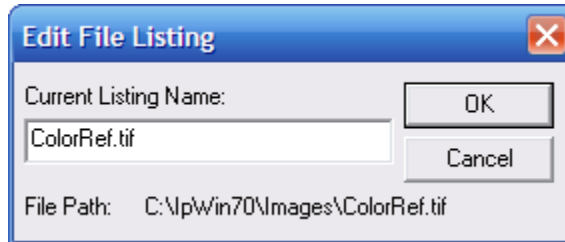
**Clear List:** Clicking this button empties all files from the **Selected Files** list box.

**Cancel:** Clicking this button returns you to the **Configure** dialog box without changing the current file list.

**Done:** Clicking this button saves your selections and returns you to the **Configure** dialog box.

*File:Remote File Transfer*

**Edit:** Clicking this button allows you to edit the presentation name of a file in the **Selected Files** list box. Clicking this button displays the following dialog box:

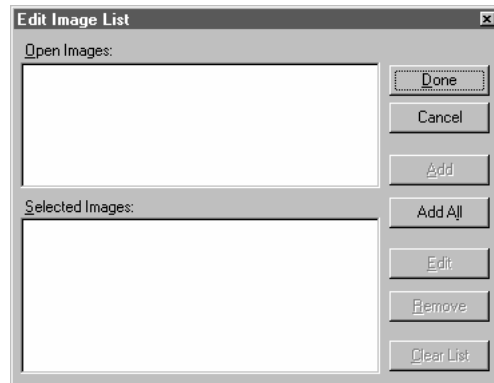


Enter the revised file name, and click **OK** to save, or **Cancel** to return to the **Edit Files** dialog.

**Refresh List:** Clicking this button refreshes the files list with the contents of the current search directory.

**Remove:** Clicking this button removes a highlighted file name from the **Selected Files** list box.

The **Edit Image List** button controls which image files the user will see when he or she connects. Clicking on this button displays the following dialog box:



**Open Images:** This list box contains the names of all the open image workspaces. Whenever an image name is highlighted, the **Add** button is enabled.

**Selected Images:** This list box contains the presentation name and workspace name of the selected images. The presentation name is usually the workspace name, except in the case of duplicates or long file names that contain spaces. In the case of duplicates, a number is appended. In the case of long file names, spaces are replaced with periods.

**Add:** Clicking this button includes the highlighted image name in the **Selected Images** list.

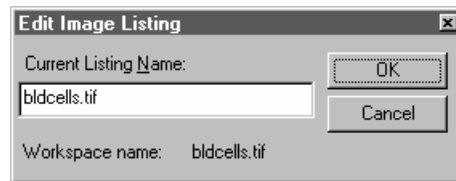
**Add All:** Clicking this button includes all the images from the **Open Images** list in the **Selected Images** list.

**Clear List:** Clicking this button empties all images from the **Selected Images** list box.

**Cancel:** Clicking this button returns you to the **Configure** dialog box without changing the image list.

**Done:** Clicking this button saves your selections and returns you to the **Configure** dialog box.

**Edit:** Clicking this button allows you to edit the presentation name of a image file in the **Selected Images** list box. Clicking this button displays the following dialog box:



Enter the revised image file name, and click **OK** to save, or **Cancel** to return to the **Edit Files** dialog

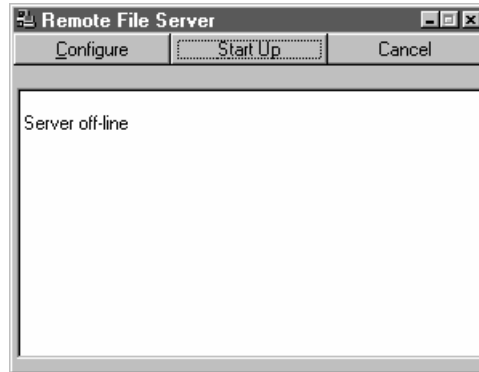
**Remove:** Clicking this button removes a highlighted image name from the **Selected Images** list box.

When you have finished editing the files and images, click **Done** to return to the **Configure Personal file server** dialog.

The **Server Identification** area allows the system administrator to edit the “welcome message” that the user sees when he or she logs into the system.

When you have finished configuring the system, click **OK** to return to the main **Remote file server** dialog.

*File:Remote File Transfer*



**Start-Up:** Clicking this button puts your personal FTP server online, and ready to receive user connections. While the server is online, the **Start-Up** button changes to **Shut Down**.

**Shut Down:** Clicking this button takes the server off-line.

**Cancel:** Clicking this button returns you to the main *Image-Pro* application area.

## **Remote Conference**

---

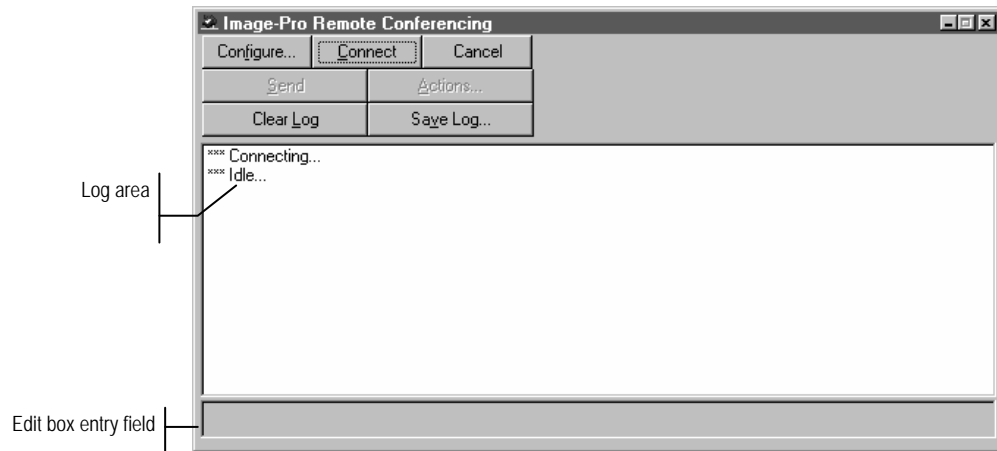
Use the *Remote Conference* command to communicate with other on-line individual using the Internet Relay Chat (IRC) protocol. You may send image files in TIF format to other users. For Image-Pro users, these files will be opened automatically. Other IRC client users will be able to save the image to a file. When you select the *Remote Conference* command, you will have two options:

- ◆ Open Remote Conference
- ◆ Personal Conference Server.

Each of these options will be discussed in the following section.

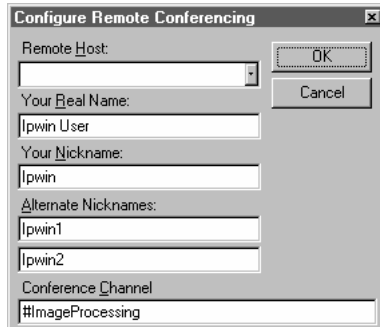
## Open Remote Conference

This option allows you to initiate a link between your computer and any other users currently logged-in to the remote server. Clicking on the **Open Remote Conference** option displays the following dialog:



Messages typed in to the edit box entry field at the bottom of the dialog will be sent to the current channel whenever you press the **<Enter>** key or click the **Send** button. Messages that you send through the remote conference channel appear in the message window preceded by the ">" character. Messages from other users will appear preceded by their nicknames. Status or informational messages appear preceded by three asterisks (\*\*\*) ; IRC actions are preceded by two asterisks (\*\*); and other types of messages by one asterisk (\*).

**Configure:** This button lets you specify the name of the remote host, your name and nickname, and the conference channel used for communication between machines. Clicking on the **Configure** button displays the following dialog box:



**Remote Host:** The remote IRC host can be specified using an IP address, such as 172.17.0.8, or as a domain name, such as ImagePro.mediacy.com. Once you have entered and saved a remote host name, it can be selected from the pull-down box.

**Your real name:** Enter your real name, including spaces and abbreviations here.

**Your nickname:** The IRC protocol requires each person to connect using a nickname. This nickname is limited to eight characters, does not allow for punctuation or spaces, and is case-sensitive.

**Alternate nicknames:** Because the nickname is limited to eight characters, there is the possibility that more than one person will try to use the same nickname, which the IRC protocol does not allow. If this occurs, Image-Pro will attempt to connect using the alternate nicknames. Failure of all three nicknames will cause the connection to fail.

**Conference channel:** The IRC protocol requires all communication to take place on a “channel.” Users can join an existing one, with #ImageProcessing being the default setting for Image-Pro. Every channel name must begin with the # character (pound sign). Other types of punctuation and spaces are not allowed in the channel name.

*File:Remote Conference*

**Connect/Disconnect:** The **Connect** button is used to connect to the IRC server currently configured. When the connection is established, this button changes to **Disconnect**. **Disconnect** is used to close an active connection at any time.

**Cancel:** Click this button at any time to close the active connection and return to the *Image-Pro* main window.

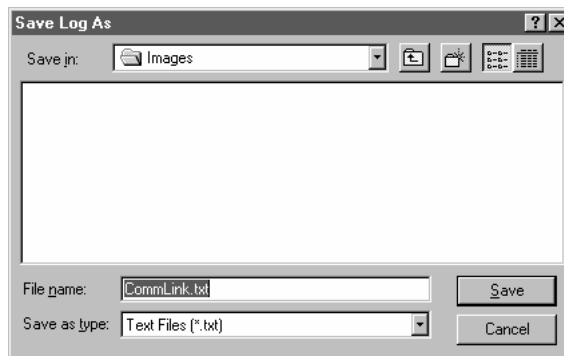
**Send:** Click this button to send your message, or press <Enter>.

**Actions:** Extensions to the IRC protocol provide the ability to indicate “actions” to the other users in the channel. Typically, these are used to extend the range of communications when not available fact-to-face. For instance, if you send the message “*wave hello to Judy*” the other users in the channel will see “*Yournickname waves hello to Judy.*” This same set of extensions can be used to transfer a file to one of the other users in the channel.

*Image-Pro* implements a special action that other *Image-Pro* systems can recognize. It allows you to place an indicator at a specific position on a file that was previously transferred via FTP or the conference facility from one user to another.

**Clear Log:** Clicking this button empties any text from the log area.

**Save Log:** Clicking this button enables you to keep the record of the communications in a file. You will see the following dialog box:

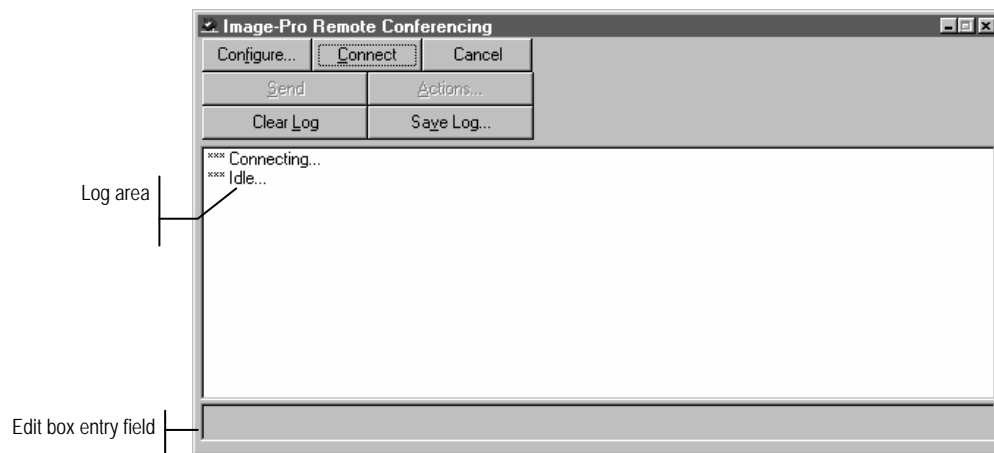


Your communications log will be saved as a text file in the directory that you specify. For more information about saving files, please refer to the section about *Open and Save dialog boxes* in the first part of this manual.



## Personal Conference Server

This option allows your *Image-Pro* system to host a conference with another *Image-Pro* system. This is useful if the IRC servers are busy, and it ensures a “private” conference. Once the connection is established, the user interface looks identical to the **Open Remote Conference** option. Clicking on the **Start-Up Remote Conference Server** option displays the following dialog:



Messages typed in to the edit box entry field at the bottom of the dialog will be sent to the current channel whenever you press the **<Enter>** key or click the **Send** button. Messages that you send through the remote conference channel appear in the message window preceded by the “>” character. Messages from other users will appear preceded by their nicknames. Status or informational messages appear preceded by three asterisks (\*\*\*) ; IRC actions are preceded by two asterisks (\*\*); and other types of messages by one asterisk (\*).

**Configure:** This button lets you specify the name of the remote host, your name and nickname, and the conference channel used for communication between machines. Clicking on the **Configure** button displays the following dialog box:

*File:Remote Conference*

**Configure Remote Conferencing**

Your current IP address:  
172.16.1.9

Your Real Name:  
Ipwin User

Your Nickname:  
Ipwin

Alternate Nicknames:  
Ipwin1  
Ipwin2

Conference Channel:  
#ImageProcessing

OK  
Cancel

**Your real name:** Enter your real name, including spaces and abbreviations here.

**Your nickname:** The IRC protocol requires each person to connect using a nickname. This nickname is limited to eight characters, does not allow for punctuation or spaces, and is case-sensitive.

**Alternate nicknames:** The alternate nicknames are not required when hosting a channel.

**Conference channel:** The IRC protocol requires all communication to take place on a “channel.” Users can join an existing one, with #ImageProcessing being the default setting for Image-Pro. Every channel name must begin with the # character (pound sign). Other types of punctuation and spaces are not allowed in the channel name.

**Connect/Disconnect:** The **Connect** button is used to put your server online. When the server is online, this button changes to **Disconnect**. **Disconnect** is used to take your server off-line.

**Cancel:** Click this button at any time to close the active connection and return to the *Image-Pro* main window.

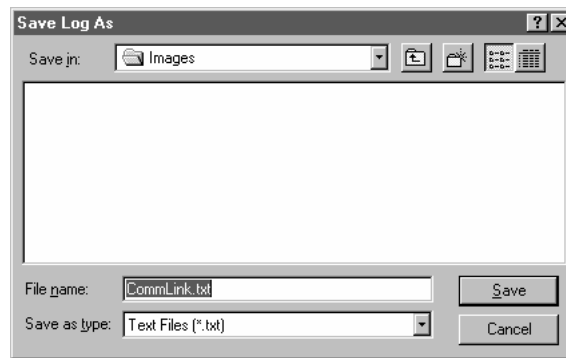
**Send:** Click this button to send your message, or press <Enter>.

**Actions:** Extensions to the IRC protocol provide the ability to indicate “actions” to the other users in the channel. Typically, these are used to extend the range of communications when not available fact-to-face. For instance, if you send the message “hello to Judy” the other users in the channel will see “*Yournickname says hello to Judy.*” This same set of extensions can be used to transfer a file to one of the other users in the channel.

*Image-Pro* implements a special action that other *Image-Pro* systems can recognize. It allows you to place an indicator at a specific position on a file that was previously transferred via FTP or the conference facility from one user to another.

**Clear Log:** Clicking this button empties any text from the log area.

**Save Log:** Clicking this button enables you to keep the record of the communications in a file. You will see the following dialog box:



Your communications log will be saved as a text file in the directory that you specify. For more information about saving files, please refer to the section about *Open and Save dialog boxes* in the first part of this manual.

## Screen Capture

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Use the *Screen Capture* command to specify the options for *Image-Pro's* Screen Capture utility. The Screen Capture utility lets you record all or part of the screen display to an image file. For example, you might want to capture one of the graphs produced by *Image-Pro* (e.g., histogram, scattergram, line profile) and import it into your word processor so it can be included in a report.

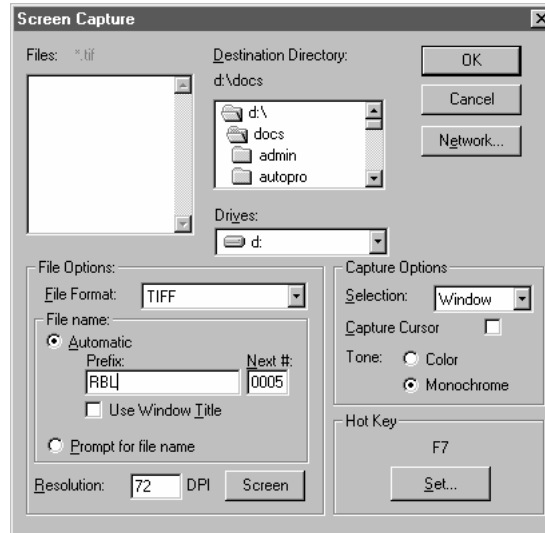
*Important - The Screen Capture utility does not function if the copy protection plug is not installed.*

To use screen capture:

- 1) Use the *Screen Capture* command to set the capture options you need (optional). These options determine how much of the screen will be captured, what key will invoke the capture and to what file the image will be stored. If you want to use the existing settings for these options, this step can be skipped.
- 2) Display the screen you want to capture.
- 3) Press the *Hot Key* (<F7>, by default).

The image file created by *Image-Pro* can be used just as any ordinary image file -- you can read it, edit it, save it or paste it into another application (the screen shots for this entire manual were captured using *Image-Pro's* screen capture facility).

When you select the *Screen Capture* command, the **Screen Capture** dialog box is presented. Tailor the options in this dialog box to suit your needs. They will become effective as soon as you close the dialog box.



**Destination Directory:** In this list box, select the folder to which you want the captured screen image saved (if the folder is on another drive, select it in the **Drive** field first).

**Drive:** In this list box, select the drive to which you want the captured screen image file saved.

**File Format:** In this list box, select the format in which you want the captured screen image saved.

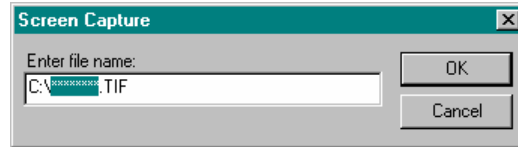
**File Name:** In this group box, select the name for the captured image. A file name can be generated automatically or manually. If you select **automatic**, Image-Pro will assign a prefix (such as GRAB) and a number to the captured image. You may also use the name of the window that you're capturing by clicking on the **Use Window Title** check box.

If the capture selection is on **Window** or **Client**, and the **Use Window Title** check box is selected, the screen capture will use the title bar of the window as the file name. If the window does not have a title bar, such as a toolbar, the screen capture tool will use the prefix.

File numbers will keep increasing to a maximum of 9999. After this maximum is reached, the file numbers will be reset to zero. You can reset the number manually by entering a number in the **Next #** edit box.

## File:Screen Capture

Selecting **Prompt for file name**, allows you to enter the name of each captured file manually. *Image-Pro* will ask you for the name of each file as part of the capture process, as shown below:



**Resolution:** You can set the resolution at which the captured image will be displayed. The default is 72 dots per inch. Clicking on the Screen button sets the image resolution to the same as the display device resolution. Setting the resolution allows you to resize the captured images without resizing the pixel width and height.

**Selection:** In this list box, specify the part of the screen you want to capture. You may select from one of the following:

- ◆ **Screen:** Select this option if you want to capture the *entire* screen.
- ◆ **Window:** Select this option if you want to capture the area occupied by the active window, including its window borders, title and menu bars.
- ◆ **Client:** Select this option if you want to capture the *client area* of the active window. This option excludes the window borders, title and menu bars from the captured image.
- ◆ **Area:** Select this option if you want to capture an area of your choice. This option lets you specify the screen area at capture time. When you invoke the screen capture utility, *Image-Pro* will provide a cross hair cursor with which you can draw a box around the portion of the screen you want to capture.

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**Note:** When you select either the **Window** or **Client** options, you will see a message prompting you to place the Window Selector tool on the window you wish to capture.

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**Capture Cursor:** Select this check box if you want the cursor included in your captured image.

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**Note:** Be sure to use either the Window, Screen, or Client Selection options when you need to include the cursor in your image. An Area screen capture will not include the cursor.

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**Tone:** You may choose to save your captured image in either color (as it appears on the screen) or monochrome. If your image will be reproduced in black-and-white, saving the captured file as a monochrome image creates smaller files.

Captured images can be converted immediately to gray scale. Monochrome images saved in TIFF file format will be compressed with LZW compression to further reduce the file size.

**Hot Key:** The currently assigned key sequence used to invoke the Screen Capture utility, and the button used to change this assignment, are enclosed in this group box.

**Set:** Click the **Set** button to change the key sequence used to invoke the Screen Capture utility. When you click **Set**, the **Set Hot Keys** dialog box is presented:



While this message box is displayed, press the key sequence you want to use to invoke Screen Capture. You may set any Character Key (e.g., a, b, c, 1, 2, 3), Function key (e.g., F1, F2, F3) or Command key (e.g., Insert, Home, Page Up), singly or in combination with one or more of the Mode keys (Shift, Alt or Ctrl).

Obviously, certain key sequences are more practical than others -- you wouldn't want to use "A" or "6" as a hot key because this would cause Screen Capture to take place any time you typed one of these keys in another application. You also want to avoid key combinations that have special meaning to Windows or your other applications. Unusual sequences such as <Ctrl> + <Shift> + <F12> or <Alt> + <Ctrl> + <End> are usually safe choices.

When you press a key combination, it appears in the **Set Hot Keys** message box. Your changes will take effect as soon as you click **OK**.

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**Note:** In *Windows Vista*<sup>TM</sup> 32- or 64- bit systems, with the *Windows Aero* theme enabled, screen capture may be excessively slow. Changing the theme from *Windows Aero* to **Windows Standard** resolves the problem.

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## Print Screen

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Use the *Print Screen* command (<F6>) to print your screen exactly as it is. You might want to do this if you have the capture options for your *Screen Capture* command set for a selection other than the entire screen, or if you want only the print copy and do not want to retain a copy of the file that *Image-Pro* creates.

When you issue the *Print Screen* command, using either the menu command or the <F6> function key, *Image-Pro* immediately scans the screen and creates a file holding the image information. Settings, such as where the file is placed, its format, and whether or not the cursor is captured, are determined by the *Screen Capture* command's current settings.

Then the **Print** dialog box appears, allowing you to set the print and other controls as you need them. These controls function just as they do in the *Print* command.

Click **Print** to initiate the print. When you have completed the print process (the Message Box reports progress), or if you want to abort the command, click the **Close** button. *Image-Pro* deletes the image file that it created.

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**Note:** You will not be able to use the **Print Screen** command from the **File** menu when certain types of dialog boxes are open. These include **Open**, **Save As**, **New**, and any other command that requires information before processing can continue.

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## Print

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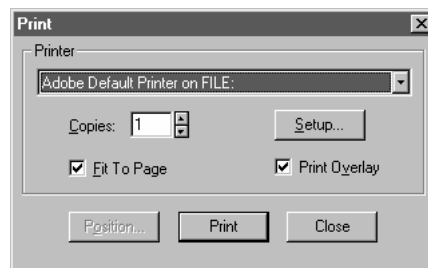
Use the *Print* command to print one or more copies of the current image, or the current AOI, to the selected output device. *Image-Pro's Print* command lets you take full advantage of your printer's capabilities. If your printer has built-in halftoning or color dithering capabilities, you can use them. Or, you can instruct *Image-Pro* to perform these processes before sending the image to the device.

The *Print* command also has facilities that let you adjust the size and position of your image on the printed page.

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**Note:** If you want to print just part of your image, use one of the AOI-defining tools to define the area you want to print before you select the **Print** command.

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**Printer:** In this list box, select the printer to which you want your image printed.

**Setup:** Click this button to access the setup panel for the printer you have selected. *Image-Pro* will present the standard setup panel for your particular printer (this is the same panel you would receive if you were setting up your printer from the Windows Control Panel). Change your printer's setup to suit your needs, then click its **OK** button to return to *Image-Pro's Print* dialog box. Note that changes to the DPI for color printing need to be made in the printer's setup page, not in IPP or on the image.

**Copies:** Enter the number of printed copies you want. If your printer supports multiple copy printing, and its buffer is large enough to contain the entire image, the additional copies will be created by the printer; otherwise, *Image-Pro* will copy the image to the device multiple times.

*File:Print*

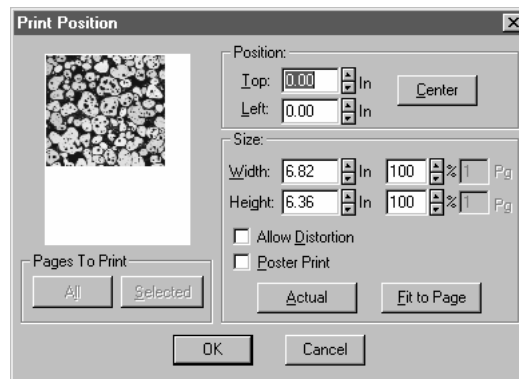
**Print:** Click the **Print** button when your settings are made to your satisfaction. The Message Box in the Status Bar announces the file it is printing and registers progress.

**Print Overlay:** Click this button to print overlaid text or drawings with your image.

**Fit to Page:** Check this box to automatically scale your image to its largest possible dimensions given the current page size and orientation.

**Close:** Click the **Close** button when printing is complete or if you decide not to print anything. If you click **Exit** during print processing (while the progress indicator shows the job is incomplete), *Image-Pro* may print all, none, or a portion of your image.

**Position:** Click this button to access the print size and position options.



**Position:** Use these options to specify the position of your image upon the printed page. Use the **Center** button to automatically place the image in the middle of the page. Or, use the **Top** and **Left** option boxes to specify a margin from the top and left edges.

**Size:** Use the options within this box to specify the printed size of your image.

**Width/Height:** Use the **Width** and **Height** selectors to scale the image to a specific height and width. If the **Allow Distortions** option is checked, you can set the height and width values independently, i.e., you can stretch or shrink the width without affecting the height and vice versa. If **Allow Distortions** is not

selected, the **Width** and **Height** values are linked. When either value is changed, a proportional change is automatically made to the other dimension to maintain the aspect ratio of your image.

**Width** and **Height** values can be set in your unit-of-measure, or in percentage form. The **Pg** option boxes are used to specify the height and width dimensions (in pages) when the **Poster Print** option is enabled.

**Actual:** Click this button to return your image to its original dimensions after you have modified its size using either the **Width**, **Height**, **Fit to Page**, or **Poster Print** options.

**Allow Distortion:** Select this option if you need to set the **Width** and **Height** dimensions independently. While this gives you full flexibility, it may result in some image distortion as *Image-Pro* stretches or shrinks the edges to achieve the specified dimensions. If **Allow Distortions** is not checked, the height and width values are linked, to maintain your image's proportions. When one dimension is changed, the other is automatically adjusted to preserve the ratio between height and width (the aspect ratio).

**Poster Print:** Select this option if you want to enlarge your image beyond a page. To produce the oversized image, *Image-Pro* “tiles” the image across an array of pages.

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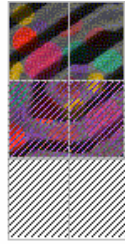
*Note:* The Position options affect the position of the entire image with respect to the entire page array. There are no mechanisms for modifying the margins of each page within the array individually. When you click the Center button, for example, the entire image is centered within the array. *Image-Pro* does not center each tile within its respective page.

---

To print selected pages of the array, use the **Pages to Print** options.

**Fit to Page:** Click this button to automatically scale your image to its largest possible dimensions given the current page size and orientation. If the **Allow Distortions** option is checked, the image will fill the entire page, from edge to edge; stretching will occur where necessary to accomplish this. If **Allow Distortions** is not checked, the image will be scaled to its largest dimensions without changing the aspect ratio between its height and width. This is the *Image-Pro* default setting.

**Pages to Print:** Use these options when printing a postered image (see **Poster Print** above) to specify the pages you want printed. If you want to print only a few pages of the array, click the **Selected** button to deactivate all the tiles. This will block them all with patterned tiles. Then use your mouse button and click on the pages to uncover just those you want to print.



If you want to print all but a few pages, click the **All** button to activate the entire array. Then use your mouse button and click just those pages you want skipped during the print process. The **All** and **Selected** buttons are simply efficient ways to activate or deactivate the

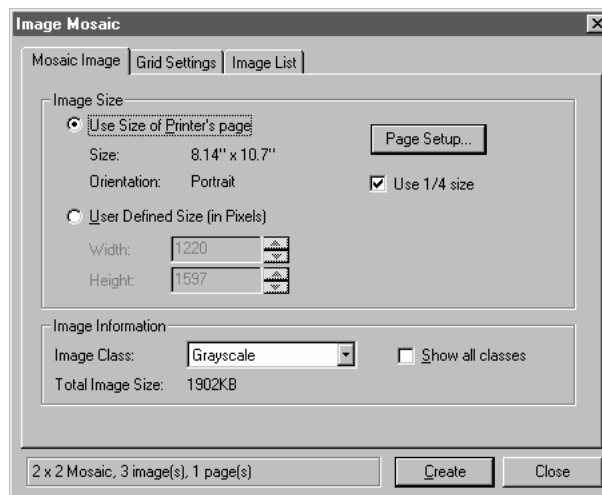
entire array in one step; they do not have to be used to select pages for print. You can manually toggle the status of a page by simply clicking upon it with the left mouse button -- if the page is already active, it will be deactivated (covered); if it is inactive, it will be activated (cleared).

The **Pages to Print** option will not be active unless the **Poster Print** option has been selected.

## Mosaic Image

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Use the *Mosaic Image* command to create a collage image made out of several open images. The resulting image can then be printed or saved to a file. When you select this command for the first time, you will see the following tabbed dialog:



The *Mosaic* tool is a **modeless** dialog with three tabs for specifying the elements of the mosaic. These tabs are:

- Mosaic Image
- Grid Settings
- Image List.

Each of these pages will be discussed in the following pages. Initially, the first page, **Mosaic Image**, will be displayed, but when the tool is opened after that first usage, the last page used will be displayed.

At the bottom of the dialog box are 3 controls that are shared by all three pages, as shown here:



*File:Mosaic Image*

The message box at the far left contains a text field indicating the current configuration of the mosaic being created. This field is updated whenever any of the pertinent settings change. The first portion of the field indicates the layout of the mosaic, in this case 2 horizontal rows and 2 vertical columns. The second portion of the field indicates the number of images currently selected for inclusion in the mosaic, in this case 3.

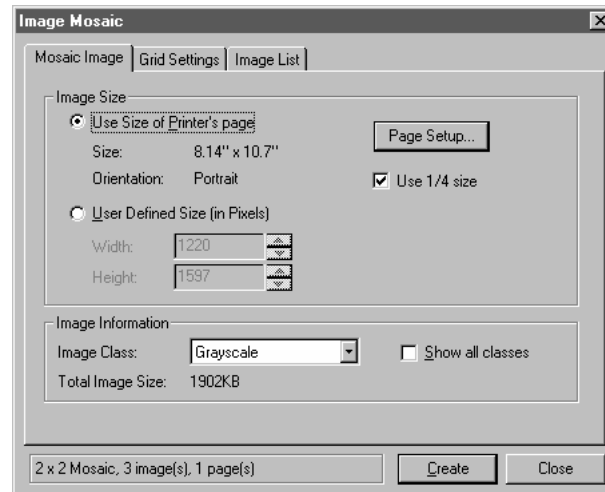
The third portion indicates the number of pages (workspaces) that will be created. In this example, all the images fit on one page.

**Create:** Click this button to start the process of creating the mosaic. This button will only be enabled when there is at least one selected image/frame. The dialog will not close when the mosaic is created.

**Close:** Click this button to close the dialog. If images have been added but a mosaic has not been created, you will see a message asking you if you want to continue.

## Mosaic Image

The **Mosaic Image** page specifies the format of the new image, as shown here:



**Use Size of Printer's page:** Click this button to use the selected printer's page size.

**Page Setup:** Click this button to change the default paper size and orientation. The page sizes available depend on the options supported by your printer.

**Use 1/4 size:** This checkbox can be used in conjunction with **Use Size of Printer's Page** to generate an image in the proper orientation and aspect ratio as the printer's page but using less memory. The size of the image will be 1/4 of the printer's width and height dimensions, resulting in an image requiring 1/16<sup>th</sup> the memory. The DPI of the mosaics created will be adjusted so that the image fills the printer page by default.

**User Defined Size:** Click this button to specify the page size in pixels.

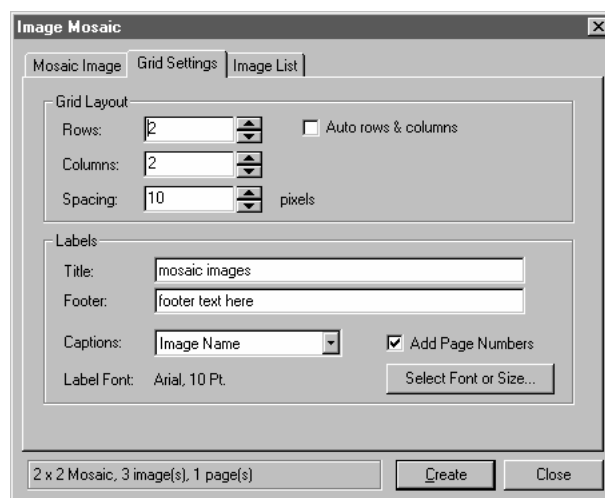
**Image Class:** Use this combo box to select the destination image class. By default, this list only displays **Best Class**, **Grayscale**, and **True Color**. When **Show all classes** is checked, the remaining image classes will also be listed. When "**Best Class**" is selected, the highest precision class of the selected images will be used for the new image,

*File:Mosaic Image*

where the order by precision is: Grayscale, Palette, Gray 12, Gray 16, Float, RGB, RGB 36, and RGB 48.

## Grid Settings

The **Grid Settings** page specifies the layout of the new image, and is illustrated below:



**Rows:** Use the spin buttons to indicate the number of horizontal rows in your image mosaic.

**Columns:** Use the spin buttons to indicate the number of vertical columns in your mosaic image.

**Auto rows & columns:** Check this box to have Image-Pro determine the number of rows and columns automatically.

The number of rows and columns can be automatically calculated if the **Auto rows & columns** is checked. If checked, the tool attempts to create a single mosaic with the largest possible square cell for each image in the Selected Workspaces list. If this is not checked, the tool will create however many pages are necessary to accommodate the number of selected images.

**Spacing:** Use the spin buttons to set the distance in pixels between the rows and the columns.

**Title:** Enter text for a title to be displayed across the top of the image mosaic.



**Footer:** Enter text for a footer to be displayed across the bottom of the image mosaic.

**Captions:** The **Captions** combo box allows you to select the type of caption to be displayed under each image. The choices include:

- ◆ <None>
- ◆ Image Name (workspace name)
- ◆ File Name (full path name)
- ◆ Date / Time (of the displayed frame, in the same format as the Image Info dialog),
- ◆ Description (one line only)
- ◆ Frame number.

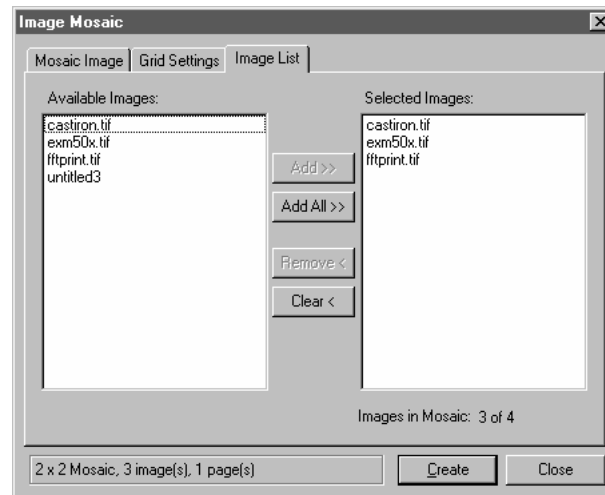
If <None> is selected for the Caption, the caption area will not be displayed and more images may fit into the mosaic.

**Add Page Numbers:** When **Add Page Numbers** is checked, page numbers will be added to the bottom right corner of the mosaic, in the format Pg. 1/3 (page one of three).

**Select Font or Size:** The **Select Font or Size** button will bring up a standard font selection dialog to select the font and/or point size settings. The default is Times New Roman, 12 Point.

## Image List

The **Image List** page selects which workspaces to use, as shown below:



**Available Images:** The **Available Images** list on the left lists all currently open workspaces. These are images that may be combined into the mosaic.

**Selected Images:** The **Selected Images** list on the right lists selected workspaces. These are the images you have chosen to include in the mosaic. The selected workspace may be listed in three different formats:

- ◆ **A single image** will be listed by name only if it is a single-frame workspace.
- ◆ **Active sequences** will be listed in a name ( X/N ) format if frame X is the selected frame of N frames.
- ◆ **Portions of sequences** will be listed as name ( N - M ) if the active portion of the sequence is selected (where N is the first selected frame and M is the last, inclusive).

**Add:** The **Add>>** button adds the highlighted images to the selected workspaces, and will only be enabled when one or more workspaces are selected in the available images list box.

**Add All:** The **Add All >>** button is a shortcut to add all of the open workspaces to the selected list, and will always be enabled.

*File:Mosaic Image*

**Remove:** The **Remove** < button removes the highlighted images from the selected workspaces list, and will only be enabled when one or more workspaces are selected in the selected images list box.

**Clear:** The **Clear** < button is a shortcut to clear the selected images list.

**Images in Mosaic:** The **Images in Mosaic** indicates the number of selected workspaces and the number of spaces available in the mosaic grid. If the **Auto rows & columns** check box is selected in the Grid Settings, the total number of spaces will automatically increase as the user adds more images into the mosaic.

The following picture shows an example of an image mosaic:



*File:Exit*

## **Exit**

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Use the *Exit* command to leave *Image-Pro* and return to Windows.

*Important - Be sure you save all the images that you want to keep before you exit. You can direct Image-Pro to prompt you to save modifications or untitled images when you exit. To do so, set Edit : Preferences: Application. If your preferences are set not to warn you, Image-Pro will close all files with no changes and no warning.*

Before *Image-Pro* returns to Windows, it updates its initialization file to record information such as your current filtering options and window arrangements. Next time you start *Image-Pro*, the environment will be returned to its present state.

You may also exit *Image-Pro* by double-clicking the **Control-menu** icon, clicking its **Close** button, or by pressing <Alt> + <F4>.

## **The Edit Menu**

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The *Edit* menu contains commands that help you manipulate the display of your image. You can cut, copy, and paste images or portions of images, make notes on the image itself, change the display of the image on the screen, and change the image class. The *Edit* menu also supplies information about the image and lets you adjust your *Image-Pro* display preferences.

## **Undo**

---

Use the *Undo* command (<Ctrl>+<Z>) to reverse the most recent actions. You can undo up to the last four operations (see the *Preferences* command for information on setting the number of Undo Buffers).

When you select *Undo*, a menu of the last four reversible operations is displayed. When you select an operation from this menu, you revert your image to its state prior to that operation. For example, if you had converted an image, applied a Sharpen filter, an Edge filter, then a Sculpt filter, undoing the *Sharpen* operation would revert to the way the image looked before the Sharpen but after the conversion.

You can undo an *Undo* operation by selecting *Redo* from the *Undo* menu.

## **Cut**

---

Use the *Cut* command (<Ctrl> + <X>) to copy the contents of the selected AOI to the Window Clipboard and replace the AOI in the image with white. If no AOI is active, *Image-Pro* copies the entire active image to the Clipboard and replaces it with white. *Cut* changes the contents of the image in the active window (i.e., *Cut* deletes the cut pixels). Any data already existing on the Clipboard will be replaced.

The data copied to the Clipboard can be pasted into the active window or into another open window using the *Paste* command. You can also create a new window directly from the Clipboard contents using the *Paste New* command.

## **Copy**

---

Use the *Copy* command (<Ctrl> +<C>) to copy the contents of the selected AOI to the Window Clipboard. If no AOI is active, *Image-Pro* copies the entire active image to the Clipboard. Copy will not change the contents of the image in the active window (i.e., copy does not delete the copied pixels). Any data already existing on the Clipboard will be replaced.

The copied data can be pasted into the active window or into another open window using the *Paste* command. You can also create a new window directly from the Clipboard contents using the *Paste New* command.



## Paste

---

Use the *Paste* command (<Ctrl> + <V>) to place the contents of the Windows Clipboard into the active image. You would use *Paste* to transfer part of one image into another.

Before you can execute the *Paste* command, you must have somehow placed valid image data on the Windows Clipboard (see the *Copy* command). If there are no data on the Clipboard, the *Paste* command will not be active. Image data can be pasted into any image class that *Image-Pro* supports.

*Image-Pro* will accept image data from other applications via the Clipboard as long as it is in Windows bitmap (BMP), Tagged Image File Format (TIF), Device Independent Bitmap (DIB) or Windows' Picture form. If you attempt to *Paste* non-image data (e.g., text or spreadsheet data) to an image window, you will receive an error message.

When you select the *Paste* command, *Image-Pro* will initially position the Clipboard data in the upper-left corner of the currently active rectangular AOI (or of the entire image if there is no active rectangular AOI). Position your cursor over the Clipboard data. Your cursor will appear as a 4-way arrow and will allow you to drag the Clipboard data to the correct position. When you are happy with its position, click the right mouse button, or press the <Enter> key, to permanently set the Clipboard data into the image. The pixels from the Clipboard will overwrite those at the specified position, according to the current **Paste Options** (see the description of **Paste Options** later in this chapter). Press <Esc> to cancel the **Paste** operation.



Drag the pasted image data when you see the 4-way cursor.  
Once the data are properly positioned, click the right mouse button, or press Enter.

If the Clipboard image is larger than the destination image, it will be cropped along the bottom and right edges. Selecting another image before confirming the *Paste* operation (left-mouse click or <Enter>) cancels the *Paste* operation.

## **Paste New**

---

Use the *Paste New* command to place the contents of the Windows Clipboard into a new image, which becomes the active image.

Before you can execute the *Paste New* command, you must have somehow placed valid image data on the Windows Clipboard (see the *Copy* command). If there are no data on the Clipboard, the *Paste* command will not be active.

The class of the new image will be the same as that of the original image.

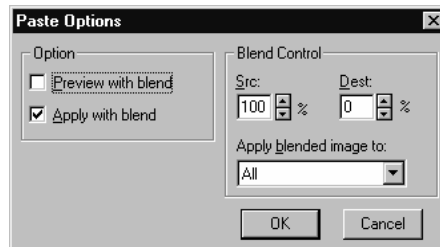
*Image-Pro* will accept image data from other applications via the Clipboard as long as it is in Windows bitmap (BMP), Tagged Image File Format (TIF), Device Independent Bitmap (DIB) or Windows' Picture form. If you attempt to *Paste* non-image data (e.g., text or spreadsheet data) to an image window, you will receive an error message.

If a nonrectangular AOI is copied into the Clipboard, *Image-Pro* uses its bounding box for the new image.

## Paste Options

---

Use the *Paste Options* command to specify settings that affect the way in which image data, pasted from the Clipboard, is combined with the target image. When you select this command, the **Paste Options** dialog box is presented.



**Preview with blend:** Enable this option to view the Clipboard data as it would appear if you were to paste it with the specified **Blend Control** options (see below). This allows you to see the effect of your blend settings while you are positioning the Clipboard data prior to actually pasting it into your image.

**Apply with blend:** Enable this option to blend the Clipboard data with the destination image. When enabled, the Clipboard and image data will be blended in accordance with the percentages and intensities specified in the **Blend Control** group box. When disabled, Clipboard data will simply overwrite the specified pixels in the destination image, regardless of the **Blend Control** values.

**Blend Control:** Use the options in this group box to control the ratio at which Clipboard data is blended with the destination image. These values are only utilized when the **Apply with blend** or **Preview with blend** options are set (see above).

**Src:** Select the percentage at which Clipboard data will be applied to the image. The larger the number, the heavier the Clipboard data is applied (e.g., if 100% is set, only the pasted Clipboard data will be visible in the result). This value is linked to the **Dest** value, such that when **Src** is changed the necessary change is automatically made to **Dest** so that the two values combined equal 100%.

**Dest:** Select the percentage of the destination image allowed to “show through” the applied Clipboard data. The smaller the

### *Edit:Paste Options*

number, the less the original image will remain visible (e.g., if 0% is set, only the Clipboard data will be visible in the result). This value is linked to the **Src** value, such that when **Dest** is changed the necessary change is automatically made to **Src** so that the two values combined equal 100%.

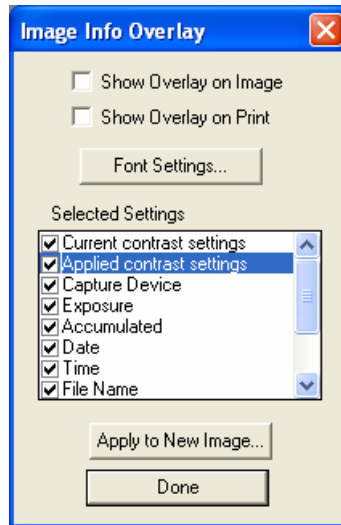
**Apply Blended Image To:** Specify the intensity values that are to be affected by the blend. Select from the following:

- ◆ **All:** The Clipboard data will be applied, in the specified blend ratio, to all pixels in the paste area, regardless of their intensity.
- ◆ **Lighter Pixels Only:** The Clipboard pixels will be pasted (in the specified blend ratio) only in places where the destination pixel is lighter than the corresponding Clipboard pixel.
- ◆ **Darker Pixels Only:** The Clipboard pixels will be pasted (in the specified blend ratio) only in places where the destination pixel is darker than the corresponding Clipboard pixel.

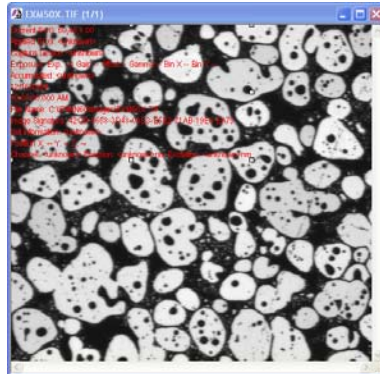
## Image Information Overlay

---

This feature of *Image-Pro Plus* allows you to display image parameter information on the active image. The “parameter stamping” can include information about the capture device used, the exposure time, image set, and channel information as well as date/time. Selecting this menu item displays the following dialog:



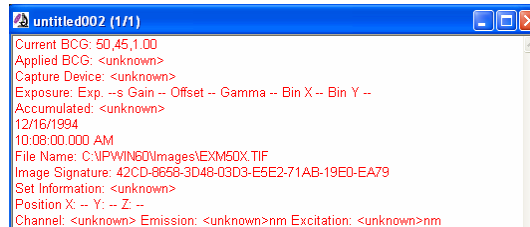
**Show Overlay on Image:** Checking this box will display the parameter information on the image, as shown here:



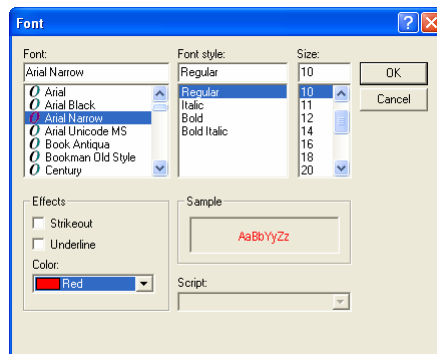
Note that the *Current Contrast Settings* and the *Applied Contrast Settings* refer to the Brightness, Contrast, and Gamma (BCG) settings used for image display.

## Edit:Image Information Overlay

**Show Overlay on Print:** Checking this box displays the parameter information on an opaque background (for printing), as shown here:



**Font Settings:** This dialog allows you to choose the font and color that will be used to display the parameter information:



Choose a font style, color, and size from the drop-down lists. A sample appears in the 'sample' box.

Click **OK** when you have finished, or **Cancel** to exit without changing any font settings.

**Selected Settings:** This box lists all the different image parameter information items that can be displayed on the active image. Check the boxes to select each one.

**Apply to New Image:** Click this button to “stamp” the parameter information on the image.

**Done:** Click this button to return to the *Image-Pro* workspace.

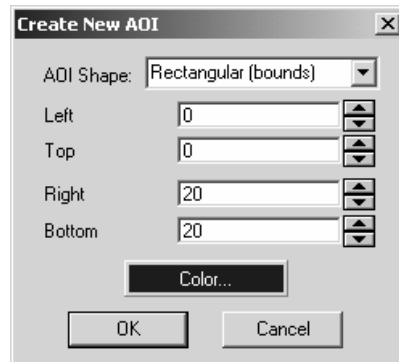
## New AOI

---

The *New AOI* command allows you to create an AOI on the active image by specifying the image coordinates. The AOI shapes available are:

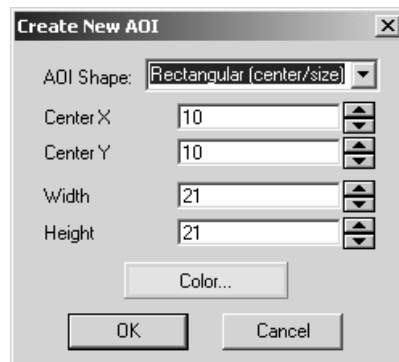
- Rectangular (bounds),
- Elliptical (bounds),
- Rectangular (center/size),
- Elliptical (center/radius).

Selecting either **Rectangular (bounds)** or **Elliptical (bounds)** displays the following dialog:



You would define your AOI by entering the pixel coordinates for the **left**, **top**, **right**, and **bottom** of the AOI shape.

Selecting either **Rectangular (center,size)** or **Elliptical (center, size)** displays a different dialog:

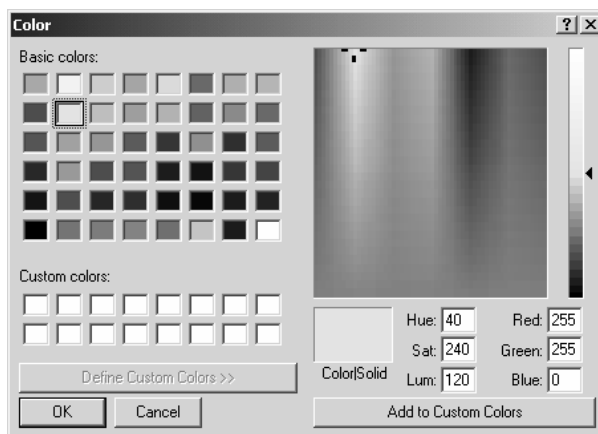


*Edit:New AOI*

You would define your AOI by entering the pixel coordinates for the **Center X** and **Center Y**, and the length in pixels for the **Width** and **Height**.

Whichever type of AOI you select will then be displayed on your active image. You can move or resize the AOI by either changing the coordinates in the dialog, or clicking and dragging the AOI on the image.

**Color:** Clicking this button displays the color selection dialog.



Here you can change the color used to preview the AOI, or define a custom color for preview. Once you apply the AOI to the image, the AOI will be displayed in the same manner as other rectangular or elliptical AOIs (a white figure within a black figure).

**OK:** Click **OK** to apply your AOI to the image.

**Cancel:** Click **Cancel** to erase your AOI.

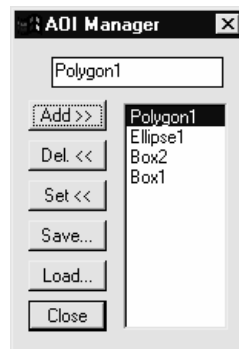


## AOI

---

Use the *AOI* command to save and recall AOI outlines. There are many instances in which it is useful to save an AOI — if your analysis involves the repetitive use of the same size AOI, you might store the AOI for use with other images. You might also save an AOI at the end of the day so that your analysis can be easily resumed at some later point. Or you might save an AOI to give to a co-worker on another workstation.

When you select the *AOI* command, the **AOI Manager** window is displayed.



The controls in this box are used to work with the current list of reusable AOIs. The names of the AOIs comprising the current list are shown in the list box on the right side of the **AOI Manager** window.

The button controls along the left side are used to add, delete and apply AOI outlines. They are also used to save/load outlines to/from AOI files. Each control is described below.

**AOI name:** Use the edit text box at the top to give your AOI the name you choose. Otherwise, *Image-Pro* provides a name consisting of the AOI type and its number in the series.

**Add:** Use this button to add the active AOI to the list of reusable AOI outlines. Before selecting this button you must have created an AOI, and it must be the active AOI (please refer to the description under *Image-Pro Tools*). When an AOI is added to the list it is given a generic name made up of its type and a number — e.g., Box1, Ellipse2, Polygon3. You may rename your AOI by highlighting its current name in the list box, then typing its new name in the edit box at the top of the **AOI Manager** window.

You will receive an alerting message if you attempt to add an AOI that has the same coordinates as one already contained in the list. You may choose to discard or save the duplicate AOI.

## *Edit:AOI*

**Del:** Use this button to delete an AOI from the current list. Before selecting this button, be sure the AOI you want to delete is the one highlighted in the list.

**Set:** Use this button to apply an AOI to the active image. Before selecting this button, be sure the name of the AOI you want to apply is the one highlighted in the list (see procedures at the beginning of this section). You may also double-click on the AOI to set it.

When an AOI is applied, it is put in the same position at which it was initially defined. If this position is outside the boundaries of the receiving image, the AOI will not appear. If parts of the AOI extend beyond the image boundaries, only part of the AOI (the part that exists within the image) will appear.

**Save:** Use this button to save the current set of AOI outlines to a file. You might do this if you use various sets of AOIs for different purposes, or if you want to use a set of AOIs on another workstation. When you click the **Save** button, the **Save AOI List** dialog box is presented. Within it, type the name of the file to which you want the outlines stored. AOI outline coordinates are stored in ASCII format to an \*.AOI file.

---

*Note:* The current AOI list is maintained from session to session, so it is not necessary to explicitly save AOI outlines to a file simply to make them available next time you work with *Image-Pro*.

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**Load:** Use this button to load a new set of AOI outlines to the current list. The new list will *replace* the current list. When you click the **Load** button, the **Load AOI List** dialog box is presented. Within it, type the name of the AOI file from which the outlines will be read, then click **OK**. The names of the AOIs contained in that file will appear in the list box in the **AOI Manager** window.

**Close:** Click this button to close the **AOI Manager** window.

*Procedures*

The following steps are required to save an AOI:

1. **Create the AOI if it has not yet been created.** You may use any of the AOI tools (rectangle, ellipse or free-form).
2. **Activate the AOI.** The AOI that is to be saved *must be active* (i.e., visible in the image) before proceeding to the next step.
3. **Select the AOI command.** This command is located on the *Edit* menu. The **AOI Manager** command window will be presented.
4. **Click the *Add* button in the AOI Manager command window.** The AOI will be given a name and will be placed in the list of reusable AOIs.
5. **Rename the AOI.** You may assign a more meaningful name than the one automatically assigned by *Image-Pro*. To do this, highlight the current name in the list, then enter a new name in the edit box at the top of the **AOI Manager** command window.
6. **Save the AOI list (optional).** If you want to use the AOI on another workstation, or if you want to organize your outlines into related groups, you can save the AOI to a file. Click the **Save** button to do this.

---

*Note:* AOI outlines are automatically saved from one session to the next. Once you add an AOI to the AOI Manager list, it will remain there until you delete it or replace the current list with one from another file.

---

## *Edit:AOI*

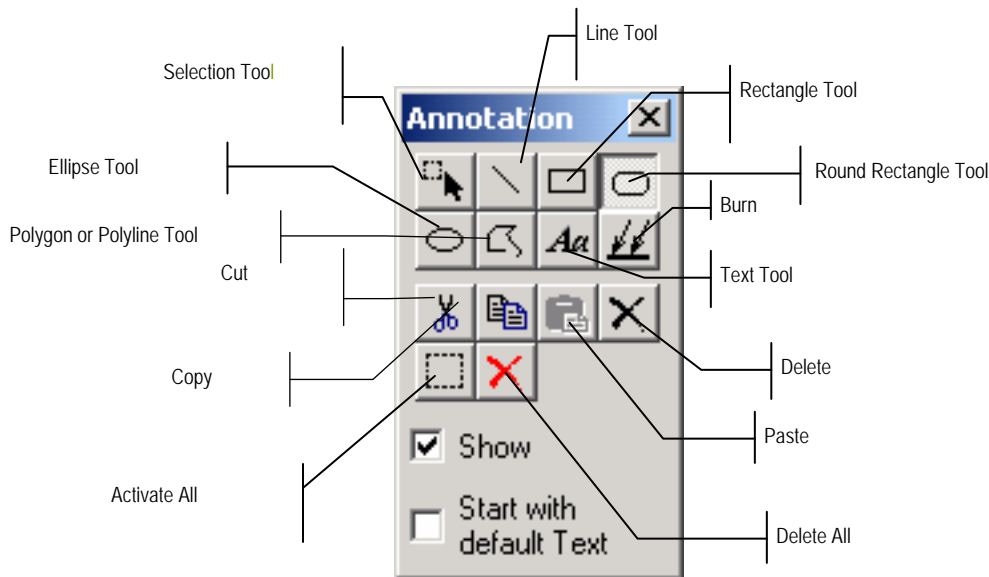
To recall an AOI that has been saved, follow the steps below:

- 1. Select the image to which the AOI will be applied.** The image into which you want the AOI placed must be *active*.
- 2. Select the AOI command.** This command is located on the *Edit* menu. The **AOI Manager** command window will be presented.
- 3. Load the AOI list from an AOI file, if necessary.** If the AOI you want to recall is not in the current list, click the **Load** button to load the proper AOI file.
- 4. Highlight the AOI in the list, then click the Set button.** This will apply the selected AOI to the active image. You may also double-click on the AOI to set it.

## Annotate

Use the *Annotate* command to add text or drawings to your images. Your annotations are in a graphics overlay that is displayed over the image without modifying the image data itself. Annotations can be stored in a .TIF or IPW file separate from the image itself. The annotations do not become part of the image until you click the **Burn** button.

When you select the *Annotate* command, you will see the *Annotation toolbar*, as shown here:

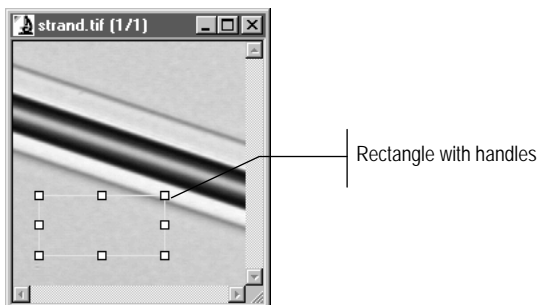


**Show:** Check this box to display the annotations on the image. If unchecked, the annotations are hidden.

**Start with Default Text:** When this box is checked new text objects will contain the current default text. This text can then be edited if necessary. Please refer to the **Properties** dialog description later in this section for information about how to use this feature.

When you select one of the shape tools (line, rectangle, ellipse, polygon, text), your cursor will change to a crosshair whenever you are over the active image. When you click on the image, that sets the top-left corner of the new object, and an outline of the object with handles appears on the image in the workspace as you drag the mouse to size

the object. Drag the handles until your drawing object reaches the size required.



If an object has a property *Zoom with Image* unchecked, one of handles has an additional cross mark inside it. This handle points to the position on the image where the object is anchored. For a rounded rectangle object, the special yellow handle is used to change roundness.

Each drawing object has its own **Properties** dialog. Click the right mouse button on the selected object, and choose *Annotation: Properties*, or double-click the object to view or edit its properties. The pages shown in the **Properties** dialog are discussed at the end of this section, and vary according to the selected object.

---

**Note-** When Annotation tool buttons are active, these tools receive all mouse movements and clicks first. This means that tools such as the AOI manager, Zoom, and Pan do not receive mouse movements or clicks. If you wish to manipulate the image using these tools, you must first de-select the Annotation tool by clicking it again. Clicking on a active tool button will release it, turning off the tools and allowing mouse messages to be passed on to other tools.

---

It is possible to save your images and annotations without burning them. If you store your annotated images in either *TIFF* or *Image-Pro Workspace* format, the transparent overlay will be stored along with, but still apart from, the image data. No other file formats are capable of storing image overlays.



The **Selection** tool is used to select a drawing object on the image. To select more than one object, hold down the <CTRL> key while clicking the mouse pointer on the objects. You may also select all the objects in an area by using the object selector to draw a rectangle to enclose them.



The **Activate All** button selects all drawing objects on the image.



The **Line** tool is used to draw straight lines and arrows. To draw a line, select this tool, and click on the location in your image where you want to start the line. Drag the mouse cursor to the ending point. A line with handles will appear.



The **Rectangle** and **Round Rectangle** buttons create similar objects. To draw a rectangle or rounded rectangle, place the cursor anywhere in the image, then drag the cursor to the appropriate size and release the mouse button. Once a rectangle has been created, its size can be modified by placing your cursor on one of the handles and dragging it to a new position. Hold down the <Shift> key when drawing a rectangle or round rectangle to draw a square.



The **Ellipse** button is used to draw circles and ellipses. To draw an ellipse, drag the cursor until an ellipse of the size and shape you need is produced. Once an ellipse has been created, its size can be modified by placing your cursor on one of the handles and dragging it to a new position. Hold down the <Shift> key when drawing an ellipse to create a circle.



To place text on an image, select the **Text Tool**. If you have default text and **Autosize bound** set (see the Settings page description below), then you can just click your left mouse button inside the image to place the text object. If you do not have default text set, you will click your left mouse button, and drag the cursor to size the text object. You will then have the opportunity to edit the text in the annotation using the Text page of the properties dialog (see the Text page description below).



Use this button to draw an open **Polyline** or closed **Polygon**. You can draw a polygon by dragging the mouse cursor along the path of the object, or by clicking on various points in the image. Double click the left mouse button to end the polyline.

*Edit:Annotate*



The **Burn** button makes your annotations a permanent part of the image. Until you click this button, your annotations exist on a transparent overlay that is displayed on top of your image. The **Show** checkbox on the Annotation toolbar controls the visibility of the transparent overlay. If the box is checked, the objects drawn on the overlay will be visible.

Once you click the **Burn** button, the objects on the overlay are permanently embedded into your image. Your image data is changed, and the annotated object destroyed.

---

**Note:** Changes to the image data can be reversed using the Undo command, but the original annotation objects cannot be restored.

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The **Cut** button will copy the selected object(s) to the clipboard and remove them from the image. You can then use the **Paste** button to paste the objects onto the original or another image.



The **Copy** button will copy the selected object(s) to the clipboard, but will not remove them from the image. You can then use the **Paste** button to paste the objects onto the original or another image.



The **Paste** button will paste any annotation objects on the clipboard to the active image.

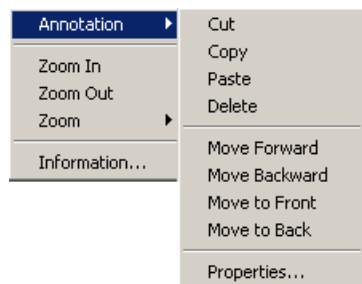


The **Delete** button will permanently delete the selected object(s) from the image.



The **Delete All** button will permanently delete all annotation objects from the image.

Click the *right* mouse button in the image area to display the image context menu:





Please refer to *Chapter 1* of this manual for descriptions of the *Zoom* and *Information* commands. When selected, *Annotation* displays a second context menu. The *Cut*, *Copy*, *Paste*, and *Delete* commands behave the same as described above for the toolbar buttons. The *Move* commands are used to set which objects will be displayed on top of other objects. If your objects do not overlap, the drawing order will not matter, but if they do, you can use the *Move* commands to assure that the objects that you want displayed on top of others are in front of those other objects. The *Properties* item displays the property dialog for the selected object.

Menu options are disabled if the menu item's operation cannot be completed. For example, if there are no selected objects, menu options *Cut*, *Copy*, *Delete*, *Move*, and *Properties* are disabled. If there is no information in the clipboard to paste, the menu option *Paste* is disabled. The *Move* commands can also be disabled if the selected object is the only annotation object, or if it is already at the front or back of the set of objects. The **Properties** dialog is accessible only if there is only one selected object.

## **Annotation Object Properties Dialogs**

All object types have default properties that will be used to define the way the object will be drawn when a new object is added to the annotation. The properties for an existing object can be edited by double-clicking the object, or selecting a single object and using the right-click popup menu's *Properties* item, either of which will display the **Annotation Object Properties**, which is a tabbed dialog of one or more pages. The contents of the property dialog will change depending on the type of object selected.

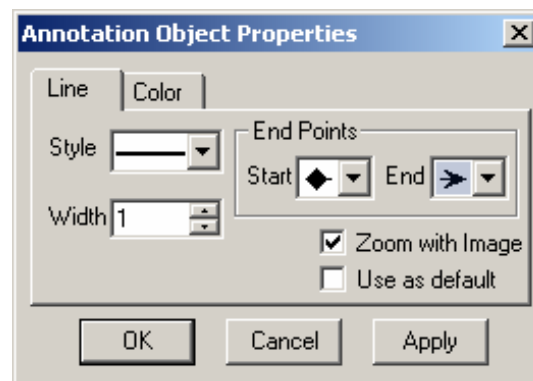
**Use as Default:** Use this command to replace the default properties with the properties that you select in the **Properties** dialog. This redefines the way that new objects of this type will be drawn. This will *not* affect the properties of any existing objects.

## Object Page

The first page of the properties dialog is named for the type of object that is selected, and the contents of this page also change.

### Line / Polyline / Polygon

The first page for line, polyline, and polygon objects allows you to set the line style and end points of the object:



Use this dialog to change the line style (solid, dotted, or dashed) and line width of your object. The **Start** and **End** selections include various shapes that can be used to mark the starting and ending points of the line.

**Zoom with image** forces the object to change size as you zoom the image in and out.

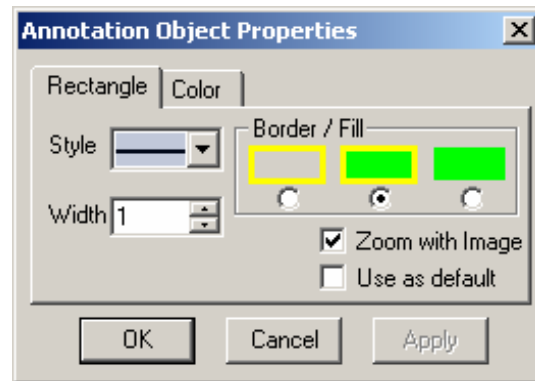
**Use as default** saves these settings as the default properties for this type of object the next time you use this tool, if you check this box.

The **Use as default** box normally is *unchecked*.

**Connect Start/End** turns your polyline into a closed polygon. The Connect Start/End option is only displayed for polyline and polygon objects.

### Rectangle / Round Rectangle / Ellipse

The first page for rectangle, round rectangle, and ellipse objects allows you to specify the line and fill properties of the object:



Use this dialog to change the line style (solid, dotted, or dashed) and line width of your object. The **Border/Fill** selections include border without fill, border and fill, or fill only. Click the appropriate radio button to indicate your choice.

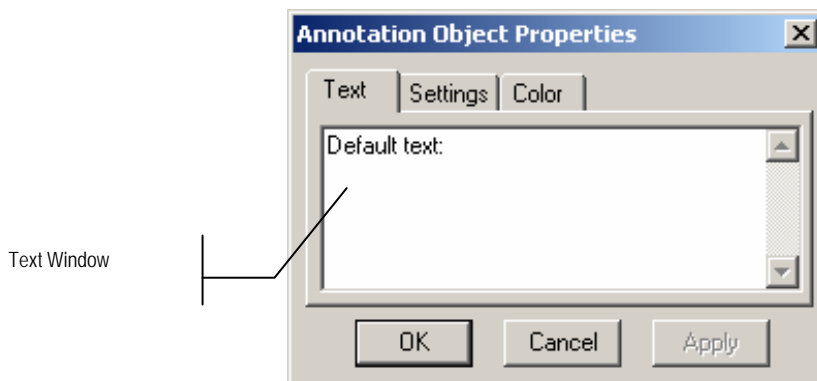
**Zoom with image** forces the object to change size as you zoom the image in and out.

**Use as default** saves these settings as the default properties for this type of object the next time you use this tool, if you check this box.

The **Use as default** box normally is *unchecked*.

### Text Page

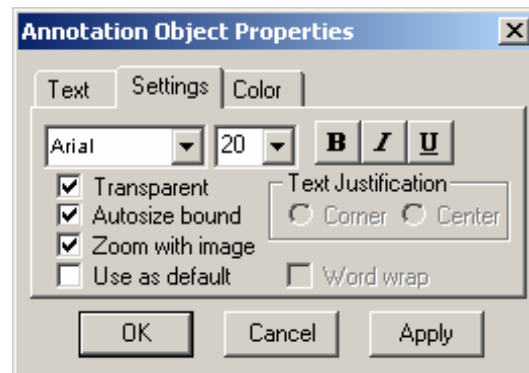
The first page for text allows you to specify the **Text** contained in the object:



The **Text** page of the properties dialog is displayed automatically each time you place a new text object. This gives you the opportunity to edit the text contained in the new object. When **Start with default Text** is selected on the main **Annotations** dialog, a new text object will be created with the current default text. When *Image-Pro* is first started, the default text is empty, so the text window illustrated above will be empty and you can enter whatever text you wish for each new object. If you want to have each text object start with particular text, first create a text object. Edit the text (if any) in the text window to what you want to appear in each new text object. Then on the **Settings** page (described below), make sure that **Use as default** is checked. After you click OK to accept the edited properties, make sure that **Start with default Text** is selected on the main **Annotations** dialog. As you create new text objects, they will be created with the default text that you entered.

### Settings Page

The properties dialog for text objects has an additional **Settings** page. The attributes on this page let you select the text characteristics, such as type font, size, style and alignment; as well as special attributes such as bold, italic, or underlined. Note that these attributes are applied to the *entire* character string that will be applied to the image.



**Transparent** lets the image show through the drawing on the image. If this box is not checked, the text is enclosed in a filled box.

**Autosize bound** allows the rectangle enclosing the text to change as the text changes.

**Zoom with image** forces the text object to change size as you zoom the image in and out.

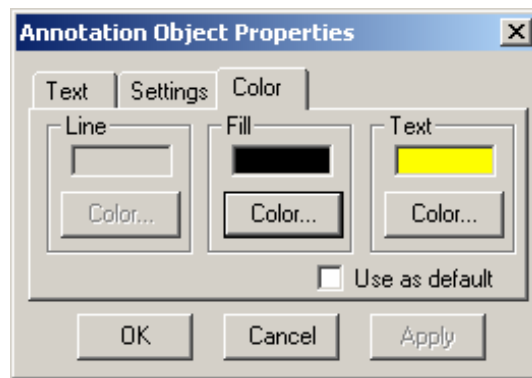
**Use as default** remembers these settings for the next time you use the tool, and resets the defaults if you check this box. **The Use as default** box normally is *unchecked*.

The **Text Justification** controls can be used to determine whether the text will appear in the top-left corner of the area that you set for the text object (when you created the object), or whether the text will be centered in the area. This option is disabled when **Autosize bound** is selected.

**Word Wrap** automatically continues the text on the next line if it reaches the edge of the bounding rectangle. This option is disabled when **Autosize bound** is selected.

### Color Page

The **Color** page is available for all drawing objects:



The **Color** page lets you select three colors: *Line* (or outline), *Fill* (or interior), and *Text*. Not all of these colors are used for all objects; unused colors will be disabled. For line and polyline objects, only the *Line* color is used. For filled objects, the *Line* color is used for the outline of the object and the *Fill* color for the object's interior. For text objects, the *Text* color is used for the typeface, and the *Fill* color for the bounding box enclosing the text, if *Transparency* (from the Settings page) is turned off.

## **Fill**

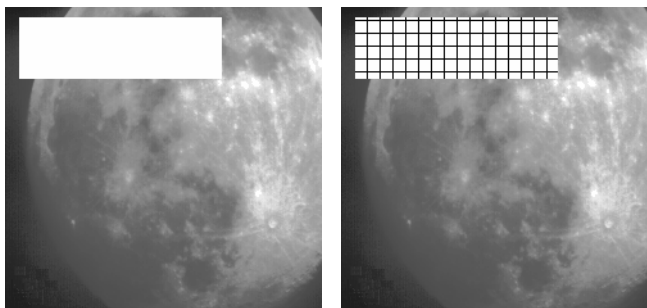
---

Use the *Fill* command to fill the active image or AOI with a solid color or a pattern from another image. You might want to use *Fill* to highlight an AOI with a color or pattern, to clear a portion of an image, or to superimpose one image over another.

*Image-Pro* will fill an AOI if you select one. Otherwise, the entire active image will be filled. When a Multiple AOI is active, *Image-Pro* will fill all the AOIs it contains.

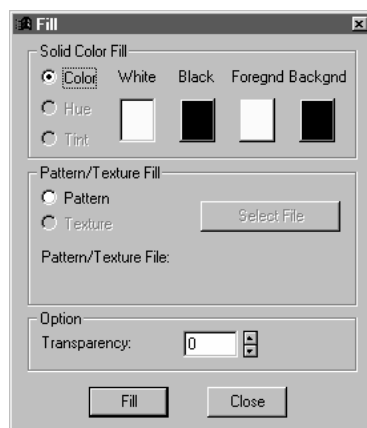
*Image-Pro* will fill with a pattern, a texture, or a color of your choice.

The AOI in the image on the left has been filled with white, the AOI on the right has been filled with a grid pattern.



If you plan to fill with a pattern, your active image must be either an RGB24, RGB36, or RGB 48 bit image, or gray scale (*Gray Scale*, *Gray Scale 12*, *Gray Scale 16*, or *Floating Point*) class. Patterns cannot be placed in *Palette* images. If your image is not gray scale or RGB, you can convert it to one of these classes using the *Convert To* command. When filling with a pattern, you must also have an image file that contains the pattern you want *Image-Pro* to fill the area with.

When you select the *Fill* command, the **Fill** dialog box is presented.



Within this dialog box, indicate whether you want to fill with a color, or with a pattern or texture. If you choose to fill with a pattern or texture, you will need to specify the image file that contains the pattern. Click the **Fill** button once you have made your choices. *Image-Pro* will fill the AOI (or the entire image, if no AOI is present) with the color or pattern you have selected. Select **Close** if you want to close the dialog box.

---

**Note:** The *Fill* dialog box is modeless. When you click **Fill**, the fill is performed, but the dialog box remains open. You may perform other **Fill** operations on the same image or other open images.

---

**Solid Color Fill:** Use this group box if you want to fill with a solid color, by choosing the appropriate color button for background and foreground. The color options (**Color**, **Hue**, and **Tint**) relate to ways in which color can be applied.

**Color:** Select this option if you want the AOI or image to be filled with a solid color. Select the color to be used by clicking on the appropriate button.

**Hue:** When filling with **Hue**, the image is internally converted to the HSV color model, and the color filling is performed on the color (hue) channel only. After filling, the filled image is converted back to the RGB color model.

The **Hue** option replaces the color values in your image. Depending on the degree of transparency you select, **Hue** mixes

## *Edit:Fill*

the colors in the original image with the color you select. Elements of the image that are white or black will not be replaced.

Visually, selecting **Hue** colors your image without affecting those areas that are black or white. Select this option if you want the pixels filled with the **Hue** value from the color selected with the **Color** buttons.

---

**Note:** The **Hue** option can only be used with RGB images. This option will be disabled if the active image is not of this class.

---

**Tint:** When filling with Tint, the image is converted to the HSV color model, and the color filling is performed on both the color (hue) and saturation channels. After the fill process is complete, the image is converted back the RGB color model.

The **Tint** option replaces the color and saturation values in your image. Depending on the degree of transparency you select, **Tint** replaces the values in the original image with new values.

Visually, **Tint** washes a color over entire image, including elements that are white or gray. Select this option if you want the image or AOI tinted with the color selected with the **Color** buttons.

---

**Note:** The **Tint** option can only be used with RGB images. This option will be disabled if the active image is not of this class.

---

**Pattern/Texture Fill:** Use this group box if you want to fill with a pattern or texture. These two options determine how a pattern file will be combined with the active image or AOI.

**Pattern:** Select this option if you want to fill the area with the contents of the image file specified in the **Pattern/Texture** group box.

Conceptually, the active image and the pattern image are aligned at the (0,0) pixel (upper left-hand corner). The portion of the pattern image that shows through will be that portion that corresponds to the active image's AOI. If the active image or AOI is larger than the pattern file, the pattern file will be repeatedly applied until the image or AOI has been entirely filled.

This button will be disabled if the active image is not of *RGB* or one of the gray scale classes.

**Texture:** Select this option if you want to give the image a textured appearance by applying the specified pattern file to just the



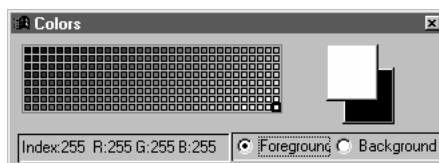
luminance channel. This results in intensity variations that give a textured look to the image.

---

**Note:** The **Texture** option can only be used with RGB images. This option will be disabled if the active image is not of this class.

---

**Foreground/Background Color:** Select **Foreground** or **Background** if you want *Image-Pro* to use the **Colors** window currently selected foreground or background color for the fill operation. If the color buttons do not currently specify the color you want to use, double-click the **Foreground** or **Background** box. The **Colors** window appears.



You can select another color by clicking on the color value currently selected and moving the cursor to the desired value.

---

**Note:** Both **Foreground** and **Background** fill according to the **Color**, **Hue**, **Tint**, and **Transparency** selections you have made.

---

**Black/White:** Use one of these color options if you want *Image-Pro* to fill with black or white.

**Select File:** Click this button to specify the file containing the pattern you want *Image-Pro* to use to fill the image or AOI. This button will only be enabled when the **Pattern** or **Texture** option is set in the **Type** group box.

When you click this button, the **Open File** dialog box is presented. Select the pattern file as you do any image file (see the *Open* command if you need assistance).

A pattern file must be an image file, and can be any class but *Bilevel*. A pattern file can contain any image you want. If the pattern file is smaller spatially than the active image or AOI, *Image-Pro* will repeat the pattern image until the entire AOI or image is filled. Pattern file opacity is determined by the value set in the **Transparency** field.

This button will be disabled if the active image is not of *RGB* or *Gray Scale* class.

**Transparency:** Set this field to indicate the level of transparency you want the color or pattern applied with. This lets you control how much of your active image shows through the applied color or pattern. A transparency of “0” indicates no transparency. A transparency of “100” indicates total transparency. The **Transparency** button will be disabled if the active image is not of *RGB* or *Gray Scale* class.

**Color/Transparency** - Set the **transparency** value to indicate how much of the original image you want to show through the color you apply.

**Hue /Transparency** - If **transparency** is set to zero, the full Hue value of the specified color will be applied to the pixels in the AOI or image. If **transparency** is set to a higher value, more of the original pixel’s Hue value will be blended with that of the specified color.

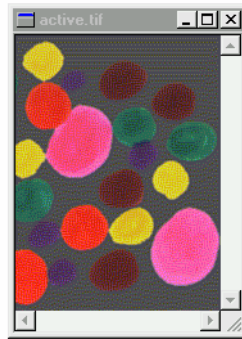
**Tint/Transparency** - If **transparency** is set to zero, the hue and full saturation of the selected color will be applied to the pixels in the AOI or image. If **transparency** is set to a higher value, the color will be applied in a less saturated form, and the hues will be mixed.

**Pattern/Transparency** - If **transparency** is set to zero, the pattern file’s pixel values will replace those in the AOI or image entirely. If **transparency** is set to a higher value, the two images will be blended so that some of the original image shows through.

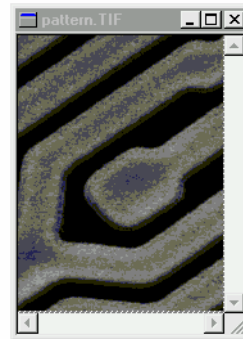
**Texture/Transparency** - Use the **transparency** value to control how much of the texture file’s intensity should be applied to the luminance channel. Set **transparency** to zero to apply the full intensity; set a higher transparency value to retain more of the original image’s intensity characteristics.

The series below shows the original image and pattern file, and the effects of applying the pattern with increasing transparency values of: 20, 40, 60 and 80.

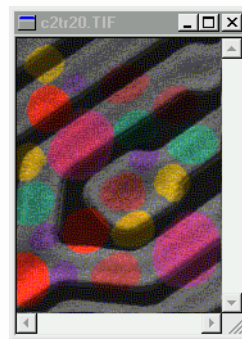
*Edit: Fill*



Active image



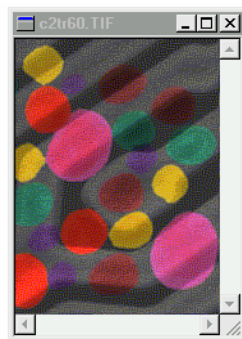
Pattern image



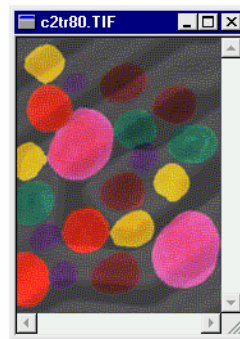
Transparency = 20



Transparency = 40



Transparency = 60



Transparency = 80

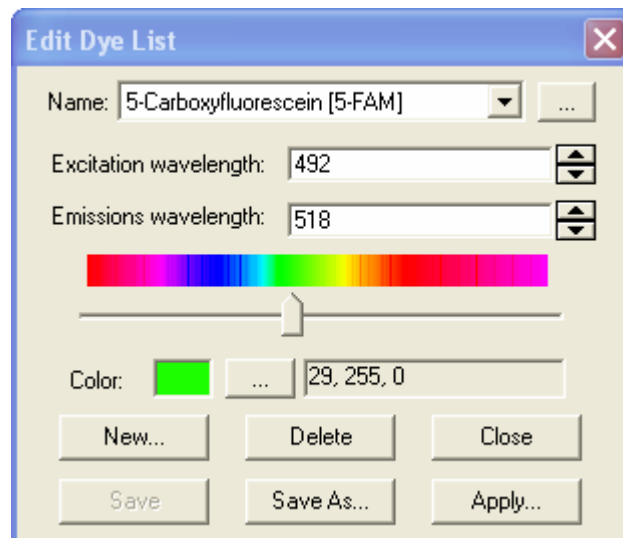
*Edit:Dye List*

## Dye List

---

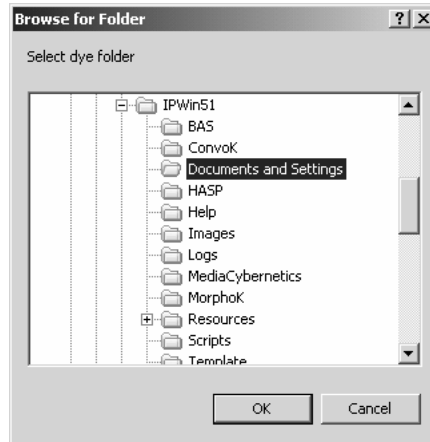
The *Dye List* feature in *Image-Pro Plus* allows you to select from a list of dyes, or add your custom dyes to the list. The list of dyes (\*.ipd) is stored in the *Documents and Settings* folder of *Image-Pro Plus*.

When you choose the *Dye List* command from the *Edit* menu, you will see the **Edit Dye List** dialog box:



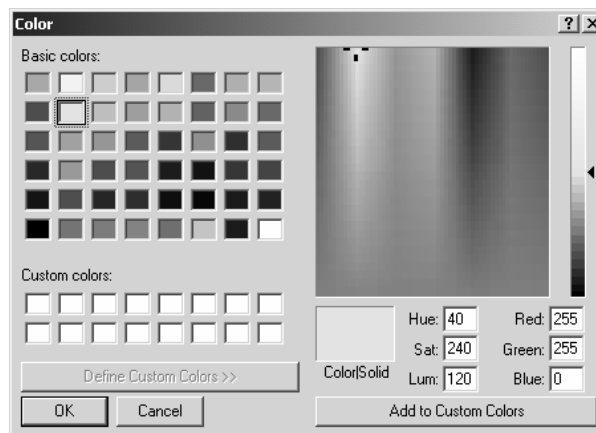
**Name:** This list includes all dye definitions found in the current dye folder. You may select a different dye from the drop-down list, and all the remaining controls will be updated to show the characteristics of the new dye.

... Clicking the folder browse button allows you to select a new dye from a different folder. This button displays a standard Windows folder selection dialog, as shown here:



**Color:** Use the slider to adjust the emissions wavelength for your dye, which also sets the default display color. (You can also use the **...button** to select a specific color using a standard *Windows* color selection dialog. ) The color will change in response to changes in the emissions wavelength.

... Clicking this button displays the color editing dialog:



Here you can change the color associated with the selected dye, or define a custom color. The color default is linked to the selection of a wavelength for your dye. If you change the emissions wavelength, the color displayed will be the standard color for that wavelength. To set a custom color, *first* you should set the wavelength and *then* edit the color.

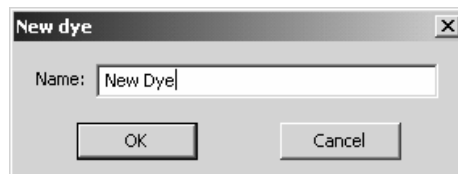
**Emissions wavelength:** This field displays the emissions wavelength for the dye you have chosen. You can adjust he

## Edit:Dye List

emissions wavelength by entering a new value. The default color will change in response to changes in the emissions wavelength.

**Excitation wavelength:** The default wavelength for your dye is displayed here. You can adjust the wavelength by entering a new value.

**New:** Click the new button to add a dye to your list of available dyes. You will see the **New Dye** dialog: Enter the name of your new dye.



**OK:** Click **OK** to save the new or updated dye definition file in the current location.

**Cancel:** Click **Cancel** to return to the *Image-Pro* workspace without saving any changes.

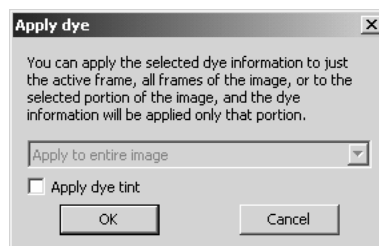
**Delete:** Click the **Delete** button to remove the selected dye from your list.

**Close:** Click this button to close the **Edit Dye List** dialog.

**Save:** Click this button to save your dye list changes.

**Save As:** Click this button to save your dye list changes to a new folder.

**Apply:** Click the **Apply** button to apply the dye to the image, or a portion of the image. You will see the **Apply Dye** dialog:



Choose a selection from the drop-down list box to indicate where you would like to apply the selected dye information. You do not have to use the Apply button; you may simply select the dye and click **OK**.

**Apply dye tint:** Check this box to apply the dye.

**Note:** Since tints such as the Brightfield, DIC, or Phase (or any tint with white as the end color), are defined as the “absence of tinting”, applying one of those tints will remove any previous tints.

---

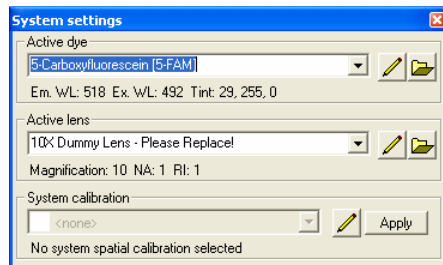
**OK:** Click **OK** apply the dye and to return to the **Edit Dye** dialog.

**Cancel:** Click **Cancel** to return to the **Edit Dye** dialog without making any changes.

## Edit System Settings

---

The system settings dialog indicates the active dye, active lens, and active system calibration, as shown here:



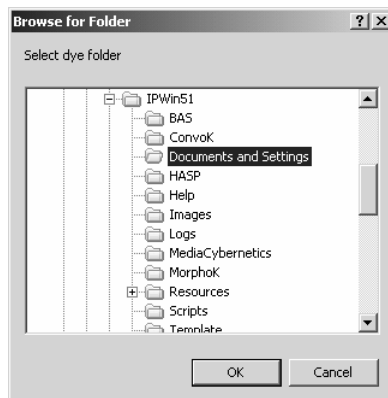
**Active Dye:** The **active dye** displays the dye currently applied to the active image.



Click this button to open the **Edit Dye List** dialog box. You can select a different dye from the drop-down list. Please see the *Edit: Edit Dye List* pages preceding this section for more information.

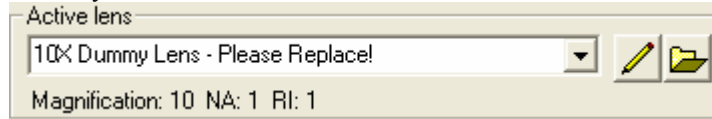



Clicking the folder browse button allows you to select a new dye from a different folder. This button displays a standard Windows folder selection dialog, as shown here:





**Active Lens:** The **active lens** box displays the name of the lens currently in use.




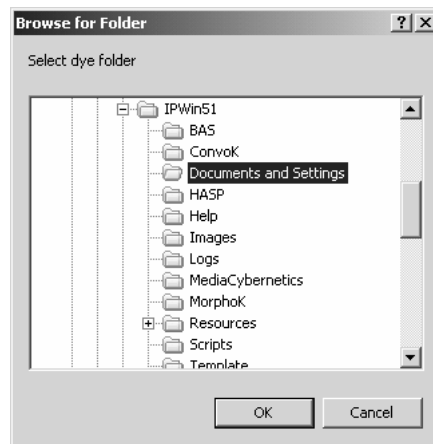
 Click this button to open the **Edit Lens List** dialog box. You can select a different lens dye from the drop-down list. Please see the *Edit: Edit Lens List* pages following this section for more information.

---

*Note:* If your calibrations have been created by the Calibration Wizard, or have been manually named so that they contain an exact match for the name of one of your lenses, selecting a new system lens will also set the correct system calibration. This will not work if you have more than one calibration for each lens.

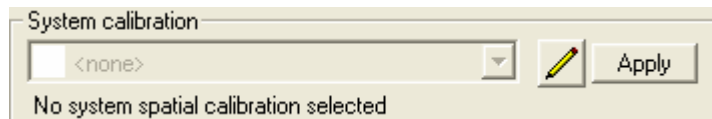
---

 Clicking the folder browse button allows you to select a new lens from a different folder. This button displays a standard Windows folder selection dialog, as shown here:



**System Calibration:** The **system calibration** is a selected reference calibration .

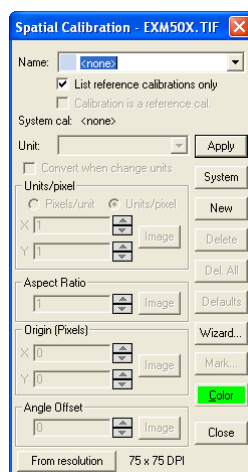
## Edit:Edit System Settings



It is applied automatically to each captured image. If you change the optics in the light path, by changing the objectives or the camera, you should select the corresponding calibration as the system calibration.



Clicking this button displays the full **Spatial Calibration** dialog, where you can modify the spatial calibration settings.



Please refer to the *Spatial Calibration* section under the *Measure* menu for more information.

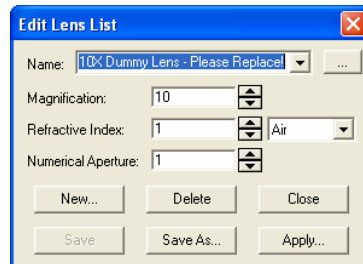
**Apply:** Click the **Apply** button to apply the dye, lens, or calibration to the active image.

## Lens List

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All of the plug-ins that are affected by lens characteristics, such as *Scope-Pro* and *SharpStack*, can now use a common set of lenses or objectives. The *Select Lens* feature of *Image-Pro Plus 7.0* enables you to choose from a centralized list of lenses. The list of lenses (\*.ipl) is stored in the *Documents and Settings* folder of *Image-Pro Plus*.

When you choose *Select Lens* from the *Edit* menu, you will see the **Edit Lens List** dialog box.

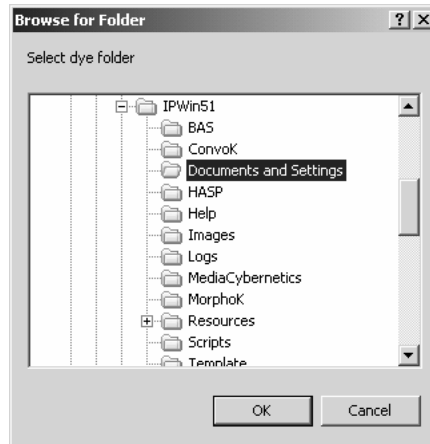


**Name:** The **Name** edit box displays the current name of the lens if you are editing an existing lens definition. If you are defining a new lens, this edit box will be empty.

You may select a different lens from the drop-down list, and all the remaining controls will be updated to show the characteristics of the new lens.

... Clicking the folder browse button allows you to select a new lens from a different folder. This button displays a standard Windows folder selection dialog, as shown here:

## Edit: Lens List



**Magnification:** Enter a **Magnification** from .001 to 3000.

**Reflective Index:** Enter a **Reflective Index** from 1.0 to 3.0; or the standard **RI** for air, water, glycerin or oil may be selected from the drop list. If a reflective index is manually entered, the drop list will be set to **Custom** (even if the value entered is one of the standard RI values).

**Numeric Aperture:** Enter a Numeric Aperture from 1.0 to 2.0.

**New:** Click the **New** button to add a lens to the list of lens definitions. You will see the **Edit Lens** dialog. Enter the name of your new lens.



**OK:** Click OK to save the new or updated lens definition file in the current lens location.

**Cancel:** Click Cancel to return to the *Image-Pro* workspace without saving any changes.

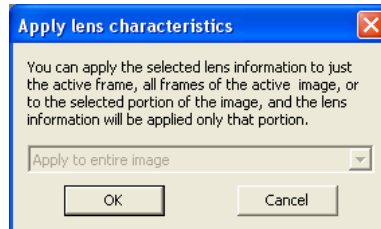
**Delete:** Click the **Delete** button to remove the selected lens from your list.

**Close:** Click this button to close the **Edit lens List** dialog.

**Save:** Click this button to save your lens list changes.

**Save As:** Click this button to save your lens list changes to a new folder.

**Apply:** Click the **Apply** button to apply the lens to the active image, or a portion of the image. You will see the **Apply Lens Characteristics** dialog:



Choose a selection from the drop-down list box to indicate where you would like to apply the selected lens information.

**OK:** Click **OK** apply the lens and to return to the **Edit Lens** dialog.

**Cancel:** Click **Cancel** to return to the **Edit lens** dialog without making any changes

## **Convert To**

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Use the *Convert To* command to create a copy of the active image in the specified class. You might do this to convert a *RGB* image to a *Gray Scale* image if only its intensity information is required, or change a *Gray Scale* image to *Floating Point* in preparation for arithmetic operations.

When you select the *Convert To* command, you will receive a menu from which you must choose the class of image you want to create. The class of the active image will be dim in the menu. Select one from the list below:

- ◆ Gray Scale 8
- ◆ Gray Scale 12
- ◆ Gray Scale 16
- ◆ Floating Point
- ◆ Palette
- ◆ RGB 24
- ◆ RGB 36
- ◆ RGB 48

The converted image will be placed into a new image window. Your original image will not be affected by the conversion.

---

*Note:* Bilevel images are no longer supported by version 7.0 of *Image-Pro Plus*.

---

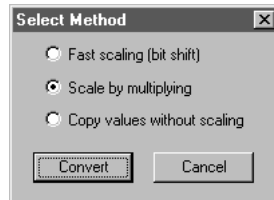
Two new options have been added to the image conversion feature: scale by multiplying and direct value copy.

**Scale by Multiplying** is an option upon integer promotion, multiplying by 257 rather than 256 for the 16 bit conversion. This means that an 8-bit value of 255 will become a 16-bit value of 65535, rather than 65280. If converted to a 12-bit value, 255 will become 4095, rather than 4080.

**Direct value copy** will turn a 255 value in an 8-bit image to 255 in either a 12 or 16-bit image. Direct value copy will copy values directly, clipping if they are above the dynamic range of the target image type.

### *Edit:Convert To*

When your image conversion results in an *increase* in dynamic range, including 8-bit grayscale to 12 or 16, 12-bit grayscale to 16, RGB to RGB 36 or RGB 48, and RGB 36 to RGB 48, you will be able to control the conversion with the following dialog:



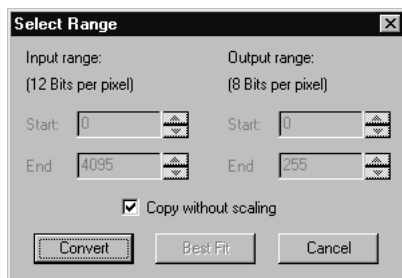
This dialog is displayed upon promoting any integer image to another integer image type. It also appears for RGB promotions.

Use the radio button to select the required conversion method:

- ◆ **Fast scaling:** This feature scales images by bit-shifting them to the left and filling with zeros. This is the method used by previous versions of *Image-Pro Plus*.
- ◆ **Scale by multiplying:** This feature multiplies images by 257 for 8 to 16 bit, and 17.0625 for 12 to 16 and 8 to 12 bit conversions. The effect is to shift the values to the left the appropriate number of bits and add the most significant bits of the original value into the now blank least significant bits of the new image. Effectively, full range remains full range, and values between zero and full range scale accordingly.
- ◆ **Copy values without scaling:** This feature copies values directly. Note that the result of this conversion will usually look quite dark or even black, as the direct copy of values results in images whose values are only a small fraction of the dynamic range.

## Edit:Convert To

When your image conversion results in a *decrease* in dynamic range, including 12- or 16-bit grayscale to 8-bit, 16-bit grayscale to 12-bit, RGB 36 or 48 to RGB 24, or RGB 48 to 36, you will be able to control the process using the following dialog:



The demotion dialog does not list the scaling options: **Fast Scaling** (scaling by bit shift) and **Scale by Multiplying** are treated identically for demotion.

The options in this box let you determine how much of the 12-bit data is scaled to 8 bits. This feature is useful for converting images with limited dynamic range. If for example, much of your 12-bit data existed in the 0 to 600 range, you might want to convert just this range to 8 bits. This measure will reduce the amount of data that is lost when the 12-bit values are rescaled.

---

**Note:** If you don't know the dynamic range of your 12-bit image, click the **Best Fit** button. *Image-Pro* will automatically set the range based upon a statistical analysis of the values in your image. See *Best Fit*, below.

---

**Input Range:** In the **Start** and **End** fields, set the beginning and ending values of the range that is to be converted to 8 bits. If you do not know the range, click the **Best Fit** button and *Image-Pro* will automatically set it for you (see *Best Fit*, below).

**Output Range:** In the **Start** and **End** fields, set the beginning and ending values of the range to which you want the 12-bit data scaled. In most cases you will want to scale to the largest possible 8-bit range, so the default values of 0 and 255 should be used.

**Best Fit:** Click this button to automatically set the **Input Range** values. *Image-Pro* will automatically set the **Start** and **End** fields to define a range that encompasses 98% of the values in the image (the highest 1% and lowest 1% are ignored).

**Copy without scaling:** This feature copies the values into the new image without scaling them down, clipping any values that exceed



the maximum range of the new image type to that maximum value. Note that when this checkbox is set the variable range control elements are disabled.

- 
- ◆ **Select *Gray Scale 8* from the *Convert To* pop-out menu** if you want to convert your image to an 8-BPP monochrome image. You might want to convert a *RGB* image to *Gray Scale* to work with just its intensity information.

A progress indicator will be displayed in the Message Bar as the conversion take place.

A few things to keep in mind when you convert to *Gray Scale 8*:

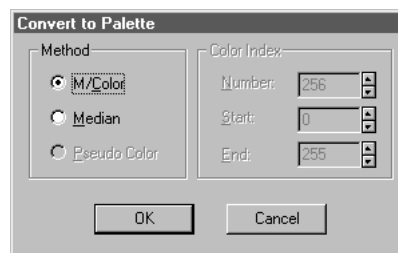
- ◆ **Palette Images** are converted by converting each pixel to its palette-specified intensity value. Intensity is calculated as follows:  $(.3 \times \text{Red} + .59 \times \text{Green} + .11 \times \text{Blue})$ .
- ◆ **RGB 24 (True Color) Images** are converted by converting each pixel to its intensity value. Intensity is calculated as follows:  $(.3 \times \text{Red} + .59 \times \text{Green} + .11 \times \text{Blue})$ .
- ◆ **RGB 36 Images** are converted in the same manner as *True Color* images, by converting each pixel to its intensity value. Intensity is calculated as follows:  $(.3 \times \text{Red} + .59 \times \text{Green} + .11 \times \text{Blue})$ .
- ◆ **RGB 48 Images** are converted in the same manner as *True Color* images, by converting each pixel to its intensity value. Intensity is calculated as follows:  $(.3 \times \text{Red} + .59 \times \text{Green} + .11 \times \text{Blue})$ .
- ◆ **Gray Scale 12 Images** will be converted according to the options set in the **Select Range** dialog, discussed previously.
- ◆ **Gray Scale 16 Images** will be converted according to the options in the **Select Range** dialog box discussed previously.
- ◆ **Floating Point Images** will be converted by scaling the range to 256 values. You may narrow the range of values to be converted by setting the **Range** values with the **Info** command. Floating Point values less than the specified range will be converted to 0; values greater than the end value in the specified range will be set to 255.

- 
- ◆ **Select *Palette* from the *Convert To* pop-out menu** if you want to convert your image to an 8-BPP, *Palette-class* image. You might

## *Edit:Convert To*

want to convert an *RGB 24* image to a *Palette* image to reduce its size (*Palette* images require only 8 BPP, whereas *RGB 24* images require 24 BPP). You might also convert a *RGB 24* image to *Palette* to create an image that can be displayed on a 256-color device.

You can convert an existing *Palette* image to *Palette*, too. This might be done to reduce the number of colors in the existing image, or to change from one type of palette to another (see **Median** and **M/Color** below):



**Method:** Within this box, select the method you want *Image-Pro* to use when creating a palette for this new image.

**M/Color:** Select this option if you want to apply Media Cybernetics' proprietary M/Color palette to the image. The M/Color palette is a 256-color palette that simulates the range of colors in a natural color image. A dithering technique is used to convert pixel values in the source image, to indices into the M/Color palette. This produces the smoothest possible result in the output image.

You might also want to use the **M/Color** palette if you are converting several images to *Palette* class, and you want them all to have the same palette. If you use the **Median** method for multiple images, a unique palette would be generated for each image. Consistent palettes across images are important if you need to use your images in an application that will display several images simultaneously. Many systems allow only a single active palette for display purposes.

If you are converting a *RGB 24* image with many varying levels of color (as in a natural image), you will want to use this technique to perform the conversion.

The **Color Index** options will not be active when the **M/Color** method is selected.

*Note - The M/Color palette does not have a wide range of gray colors in it. If your image is primarily gray, you might want to convert it with the **Median** option.*

**Median:** Select this option if you want to create a palette based upon the most frequently occurring colors in the source image. Set the options in the **Color Index** box to specify the number of colors you want *Image-Pro* to put in the palette, and indicate where they should be placed within the palette.

Because the **Median** method allows you to specify the number of colors that remain in the image, you can reduce your image to far fewer color levels than with the **M/Color** method. This can be important when image size is more important than image quality -- the fewer colors in the image, the better it will compress because more of its pixel values will be repeated.

For an artistic effect, you might want to use the **Median** method, with a small **Number** value, to achieve a “posterized” look.

**Pseudo-Color :** Select this option to convert a pseudo-colored image to a palette image.

**Color Index:** Within this box, select the number of colors you want to place in the palette. Also, specify the position of these colors within the palette.

**Number:** Specify the number of colors you want left in the image. If you want all possible colors to remain in the image, set this value to 256. Reducing this value will place fewer colors in the palette, and will leave fewer colors in your image.

Regardless of what number you specify here, the palette itself will *always* contain 256 colors. Unused palette entries are set to black (RGB = 000).

For example, if you were trying to reduce the image for compression results, you might want to constrain the palette to the 64 most frequently occurring colors in the image. When the conversion is complete, your palette would contain 256 colors, but only 64 of them would contain color. Moreover, every pixel in your image will be changed to one of these 64 colors. Those pixels that were one of the most popular 64 will be converted to the color closest to it in value.

**Start/End** Use these fields to specify the palette positions into which you want the specified number of colors placed. For

## *Edit:Convert To*

example, if you have specified 20 colors, and you wanted the first color to be assigned to palette position 0, set 0 as the **Start** value. You will see that **End** will automatically change to 19.

Colors will be assigned consecutive palette positions. If you adjust the **End** value, the **Numbers** value will be adjusted to reflect the exact number of indices between the specified **Start** and **End** values (you are not allowed, for example, to specify 10 colors positioned from indexes 0 to 20).

Any unused palette entries will be set to black (RGB = 000,000,000).

- 
- ◆ **Select *RGB 24 (True Color)* from the *Convert To* pop-out menu** if you want to convert your image to a 24-BPP (chunky), *RGB 24* image. You might want to convert a *Palette* image to a *RGB 24* image to give you greater flexibility in working with it.

A few things to keep in mind when you convert to *RGB 24*:

- ◆ **Palette Images** are converted by converting each pixel to its palette-specified RGB value. For example, if the pixel contained a value of 10, and the contents of index 10 was RGB=255,100,255 (magenta), the converted pixel would be changed to 255,100,255 (magenta).
- ◆ **Gray Scale 8 Images** are converted by converting Black pixels to RGB=000,000,000, White pixels to RGB=255,255,255 and pixels with intermediate levels of gray to RGB values equal to that level. For example, a pixel with gray level 128 would convert to RGB=128,128,128. A pixel with gray level 222 would convert to 222,222,222, and so forth.
- ◆ **Gray Scale 12 Images** will be converted by converting the image to 8-bit *Gray Scale* (i.e., dropping the 4 low-order bits), then converting the *Gray Scale* data to RGB (see *Gray Scale* conversion above).
- ◆ **Gray Scale 16 Images** will be converted by converting the image to 8-bit *Gray Scale* (i.e., dropping the 8 low-order bits), then converting the *Gray Scale* data to RGB (see *Gray Scale* conversion above).
- ◆ **Floating Point Images** will be converted by converting the image to 8-bit *Gray Scale* (i.e., scaling the values to 256 levels), then converting the *Gray Scale* data to RGB (see *Gray Scale*

conversion above). You may narrow the range of values to be converted by setting the **Range** values with the *Info* command. Floating Point values less than the specified range will be converted to 0; values greater than the end value in the specified range will be set to 255.

- ◆ **RGB 36 Images** are converted according to the options set in the **Select Range** dialog described earlier.
- ◆ **RGB 48 Images** are converted according to the options set in the **Select Range** dialog described earlier.

- 
- ◆ **Select *Gray Scale 12* from the *Convert To* pop-out menu** if you want to convert your image to a 12 BPP, *Gray Scale* image.

A progress indicator will be displayed as the conversion takes place.

A few things to keep in mind when you convert to *Gray Scale 12*:

- ◆ **Gray Scale 8 Images** are converted by increasing their pixel depth from 8 to 12 bits. according to the options set in the **Select Method** dialog described earlier.
- ◆ **Palette Images** are converted by converting each pixel to its palette-specified Intensity value. Intensity is calculated as follows: (.3 x Red + .59 x Green + .11 x Blue). The resulting 8-bit intensity is placed into the 8 high-order positions of a 12-bit pixel, producing an image with an intensity range of 0 - 4095 (see *Gray Scale* above).
- ◆ **Gray Scale 16 Images** are converted according to the options set in the **Select Range** dialog described earlier.
- ◆ **True Color Images (RGB 24)** are converted by converting each pixel to its Intensity value. Intensity is calculated as follows: (.3 x Red + .59 x Green + .11 x Blue). The resulting 8 bit intensity is placed into the 8 high-order positions of a 12-bit pixel, producing an image with an intensity range of 0 - 4095 (see *Gray Scale* above).
- ◆ **RGB 36 Images** are converted by converting each pixel to its Intensity value. Intensity is calculated as follows: (.3 x Red + .59 x Green + .11 x Blue). The resulting 12 bit intensity is placed into the 12 high-order positions of a 12-bit pixel.

## *Edit:Convert To*

- ◆ **RGB 48 Images** are converted by converting each pixel to its Intensity value. Intensity is calculated as follows: (.3 x Red + .59 x Green + .11 x Blue). This produces an image with an intensity range of 0 - 4095.
- ◆ **Floating Point Images** are converted by scaling the intensity range contained in the image to 4096 levels (0 - 4095). You may narrow the range of values to be converted by setting the **Range** values with the *Info* command. Floating Point values less than the specified range will be converted to 0; values greater than the end value in the specified range will be set to 4095.

- 
- ◆ **Select *Gray Scale 16* from the *Convert To* pop-out menu** if you want to convert your image to a 16 BPP, *Gray Scale* image.

A progress indicator will be displayed as the conversion takes place.

A few things to keep in mind when you convert to *Gray Scale 16*:

- ◆ **Gray Scale 8 Images** are converted by increasing their pixel depth from 8 to 16 bits. These images will be converted according to the **Select Method** dialog described at the beginning of this section.
- ◆ **Palette Images** are converted by converting each pixel to its palette-specified Intensity value. Intensity is calculated as follows: (.3 x Red + .59 x Green + .11 x Blue). The resulting 8-bit intensity is placed into the 8 high-order positions of a 16-bit pixel, producing an image with an intensity range of 0 - 65,535 (see *Gray Scale 8* above).
- ◆ **True Color Images (RGB 24)** are converted by converting each pixel to its Intensity value. Intensity is calculated as follows: (.3 x Red + .59 x Green + .11 x Blue). The resulting 8 bit intensity is placed into the eight high-order positions of a 16-bit pixel, producing an image with an intensity range of 0 - 65,535 (see *Gray Scale 8* above).
- ◆ **RGB 36 Images** are converted by converting each pixel to its Intensity value. Intensity is calculated as follows: (.3 x Red + .59 x Green + .11 x Blue). The resulting 8 bit intensity is placed into the eight high-order positions of a 16-bit pixel, producing an image with an intensity range of 0 - 65,535 (see *Gray Scale 8* above).

- ◆ **RGB 48 Images** are converted by converting each pixel to its Intensity value. Intensity is calculated as follows: (.3 x Red + .59 x Green + .11 x Blue). The resulting 8 bit intensity is placed into the eight high-order positions of a 16-bit pixel, producing an image with an intensity range of 0 - 65,535 (see *Gray Scale 8* above).
- ◆ **Gray Scale 12 Images** are converted according to the options set in the **Select Method** dialog described earlier.
- ◆ **Floating Point Images** are converted by scaling the intensity range contained in the image to 65,536 levels (0 - 65,535). You may narrow the range of values to be converted by setting the **Range** values with the *Info* command. Floating Point values less than the specified range will be converted to 0; values greater than the end value in the specified range will be set to 65,535.

- 
- ◆ **Select *Floating Point* from the *Convert To* pop-out menu** if you want to convert your image to a variable depth, *Floating Point* image. You might want to convert a *Gray Scale* image to a *Floating Point* image to eliminate the clipping at 0 and 255 which occurs when arithmetic operations are performed (floating point images can contain negative values).

A progress indicator will be displayed as the conversion takes place.

A few things to keep in mind when you convert to *Floating Point*:

- ◆ **Gray Scale 8 Images** are converted by converting the pixel values to floating-point values from 0.0 to 257.0.
- ◆ **Palette Images** are converted by converting each pixel to its palette-specified Intensity value. Intensity is calculated as follows: (.3 x Red + .59 x Green + .11 x Blue). The intensity result is converted to a floating-point value from 0.0 to 257.0.
- ◆ **True Color Images (RGB 24)** are converted by converting each pixel to its Intensity value. Intensity is calculated as follows: (.3 x Red + .59 x Green + .11 x Blue). The result is converted to floating-point values from 0.0 to 257.0.
- ◆ **RGB 36 Images** are converted by converting each pixel to its Intensity value. Intensity is calculated as follows: (.3 x Red + .59 x Green + .11 x Blue). The result is converted to floating-point values from 0.0 to 4097.0

## *Edit:Convert To*

- ◆ **RGB 48 Images** are converted by converting each pixel to its Intensity value. Intensity is calculated as follows: (.3 x Red + .59 x Green + .11 x Blue). The result is converted to floating-point values from 0.0 to 65537.0.
- ◆ **Gray Scale 12** are converted by converting the pixel values to floating point values that lie within the range of 0.0 (black) - 4097.0 (white).
- ◆ **Gray Scale 16** are converted by converting the pixel values to floating point values that lie within the range of 0.0 (black) - 65537.0 (white).

- 
- ◆ **Select RGB36 from the Convert To pop-out menu** if you want to convert your image to a 36-bit RGB image.

A few things to keep in mind when you convert to *RGB 36*:

- ◆ **Gray Scale 8 Images** are converted by converting Black pixels to RGB=000,000,000, White pixels to RGB=4095, 4095, 4095 and pixels with intermediate levels of gray to RGB values equal to that level. For example, a pixel with gray level 128 would convert to RGB=2048, 2048, 2048.
- ◆ **Gray Scale 12 Images** will be converted duplicating the 12-bit gray scale pixel value for each channel.
- ◆ **Gray Scale 16 Images** will be converted by converting the image to 12-bit *Gray Scale* (i.e., dropping the 4 low-order bits), then converting the *Gray Scale* data to RGB (see *Gray Scale 12* conversion above).
- ◆ **Palette Images** are converted by converting each pixel to its palette-specified RGB value, and then scaling up to 12 bits per sample. For example, if the pixel contained a value of 10, and the contents of index 10 was RGB=255,28,255, the converted pixel would be changed to 4095,2048,4095.
- ◆ **Floating Point Images** will be converted by converting the image to 12-bit *Gray Scale* (i.e., scaling the values to 4096 levels), then converting the *Gray Scale* data to RGB (see *Gray Scale 12* conversion above).
- ◆ **RGB 24 (True Color) Images** will be converted according to the options in the **Select Method** dialog, described earlier in this section.



- ◆ **RGB 48 Images** will be converted according to the options set in the **Select Range** dialog described earlier.

- 
- ◆ **Select *RGB 48* from the *Convert To* pop-out menu** if you want to convert your image to a 48-bit RGB image.

A few things to keep in mind when you convert to *RGB 36*:

- ◆ **Gray Scale 8 Images** are converted by converting Black pixels to RGB=000,000,000, White pixels to RGB=65535, 65535, 65535, and pixels with intermediate levels of gray to RGB values equal to that level. For example, a pixel with gray level 128 would convert to RGB=128,128,128.
- ◆ **Gray Scale 12 Images** will be converted by converting the image to 8-bit *Gray Scale* (i.e., dropping the 4 low-order bits), then converting the *Gray Scale* data to RGB (see *Gray Scale* conversion above).
- ◆ **Gray Scale 16 Images** will be converted by converting the image to 8-bit *Gray Scale* (i.e., dropping the 8 low-order bits), then converting the *Gray Scale* data to RGB (see *Gray Scale* conversion above).
- ◆ **Palette Images** are converted by converting each pixel to its palette-specified RGB value, and then scaling up to 12 bits per channel. For example, if the pixel contained a value of 10, and the contents of index 10 was RGB=255,128,255 (magenta), the converted pixel would be changed to 65535, 32768, 65535 (magenta).
- ◆ **Floating Point Images** will be converted by converting the image to 8-bit *Gray Scale* (i.e., scaling the values to 256 levels), then converting the *Gray Scale* data to RGB (see *Gray Scale* conversion above).
- ◆ **RGB 24 (True Color) Images** will be converted according to the options in the **Select Method** dialog, described earlier in this section.
- ◆ **RGB 48 Images** will be converted according to the options set in the **Select Range** dialog described earlier.

## **Duplicate/Crop To AOI**

---

Use the *Duplicate* command to create an exact copy of the active image, or AOI, in a new, untitled image window.

When you select the *Duplicate* command, *Image-Pro* will generate a new copy of the active image, or AOI, in a new window. If a nonrectangular AOI is active when the *Duplicate* command is selected, the new image will contain an image made up of the pixels contained within the AOI's bounding rectangle.

The duplicate image will be of the same class and logical resolution as the image from which it was created. If a *Palette* image is duplicated, its palette will be copied to the new image too. Regardless of image class, however, descriptive attributes, such as **Description**, **Artist**, and **Date**, will not be passed to the new image (these attributes are considered unique to a single image).

When the active image is a sequence, the active portion of the sequence will be copied when the **Apply to Sequence** button is selected. Otherwise, only the current frame of the active image will be copied.

## Rotate

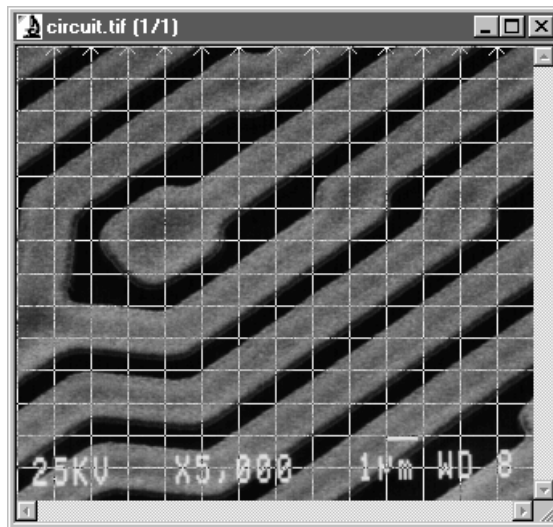
---

Use the *Rotate* command to rotate the image or the active AOI to a specified angle.

When you rotate an image or AOI, *Image-Pro* places the rotated result in a new image window; the original image is not affected.

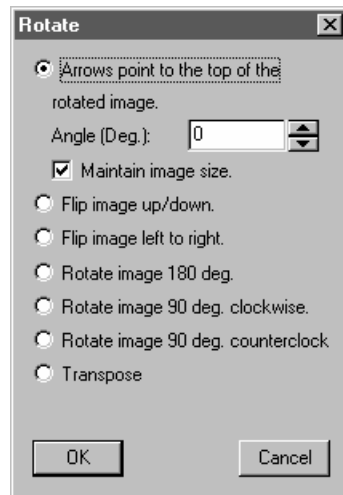


When you select the *Rotate* command, a grid will be superimposed on your image in the application area, as shown here:



## Edit:Rotate

you will see the following dialog box. Select the kind of rotation you want to perform.



- ◆ **Arrows point to the top of the rotated image.** You can specify the angle of rotation, indicating the correct angle by typing in a number or using the spin buttons. *Image-Pro* will turn the image in the application area by the specified number of degrees.
- ◆ **Maintain Image Size:** Check this box to make the rotated image the same size as the original image. If this option is not selected, the new image will be enlarged enough to contain the entire original image, with the unused corners filled with black.
- ◆ **Flip image up/down:** *Image-Pro* reverse the position of the image in the application area, so that the top right corner of the original image is now the bottom right corner, and the top left corner of the original image is now the bottom left corner.
- ◆ **Flip image left to right:** *Image-Pro* reverse the image in the application area, so that the top right corner of the original image is now the top left, and the top left corner of the original image is now the top right corner.
- ◆ **Rotate image 180 Degrees:** *Image-Pro* creates a mirror image of the original image in the application area, so that the top right corner of the original image becomes the bottom left corner, and the top left corner of the original image is now the bottom right corner.

*Edit:Rotate*

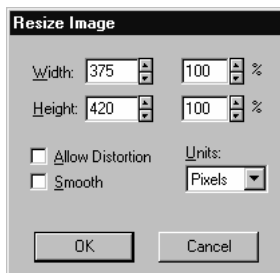
- ◆ **Rotate image 90 degrees clockwise:** *Image-Pro* turns the image in the application area to an angle 90 degrees to the right.
- ◆ **Rotate image 90 degrees counterclockwise:** *Image-Pro* turns the image in the application area to an angle 90 degrees to the left.
- ◆ **Transpose:** Select *Transpose* if you want to produce a mirror image and change the orientation of your image or AOI.
- ◆ **OK:** Click this button to save your rotated image in the application area.
- ◆ **Cancel:** Click this button to cancel the *Rotate* operation.

## Resize

---

Use the *Resize* command to create a new copy of your image at a specified size. When you resize an image, *Image-Pro* places the resized result in a new image window; the original image is not affected.

This process actually changes spatial resolution by adding (replicating) or removing (decimating) pixels to achieve the specified dimensions. If the smoothing option is used, a bilinear scaling technique is applied to reduce jagged edges.



**Units:** From this list box, select the unit of measure you want the **Width** and **Height** measurements expressed as.

**Width/Height:** Use the **Width** and **Height** selectors to scale the image to a specific height and width. If the **Allow Distortions** option is checked, you can set the height and width values independently, i.e., you can stretch or shrink the width without affecting the height and vice versa.

**Allow Distortion:** Select this option if you need to set the **Width** and **Height** dimensions independently. If **Allow Distortions** is not checked, the **Height** and **Width** fields are linked to maintain your image's proportions. When one dimension is changed, the other is automatically adjusted to preserve the ratio between height and width (the aspect ratio).

**Smooth:** Select this option if you want *Image-Pro* to use its bilinear scaling technique to re-size the image. With bilinear scaling, adjacent pixels are evaluated and interpolated to produce smooth results. Smoothing will produce the best possible re-sized image, but it may take longer to produce.

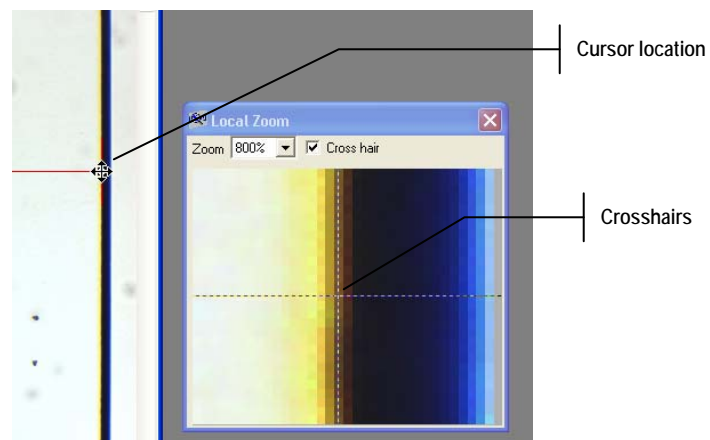
## **Local Zoom**

---

The *Local Zoom* feature on the *Edit* menu provides a tool to show a zoomed area of an image in a separate window with a given zoom factor. It can be used with manual measurements, the calibration marker, manual tagging, etc.

When you select **Local Zoom**, a new window opens in the *Image-Pro* workspace.

The cross hair in the **Local Zoom** window shows the current cursor location. This makes placing accurate manual measurements very easy, even on big images, as shown here:



**Zoom:** Choose the required zoom factor from the drop-down list.

**Cross hair:** Checking this box turns the cross hair on; unchecking the box turns the cross hair off.

---

**Note:** Overlays are NOT displayed in the *Local Zoom* tool.

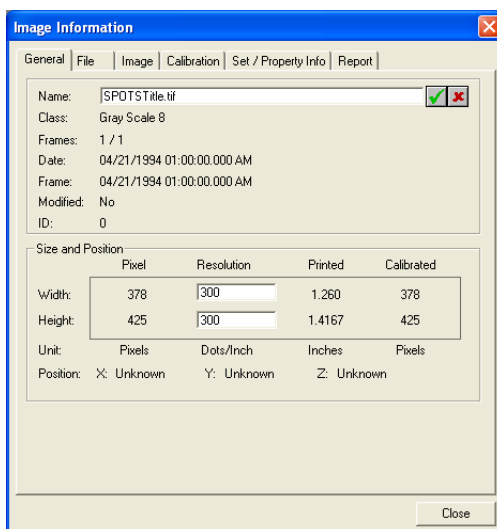
---

*Edit:Info*

## Info

---

Use the *Info* command to view and/or modify information such as resolution and description. Not all image information can be modified from this menu (the image's size, for example, is fixed). Further, not all file formats are capable of storing every information field. TIFF and *Image-Pro Workspace* formats store all the fields; other formats may store only the name. You will need to check with your file specification manual to determine which fields apply to your format. When you click on the *Info* command, you will see the following tab dialogs:



**Close:** Click this button to close the dialog and return to the *Image-Pro* workspace.



## General

The fields on this page provide general information about the active image:

**Name:** Indicates the name of the active image. This field can be modified to change the name.

**Class:** This “read-only” field displays the class of the active image. If you need to change an image's class, use the **Convert To** command.

**Frames:** Indicates the frame number and the number of frames in a sequence, i.e. the current frame is the 22<sup>nd</sup> frame in a sequence of 30 frames.

**Date:** Indicates the date the workspace was created. The date and time are displayed.

**Modified:** Indicates if the image was changed by *Image-Pro* since the last time it was loaded from a file.

**ID:** Indicates the document ID number assigned to the workspace. This is useful for *Auto-Pro* programming.

**Size:** The fields in this group box are used to view size-related values, as shown here:

Size and Position				
	Pixel	Resolution	Printed	Calibrated
Width:	325	<input type="text" value="100"/>	3.250	325
Height:	355	<input type="text" value="100"/>	3.550	355
Unit:	Pixels	Dots/Inch	Inches	Pixels
Position:	X: (unknown)	Y: (unknown)	Z: (unknown)	

The rows indicate width, height, and units. The columns display the pixel size, resolution, printed, and calibrated sizes.

**Pixel:** This field indicates the size of the image in pixels.

**Resolution:** This field displays the logical resolution associated with the active image. This field can be changed. The logical resolution can be used to determine how much space the image will occupy in its printed form. For example, if you set **Dots / Inch (width)** to 300, you can use the **Printed** column to obtain the image's printed dimensions at that resolution.

*Edit:Info*

The logical resolution is not stored in all file formats. When it is, it is used by many applications to determine how to scale the image's dimension.

**Printed:** This field describes how much space the image will occupy in its printed form.

**Calibrated:** This field describes how much space the calibrated image will occupy. Changes in the spatial calibration of the image affect this field.

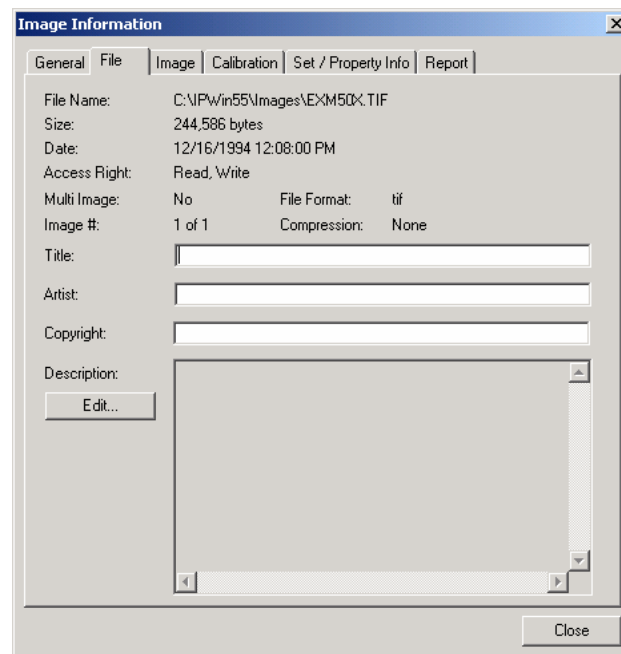
**Units:** Indicates the units in each column.

**Position:** Indicates X, Y, and Z positions of the image. This information will be provided by the *Scope-Pro* and the *3-D Constructor* plug-ins.

*Example: Your video camera captures an image of a cell at 320 x200 pixels, and the picture is spatially calibrated to represent a few microns. To print the picture, adjust the resolution so that the printed size is indicated in inches.*

## File

The fields on this page display the file information associated with your image. Not all file formats accept such information. For example, TIFF accepts all information fields; JPEG accepts only the description field. Refer to your file format specification to determine which fields are used.



**File Name:** This “read-only” field displays the name of the image file.

**Size:** This “read-only” field displays the file size in bytes.

**Date:** This field displays the date and time the image was created.

**Access Right:** Indicates if the file is stored with Read Only or Read/Write properties.

**Multi Image:** Indicates if there is more than one image stored in the file.

**File Format:** Indicates the format of the stored image: *Image-Pro Workspace*, TIFF, JPEG, sequence, etc.

### *Edit:Info*

**Image #:** If this is a multi-image file, the active image will be designated as 2 of 5, for example, or 1 of 3. Single image files are always 1 of 1.

**Compression:** Indicates the compression method used to store the image, i.e. JPEG, LZW, etc.

**Title:** This field is generally used to store a brief name for your image (distinct from its file name). For example, you might want to store an experiment name here, or store process information (e.g., segmentation performed with edge filter).

**Artist:** This field is generally used to record the name of the artist who produced the image. You might want to use this field to store the name of the operator, device, or laboratory that generated a particular image.

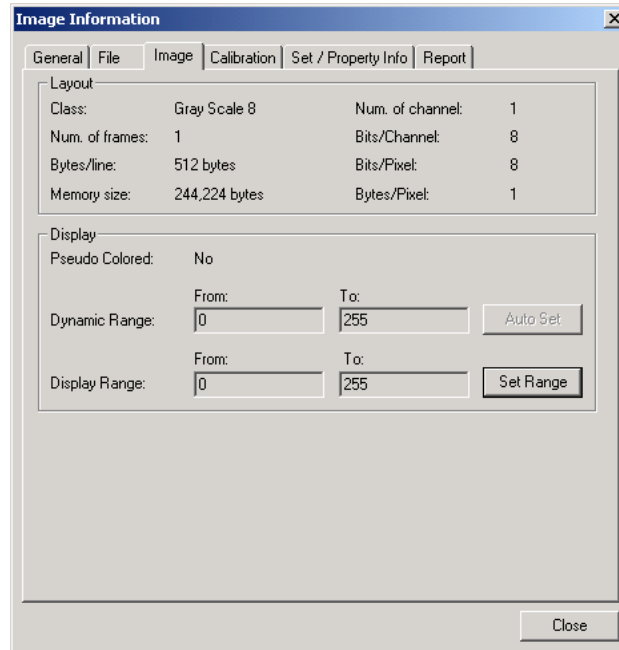
**Copyright:** If this image is copyrighted, the information will appear here.

**Description:** Enter a brief text description associated with this image.

**Edit:** Click the **Edit** button to revise the text description associated with this image.

### **Image**

The fields on this page display information about the image's characteristics:



**Layout:** The fields in this group box provide information about the layout of the image in memory.

**Class:** This “read-only” field displays the class of the active image. If you need to change an image's class, use the **Convert To** command.

**Number of Channels:** This “read-only” field displays the number of color channels in the image

**Number of Frames:** The number of frames in a image sequence is displayed here.

**Bytes per Line:** This “read-only” field displays the number of bytes in each line of the image.

**Memory Size:** This “read-only” field displays the amount of memory the current image requires.

**Bits/Channel:** This “read-only” field displays the active image's depth per color channel.

**Bits / Pixel:** The bits per pixel is equal to the bits per channel multiplied by the number of channels. This field displays the active image's bit depth (the number of bits per pixel).

## *Edit:Info*

**Bytes/Pixel:** Indicates the number of bytes required to store one pixel.

**Display:** The fields in this group box provide information about the image display.

**Pseudo-Color:** Indicates if the image is pseudo-colored or not.

**Dynamic Range:** This field displays the dynamic range of the image. Most images have a dynamic range of 0 - 255; 12-bit channel images have a range of 0 - 4095; 16-bit channel images have a range of 0 - 65535. Floating-point images have no fixed range. If the range of a floating-point image contains values greater than the maximum, they will be displayed as white. If you have a floating-point image, you can edit this field.

**Auto-Set:** Clicking on the **auto-set** button sets the dynamic range for a floating-point image with the actual minimum and maximum values found in the image.

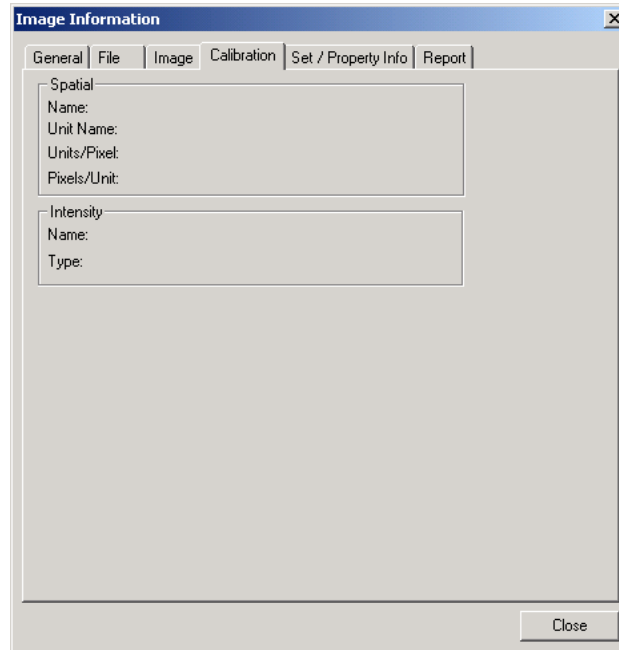
**Display Range:** This field displays the white/black designation for the intensity range associated with the active image. The values will be displayed but cannot be changed.

**Set Range:** Clicking this button brings up the Display Range dialog where you can set the active range.

## **Calibration**

The fields on this page display information about the type of calibration used on this image:

*Edit:Info*



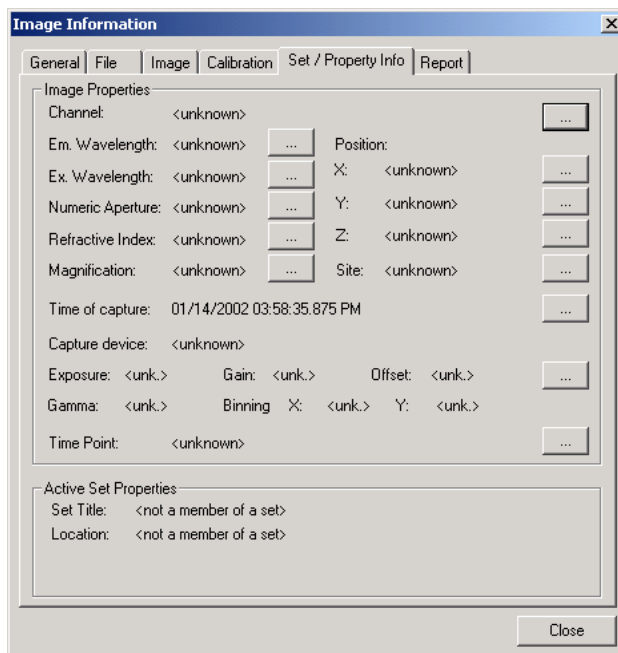
**Spatial:** The fields in this group box contain information about the type of spatial calibration used with this image. For more information about these fields, please refer to the *Calibration* Command on the *Measure* menu.

**Intensity:** The fields in this group box contain information about the type of intensity calibration used with this image. For more information about these fields, please refer to the *Calibration* Command on the *Measure* menu.

Edit:Info

## Set/Property Info

The *Set/Property Information* feature reports information about the currently active image and the set in which it belongs. When you select *Set /Property Info*, you will see the following dialog:



The information on this dialog comes from the image's properties, and the information collected by the **Set Manager**.

**Image Properties:** This group box contains information about the currently active image:

- **Channel**
- **Em Wavelength (Emissions wavelength)**
- **Ex Wavelength (Excitation wavelength)**
- **Numeric aperture**
- **Refractive Index**
- **Magnification**
- **Position (X, Y, Z)**



- Site
- Time of capture
- Capture device
- Capture properties (exposure, gain, offset, gamma, binning)
- Time point.

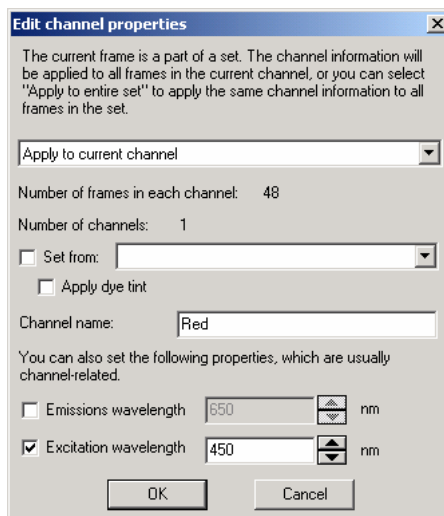
---

**Note:** Editing some of the image properties directly can give a plain sequence set-like multi-dimension characteristics. For example, dividing a sequence into two or more channels using the **Channel Properties** dialog, or creating two or more Z stacks using the **Edit Z Plane Spacing** dialog, automatically gives the image additional dimensions.

---

Next to each of these properties is an edit button that allows you to add or modify the information for each property. Each of these edit dialogs provides you with a set of options for applying the property changes to the whole image or the entire set, or to just a portion of the image or set. These options, particularly the entire set options, are usually only appropriate for a few types of properties that really do apply to every frame of the image, or every frame of every image in the entire set.

**Edit Channel:** The **Edit Channel Properties** dialog allows you to set the channel name, and other channel-related properties.



The drop-down list allows you to select the option most appropriate for the property being edited. These options include:

*Edit:Info*

- **Apply to whole image**
- **Apply to active portion of image,**
- **Apply to current Z stack,**
- **Apply to current channel, and**
- **Apply to entire set.**

**Number of frames in each channel:** Select the number of frames in each channel from the drop-down list box.

**Number of channels:** Indicates the current number of channels.

**Set From:** Indicates if the channel name, emissions wavelength, and excitation wavelength will be set from the currently selected dye. If there are no dye files selected, this option is not available.

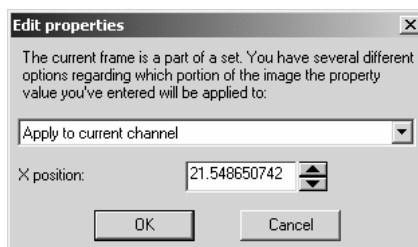
- **Apply Dye Tint:** If you have chosen to apply the channel characteristics of the selected dye, you can check this box to apply the dye's display color to the selected portion of the image.

**Channel name:** Enter a name for the current channel here.

**Emissions wavelength:** Indicates whether to set the emissions wavelength. You can edit the wavelength directly, or use the spin buttons to make adjustments.

**Excitation wavelength:** Indicates whether to set the excitation wavelength. You can edit the wavelength directly, or use the spin buttons to make adjustments

**Edit Position:** If you want to edit the X or Y position, you will see **Edit Properties** dialog, shown here:

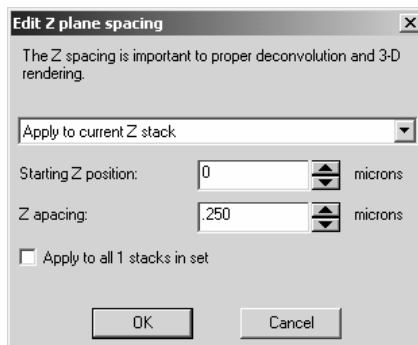


- **Apply to current channel**
- **Apply to current Z-stack,**
- **Apply to entire set**
- **Apply to whole image**

- **Apply to active frame.**

**X (or Y) position:** You can edit the position directly or use the spin buttons to set the correct X or Y location.

**Edit Z-plane spacing:** The **Edit Z-plane spacing** dialog allow you to divide a sequenced into a series of Z-stacks, and automatically set the Z position of each frame, based upon a known spacing.



The **Edit Z plane spacing** dialog offers these choices:

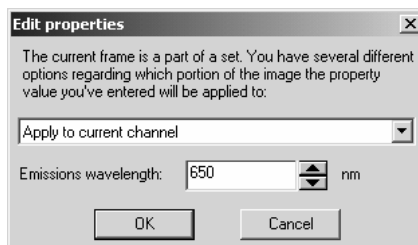
- **Apply to current Z-stack,**
- **Apply to entire set**
- **Apply to whole image**

**Starting Z position:** The **Starting Z-position** is the absolute position in microns that is assigned to the first frame of each Z-stack.

**Z spacing:** The **Z-spacing** is used to calculate the absolute Z-position of all the frames in the stack after the first frame. In the example shown, frame 1 would have a position of 0, 0; frame 2 would have a position of .25 microns; frame 3 a position of .5 microns, etc.

**Apply to all #stacks in set:** The size of the selected Z-stack may indicate that the sequence has multiple Z-stacks. This option allows you to set the same Z-stack spacing for all the of the stacks in the sequence.

**Edit Emissions Wavelength:** Selecting this option displays the following dialog:

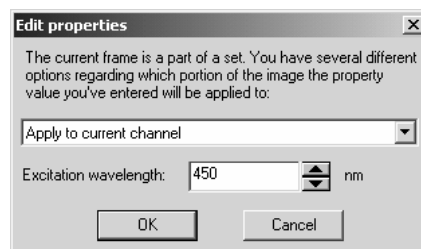


You have the following choices:

- **Apply to current channel**
- **Apply to current Z-stack,**
- **Apply to entire set**
- **Apply to whole image**
- **Apply to active frame.**

**Emissions wavelength:** Indicates the emissions wavelength. You may edit the wavelength directly , or use the spin buttons to make adjustments.

**Edit Excitation Wavelength:** Selecting this option displays the following dialog:

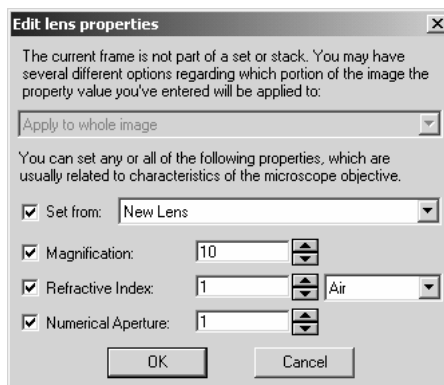


You have the following choices:

- **Apply to current channel**
- **Apply to current Z-stack,**
- **Apply to entire set**
- **Apply to whole image**
- **Apply to active frame.**

**Excitation wavelength:** Indicates the excitation wavelength. You may edit the wavelength directly or use the spin buttons to make adjustments

**Numeric Aperture:** Editing the numeric aperture displays the following version of the **Edit Lens Properties** dialog:



You will have the following choices:

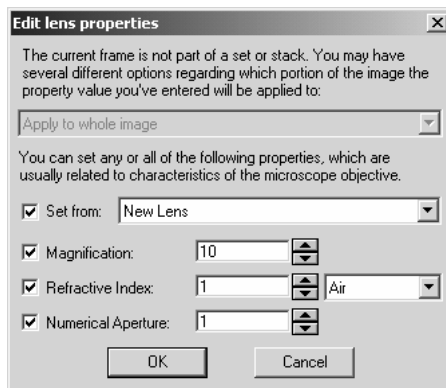
- **Apply to current channel**
- **Apply to current Z-stack,**
- **Apply to entire set**
- **Apply to whole image**
- **Apply to active frame.**

**Set From:** Indicates if the channel name, emissions wavelength, and excitation wavelength will be set from the currently selected lens. If there are no lens files selected, this option is not available

**Numeric Aperture:** Indicates the current value of the N.A. You may edit the N.A. directly or use the spin buttons to make adjustments

*Edit:Info*

**Refractive Index:** Selecting this option displays the following dialog:



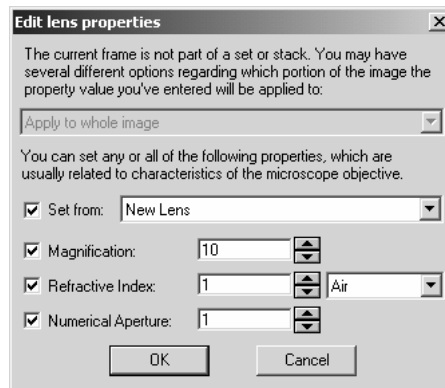
You will have the following choices:

- **Apply to current channel**
- **Apply to current Z-stack,**
- **Apply to entire set**
- **Apply to whole image**
- **Apply to active frame.**

**Set From:** Indicates if the channel name, emissions wavelength, and excitation wavelength will be set from the currently selected lens .  
If there are no lens files selected, this option is not available

**Refractive Index:** Indicates the current value of the refractive index (R.I.). You may edit the R.I. directly or use the spin buttons to make adjustments

**Magnification:** Selecting this option also displays the **Edit lens properties** dialog (shown above).



You will have the following choices:

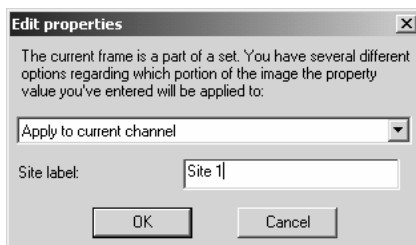
- **Apply to current channel**
- **Apply to current Z-stack,**
- **Apply to entire set**
- **Apply to whole image**
- **Apply to active frame.**

**Set From:** Indicates if the channel name, emissions wavelength, and excitation wavelength will be set from the currently selected lens . If there are no lens files selected, this option is not available.

**Magnification:** Indicate the magnification associated with the current lens.

*Edit:Info*

**Site:** Editing the **Site** allows you to create a label for the site, as shown here:

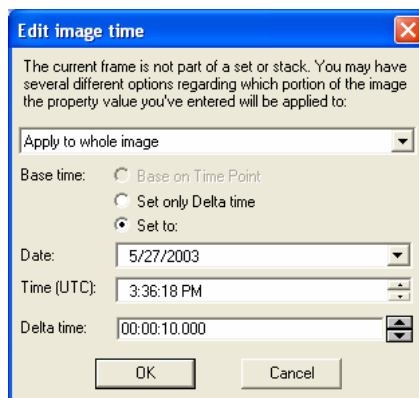


You will have the following choices:

- **Apply to current channel**
- **Apply to current Z-stack,**
- **Apply to entire set**
- **Apply to whole image**
- **Apply to active frame.**

**Site Label:** Enter text to label the site.

**Edit Time of Capture:** Editing the **Image Time** lets you adjust the time of capture for an image or sequence, as shown here:



You will have the following choices:

- **Apply to current channel**
- **Apply to current Z-stack,**
- **Apply to entire set**
- **Apply to whole image**



- **Apply to active frame**
- **Apply to current time point**
- **Apply to all time points**

**Base time:** Indicate how you want to set the base time by selecting one of the following:

**Base on Time point:** The image time can be based on the current time point (if one is found)

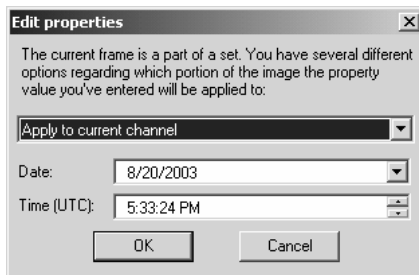
**Set only Delta time:** the current image time of will be based on the time of capture. This option is disabled if the current image is not part of a set or sequence.

**Set to:** Select the **Date** and **Time (UTC)** using the appropriate spin buttons..

**Date:** Enter the date in month, day, and year format. If you need a calendar, one will appear when you click the drop-down button.

**Time (UTC):** Enter the time in hours, minutes, and seconds. Use the spin buttons to set the correct time

**Edit Time Points:** Editing the **Time Points** lets you select the appropriate date and time, as shown here:



You will have the following choices:

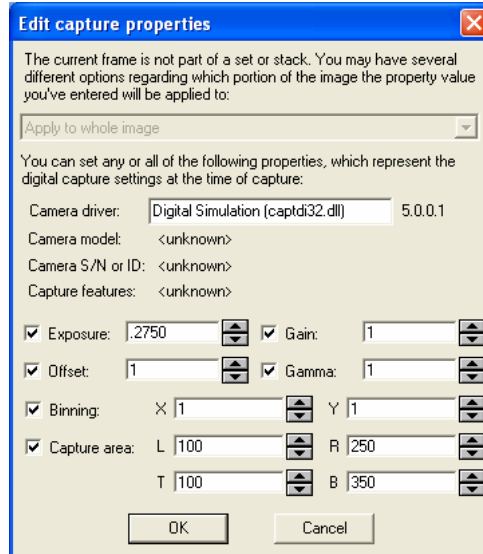
- **Apply to current channel**
- **Apply to current Z-stack,**
- **Apply to entire set**
- **Apply to whole image**
- **Apply to active frame**

**Date:** Enter the date in month, day, and year format. If you need a calendar, one will appear when you click the drop-down button.

*Edit:Info*

**Time (UTC):** Enter the time in hours, minutes, and seconds. Use the spin buttons to set the correct time.

**Edit Capture Properties:** This dialog displays capture information that is not otherwise available.



The camera driver, camera model, camera ID and camera features may be viewed in this dialog, but not modified. The following capture properties may be modified or adjusted by checking the box by each time and using the spin buttons to select the appropriate number:

- Exposure
- Gain
- Offset
- Gamma
- Binning
- Capture area

*Edit:Info*

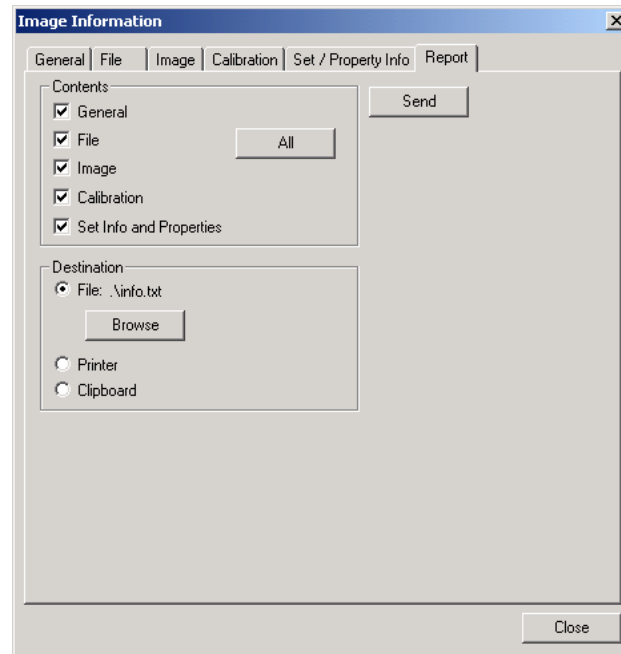
**Active Set Properties:** Information about the active set is contained in the **Active Set Properties** group box.

- **Set Title**
- **Location**

You may not modify the active set properties.

## Report

The fields on this page allow you send image information to the printer, a text file, or the Windows Clipboard.



**Contents:** Use these fields to select the image information for the report, or click **All** to print all of it. The report is stored in text format.

**Destination:** Use these buttons to select a destination for the report.

**File:** Click on this button to store the image information in a text file. The name of the file is displayed next to the button. To select a file, click on the **Browse** button.

**Printer:** Click on this button to direct your report to the printer.

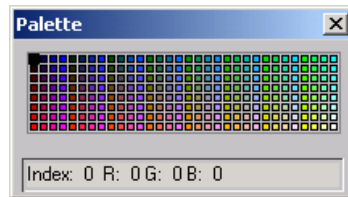
**Clipboard:** Click on this button to direct your report to the Windows Clipboard.

**Send:** Click the **Send** button to send the file to the selected file, printer, or Clipboard.

## Palette

---

Use the *Palette* command to display the 256-color palette associated with a palette image. These colors can be edited, and changes made by editing them will affect the colors seen within the image (e.g., if you change palette entry 15 from Red to Green, all pixels in your image with a value of 15 will change to Green).

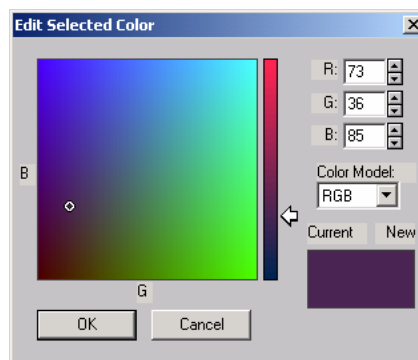


The palette window is a floating window. Once it has been opened via the *Palette* command, it will remain visible until you close it.

**Index:** The **Index** field displays the palette index value and the RGB values it contains.

When a *Palette* image is active, you can edit the color values in the palette. For example, you might want to add an entirely new color to the palette (keep in mind you cannot really “add” another color: the palette has a fixed number of entries; a color is added by replacing an existing color). When you change the value of an index in a *Palette* image, the new color will actually be applied to your image all pixels containing that index will be displayed with the new color.

To change the values on the palette, simply double-click on the index you want to change. This action will activate the **Edit Selected Color** dialog box.



## *Edit:Palette*

Use this dialog box to specify the new color values you want assigned to the selected index. You may set your values using either the RGB or HSV color models. Component values may be entered directly into the value fields, or can be set interactively using the color window on the left-hand side of the dialog box (procedures below). When you are satisfied with the color you have specified, click **OK** to return to the palette window.

**Color Model:** From this list box, select the color model you want to use to specify your color values. If you are making minor adjustments to the existing color, you might want to use the RGB model, and simply tinker with the values in the RGB fields until they create the color you need. But, if you are looking for an entirely new color, your search will be easier if you change the model to HSV.

**R/G/B:** In these fields, specify the amount of Red, Green or Blue (from 0 to 255) in your color. All three set to 0 will create black, all three set to 255 will create white. All three set to the same intermediate level (somewhere between 0 and 255) will create a level of gray.

You may either type the values directly, or select them using the color window on the left. To specify an RGB color using the color window:

1. **Select your Red value** using the arrow and the vertical scale. Move the arrow up to increase the Red value, and down to reduce it.
2. **Select your Green and Blue values** using the circlet within the color window. Move the circlet up and down to increase or decrease the Blue component, and right and left to increase or decrease the Green component.

Watch the **New** color swatch to monitor the color as you create it.

**H/S/V:** In these fields, specify the Hue, Saturation and Value settings. The H/S/V fields will only be present if HSV has been selected in the **Color Model** field. Setting **Value** to "0" will create Black. Setting **Value** to 100 and **Saturation** to 0 will create White. Setting **Saturation** to 0 with a **Value** less than 100 will create a level of gray.

### *Edit:Palette*

Colors are created by specifying a Hue value, its level of saturation (0 "Saturation" indicates that none of the Hue is present) and its intensity in the Value field.

You may either type the values directly, or select them using the color window on the left. To specify an HSV color using the color window:

- 1) **Select your Hue** using the arrow and the vertical scale. Move the arrow to the color you want to work with.
- 2) **Select your Saturation** component by moving the circlet within the color window from right to left to increase and left to right to decrease the Saturation. Higher saturation displays a purer color; lower saturation mixes the color with white.
- 3) **Select your Value** component by moving the circlet within the color window up and down to increase and decrease the Value component. Higher values display lighter colors.

Watch the **New** color swatch to monitor the color as you create it.

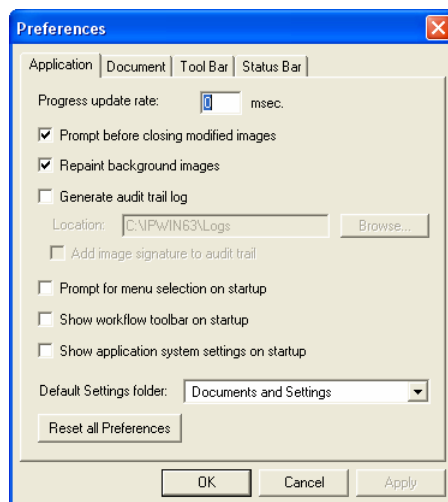
**Current:** The color swatch in this field will display a sample of the original color assigned to the index you are editing.

**New:** The color in this swatch will display a sample of the color generated by the values specified in the value fields. Use this field to monitor the effects of the changes you make, or to see the color you've selected using the color window controls.

## Preferences

---

Use the *Preferences* command to specify certain default settings that affect the way in which *Image-Pro* operates. When you select the *Preferences* command, you will be presented with the **Preferences** tab dialog.



### Application

Click on this page to select options related to the current application:  
*Image-Pro Plus v. 7.0.*

**Progress update rate:** Set the frequency at which you want the progress indicator to be updated. A value between 1 and 2000 milliseconds (inclusive) can be specified. A high value will result in fewer updates to the progress indicator, thus allocating more time to the process being tracked. A low value will result in more frequent updates of the progress indicator.

This will take time away from the task at hand, but will result in very precise progress measurement.

**Prompt before closing modified images:** Check this box if you want *Image-Pro* to prompt you before closing an image that has been modified since it was last saved. Disable this box if you want *Image-*



*Pro* to close a modified image immediately (the image will not be saved, and *Image-Pro* will issue no warning).

**Repaint background images:** Check this box to maintain the color integrity of all open images. Enabling this option causes *Image-Pro* to repaint every open image whenever you switch image windows.

Disabling this option allows *Image-Pro* to ignore color integrity for all but the active image. Because *Image-Pro* does not have to spend time repainting other image windows, performance is faster. However, it may produce some unexpected color distortion in the inactive images (to demonstrate this, load a *Gray Scale* and an *RGB* image and switch between the two).

This distortion is simply an artifact of the display process — your actual image data are not affected. A distorted image will be correctly rendered once it becomes the active image.

**Generate Audit Trail log:** Check this box to create an audit trail of all the recordable events during your image processing session. This enables you to reconstruct your actions should your session terminate unexpectedly. Audit trail logging will be turned off during macro execution, except that the act of running the macro will be recorded. Otherwise, the **Audit Trail** default is **on**.

**Location:** The daily audit trail log file can be found in the *Logs* folder of the *Image-Pro Plus* folder on your hard drive.

**Prompt for Menu Selection on Start-Up:** Check this box to display a selection box of menu choices when you first start *Image-Pro Plus*. Your choices are: basic, complete, industrial, or biological. There is also a check box to turn this selection off.

**Show Workflow Toolbar on Start-Up:** Check this box to display the **Workflow Toolbar** when you first start *Image-Pro Plus*. The **Workflow Toolbar** keeps track of your image processing operations, making it easier for you to retrace your steps.

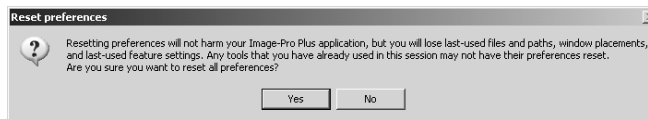
**Default Settings Folder:** This drop-down list box allows you to choose the default location of your *Image-Pro* application settings files. You may select one of the following:

- The *IPP Documents and Settings* folder
- A folder in your current *My Documents* folder
- A folder of shared settings for all *Image-Pro* users.

**Reset All Preferences:** Clicking this button resets all your application and feature preferences to their original (factory)

## *Edit:Preferences*

installation settings. Clicking this button displays the following warning message:

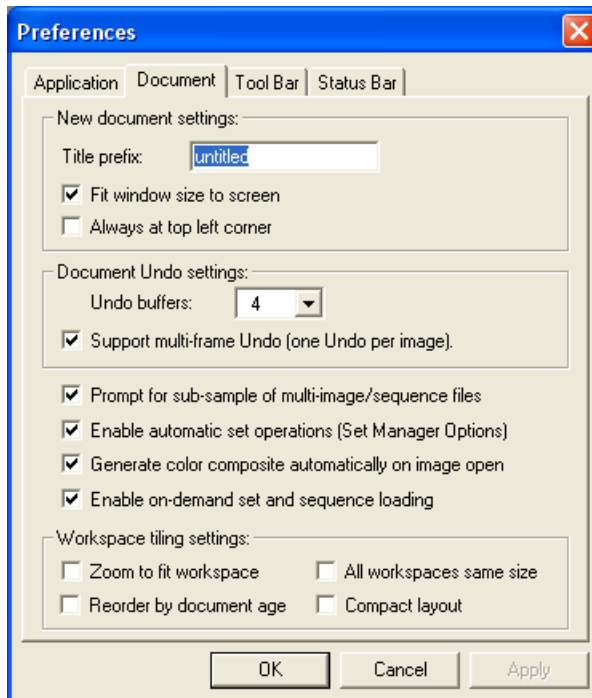


Click **Yes** to reset your preferences, or **No** to cancel.

This button cannot reset the preferences for any active features, but will be useful in resetting paths for settings and data files after changes to the preferences in the **Default Settings Folder** (above).

## Document

The selections on this page allow you to specify settings for a new document or image window in the application area.



**New Document Settings:** The options in this group box specify the name and position of the new document or image window.

**Prefix:** Indicate the name of the new document or image window. For example, if you select *Test* as the prefix, your new documents will be named Test0, Test1, Test2, etc.

**Fit window size to screen:** If you have a very large image, checking this box will automatically fit the entire image to the screen. If you leave this box unchecked, your image will be displayed with a 100% zoom factor (you may not be able to see the entire image on your screen).

**Always at top left corner:** Checking this box places the new document or image window in the top left corner of the application area.

**Undo Buffers:** Select the number of buffers you want reserved for Undo operations (up to four). The number you set here determines the number of operations you can Undo. Undo buffers use memory, and when you are working with large images, undo buffers can increase the time it takes *Image-Pro* to perform some operations. Depending on your memory availability, and the speed of your CPU, you may want to change the buffer value from its default level of three.

**Support Multi-Frame Undo:** allows you to undo operations on image sequences (one undo operation per image frame in the sequence, due to the amount of memory required).

**Prompt for sub-sample of multi-image/sequence files:** If this box is checked, *Image-Pro* will ask you if you want to open all the frames in a sequence, or only a partial sequence.

**Enable Automatic Set Operations:** If this box is checked, *Image-Pro* will automatically generate sets when you open a multi-dimensional image. See the *Set Manager Options* section for more information.

**Generate Color Composite Automatically on Image Open:** If this box is checked, *Image-Pro* will automatically generate color composites when you open a multi-dimensional image.

**Enable On-Demand Set and Sequence Loading:** Check this box to allow *Image-Pro Plus* to delay loading for large images and image sets.

**Workspace Tiling Settings:** This group of settings affects how the image workspaces are resized and arranged when you select **Tile** from the *Image-Pro Window* menu.

**Zoom to fit workspace:** When this box is selected, IPP will try to adjust the zoom factor so that the majority of the image is visible in the resized workspace.

**Reorder by document age:** When this box is selected, the workspaces will be arranged in order in which they were opened, created, captured, etc., by columns first and then by rows.

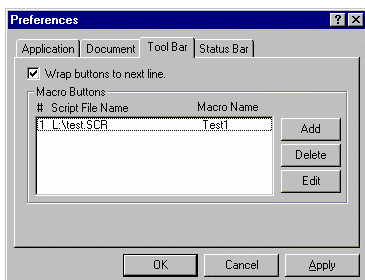
**All workspaces same size:** When you choose this option, the workspaces will all be resized to the size of the smallest workspace. The workspaces may be different sizes after tiling if the images are different sizes, or in some cases when the tiled layout results in some columns having more images than others.

**Compact layout:** When this option is selected, the workspaces will be moved so that they are next to each other in the columns and rows, such that any unused space in the *Image-Pro Plus* workspace will be found at the bottom and/or right side.

**Compact layout** is only enabled when All workspaces same size is also enabled.

## Toolbar

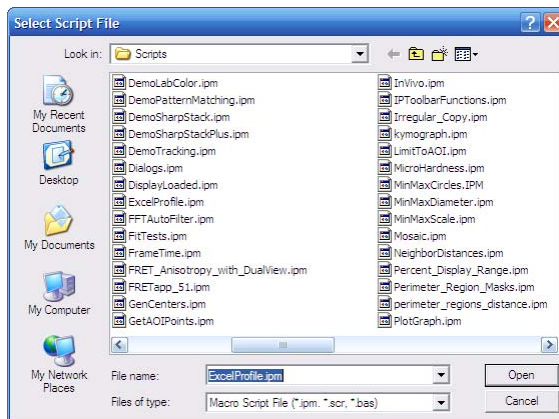
The options on this page allow you to change the look of the *Image-Pro* toolbar



**Wrap buttons to next line:** Check this box to allow *Image-Pro* to wrap the toolbar to the next line when you resize the application window.

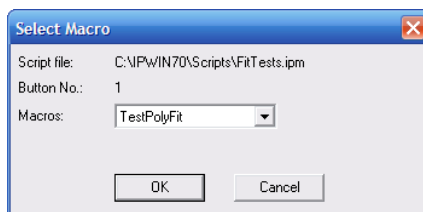
**Macro Buttons:** This area allows you to associate a macro script file with a button on the toolbar. You may designate up to ten macro buttons, and each button is associated with a different script file.

Clicking the **Add** button brings up the following dialog:



## Edit: Preferences

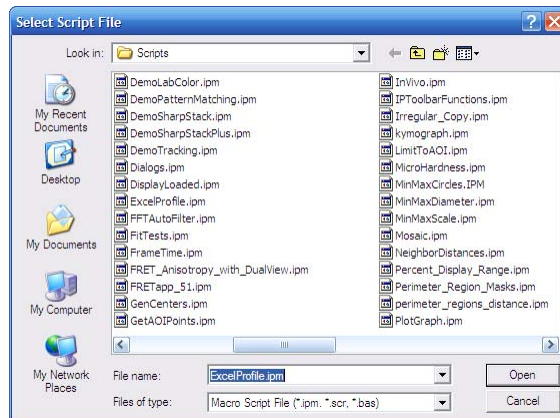
Once the script file is selected, *Image-Pro* displays the *Select Macro* dialog which lists all macros in the script file. Indicate which macro script should be associated with button #1.



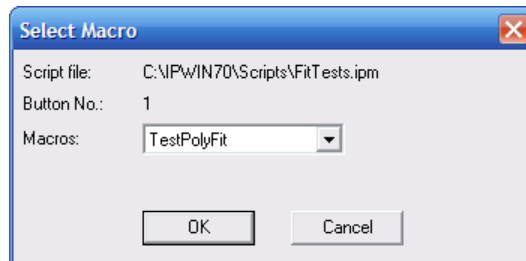
Click **OK** to assign the macro script to a button, or **Cancel** to change your mind.

The **Delete** button removes the macro button assignment.

The **Edit** button allows the user to select another script file or another macro from the same script file. To change to a different macro from the same script file, highlight the script file name, and click the **Edit** button. You will see the *Open* dialog, with the current script file selection.



Click **Open**. *Image-Pro* displays the *Select macro* dialog, showing the current macro name.



Click **OK**. Then you may select another script or button.

Click **Apply**. The macro buttons on the *Image-Pro*'s toolbar will appear or disappear immediately. You should not have to restart *Image-Pro* in order to see the changes.

The *Image-Pro* toolbar does not disable the button when the button is not connected to a valid macro. Instead, you will see error message when you click an "unassigned" button.

The macro button can be assigned to a macro in a different script file anywhere in the system. After executing the macro, the current active script file will be reactivated.

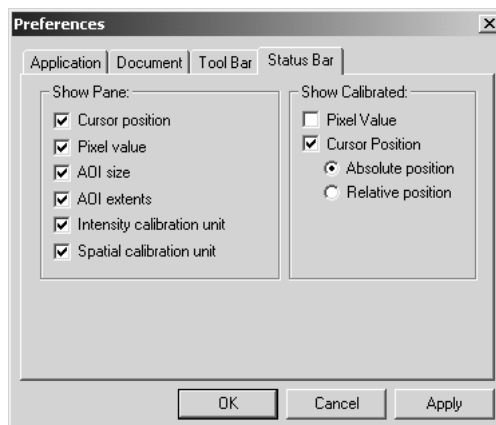
---

**Note:** The *Edit Macro* feature from the *Window* menu provides additional options for customizing the menu and toolbar, include an alternative method for adding macro toolbar buttons.

---

## Status Bar

The selections on this tab relate to the display of the status bar at the bottom of the application area.



**Show Pane:** Check options in this group box to display the selected information within the status bar. If necessary, *Image-Pro* will create a new pane for placing the information. You can hide or show the panes by right-clicking on the status bar, and selecting or deselecting the pane.

---

**Note:** If the information you have selected (Free memory available, for example) is not displayed on the status bar, your application window may be too small to display it. Widen the window to display the full complement of panes. You may also adjust the size of the individual panes.

---

**Cursor Position:** Check this option if you want *Image-Pro* to report the X and Y coordinates of the mouse cursor in its message bar. If **Cursor Position** in the **Show Calibrated** group box is selected and a spatial calibration has been set, *Image-Pro* reports the *calibrated* cursor position. Otherwise, cursor position is expressed in pixels.

**Pixel Value:** Check this option if you want *Image-Pro* to report the intensity value of the pixel identified by the cursor. If **Pixel Value** in the **Show Calibrated** group box is selected and an intensity calibration has been set, *Image-Pro* reports the *calibrated* pixel value. Otherwise, *Image-Pro* reports the actual pixel value.

**AOI Size:** Check this option to display the width and height (in pixels) of the active image or AOI.



*Edit:Preferences*

**AOI Extents:** Check this option to display the coordinates of the upper-left and lower-right corners of the active AOI.

**Intensity Calibration Unit:** Check this option to display the name of the intensity calibration in use.

**Spatial Calibration Unit:** Check this option to display the name of the spatial calibration in use.

**Free Memory Available:** Check this option to display the amount of system memory free.

**Show Calibrated:** Check options in this group box to change the way information is displayed.

**Pixel Value:** Check this option if you want *Image-Pro* to report *calibrated* intensity in the pixel value pane (see the *Calibration* command for more about intensity calibration). If no intensity calibration has been set, *Image-Pro* displays the actual pixel value.

**Cursor Position:** Check this option if you want *Image-Pro* to report *calibrated* X and Y coordinates in the pixel value pane (see the *Calibration* command for more about spatial calibration). If no spatial calibration has been set, *Image-Pro* displays the coordinates in pixels.

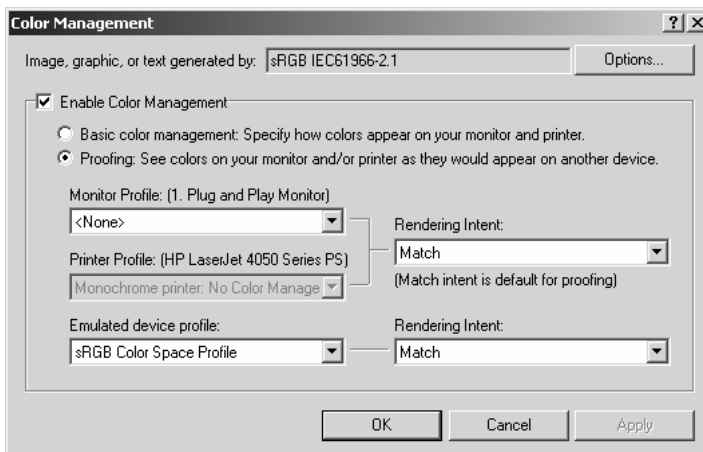
## Color Management

---

Color management, for the *Image-Pro* family of products, enables you to establish color consistency for all your images, using specific ICC color profiles for your display monitor, camera, printer, and light source. This process will produce greater color accuracy while viewing and editing your images on a CRT or LCD monitor.

### The Color Management Dialog

When you select *Color Management* from the *Edit* menu, you will see the following dialog:



The drop-down list box at the top of the dialog indicates the color profile currently in use. You can use the **Options** button to select a different profile.

The **Enable Color Management** check box allows you to enable or disable color management on your desktop system. Your choices are:

- **Basic color management.** Click this radio button to specify how the colors appear on your monitor and/or printer.
- **Proofing.** Click this radio button to specify how the colors will appear on another device, such as your printer.
- **Monitor Profile:** The **Monitor Profile** list box helps you select a color profile for the monitor from the list of available profiles associated with that monitor. The default profile is the system default profile for the current monitor.

- **Rendering Intent:** The **Rendering Intent** list box lets you choose a rendering intent. Possible rendering intents are shown in the following table.

<b>Intent</b>	<b>ICC Name</b>	<b>Description</b>
Picture Perceptual	al	For images and photographs; attempts to maintain the relationship between colors.
Graphic Saturation	aturation	For charts and business graphics; attempts to maintain a color's saturation, rather than its hue or lightness.
Proof Relative Colorimetric	Colorimetric	Does not preserve the white point; if the output gamut does not support a given color, the nearest supported color is used.
Match Absolute Colorimetric	Colorimetric	Preserves the white point; if the output gamut does not support a given color, the nearest supported color is used.

The default intent is *Picture*.

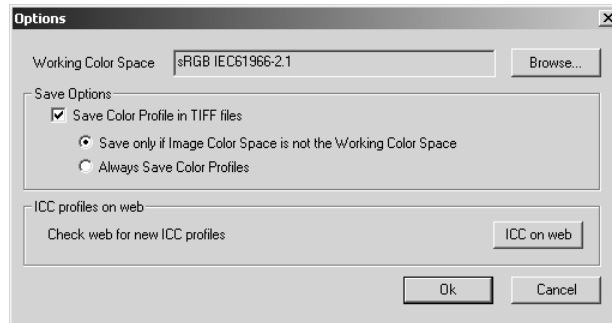
- **Printer Profile :** The **Printer Profile** list box lets you select a specific profile for the current printer. The default value that appears in this box is the default profile for the current printer's current configuration.

- **Proofing:** The **Proofing** check box allows you to enable soft (electronic) or hard (paper) proofing. When proofing is enabled, the **Emulated Device Profile** list box defaults to the current printer profile. However, all profiles installed on the system are available in this list box.

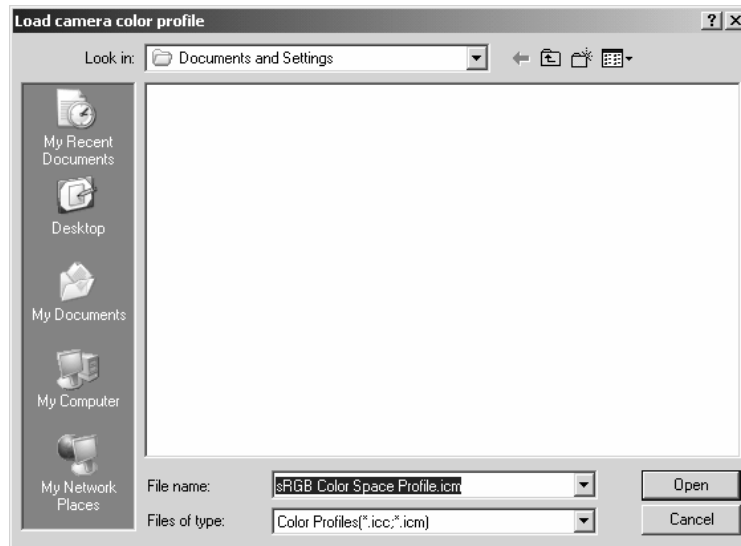
When proofing is enabled, the **Rendering Intent** list box under **Proofing** defaults to the intent set in the **Rendering Intent** list box. The upper **Rendering Intent** list box is set to the **Match** intent. You may select a different proofing intent. It would be very unusual, however, for you to choose anything other than **Match** in the upper list box at this time.

## The Color Management Options Dialog

When you click the **Options** button on the **Color Management** dialog, you will see the following choices:



**Working Color Space:** This list box defines the color space assigned to images without color profiles. To choose a different space, click the **Browse** button. You will see the **Load camera color profile** dialog:



Choose the correct profile from the list, and click **Open**. The selected profile should appear in the **Working Color Space** box.

**Save Options:** This portion of the dialog allows you to save your color profiles.

- **Save Color Profile in TIFF files :** When you check this box, the color profiles associated with images can be saved in TIFF files.
- **Save only if Image Color Space is not the Working Color Space:** When this option is selected, the profiles are saved only if they are different from working color space.
- **Always Save Color Profiles:** When you choose this option, you may save any color profile, including the working color space, associated with the image.

**ICC profiles on web:** New ICC color profiles will be available for download from the Media Cybernetics website. These profiles will work in conjunction with *Color-Pro* to ensure color fidelity for the most recent color cameras.

- **ICC on web:** Click this button to download the newest ICC color profiles

Once you have made your selections, click **OK**. Click **Cancel** to return to the **Color Management** dialog without making any changes.

*Edit:Color Management*

## The Acquire Menu

---

Use the *Acquire* menu commands and tab dialogs to capture an image directly from a digital camera, video camera, or other video input device (e.g., VCR). The *Acquire* commands let you monitor the video as you make camera and/or subject adjustments (refocusing, relighting, repositioning, and so forth). It also lets you monitor the video for the right subject matter to come into view. Once the conditions are as you want them, you can manually capture an image from the continuous video feed.

These commands also help you to select the appropriate hardware and software drivers, and configure your setup correctly.

---

**Note:** The form of the **Acquire** controls vary slightly according to the capture board you use. With certain boards, only the controls available for your driver will be present.

---

The procedure for acquiring an image involves the following basic steps:

- 1) **Setting up your digital camera or video input device and frame grabber** using the **Setup Tab**. *Image-Pro* stores these settings, so your next usage of the same hardware will not require resetting.
- 2) **Open Capture in the Main dialog.**
- 3) **Click the Auto-Set button to reset the default, auto-exposure, and white balance.**
- 4) **Start the Live Preview to focus your capture settings.**
- 5) **Click Snap to acquire an image.**

---

**Note:** **Auto-Set** will do the best job that it can to generate a good quality images from your camera hardware and the source. Under some circumstances, you may get unacceptable results if the source conditions are outside of the capabilities of your camera. Also, **Auto-Set** may not be available with your camera; it only applies to digital cameras.

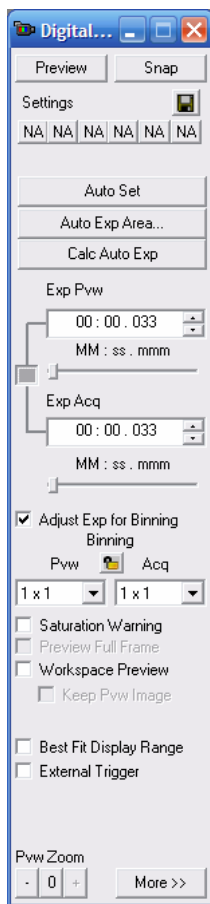
---

## The Digital Capture Dialog

---

In *Image-Pro Plus v. 7.0*, the capture dialog has been enhanced and improved. The “basic” dialog has been revised so that it contains a selection of tabs, allowing you to have access to all the capture features. There is no “advanced” dialog available anymore. The *digital* capture dialog is described in the following section, and the *analog* capture later in this chapter.

**Main Dialog:** Clicking on this button presents a reduced form of the *Signal* dialog, as shown here:



**Preview/Stop:** Click **Preview** to preview the image before capturing it to a file. Click **Stop** to deactivate the live-video display.

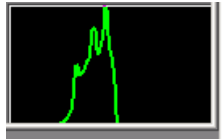


**Snap/Cancel:** Click this button to initiate the snap. The number of images, destination, and averaging are determined by the controls on the other tab dialogs. Click **Cancel** to stop the snap.

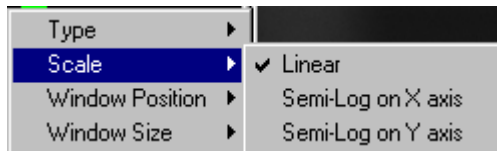
**Settings:** These buttons can be used to load your saved capture settings from *Image-Pro's Documents and Settings* folder. Click the right mouse button to see a pop-out menu displaying the selections to set a new file, review the current settings, un-assign the buttons, save the current settings into a file, or assign a button to that file.

Passing the cursor over one of the buttons will display the settings assigned to that button in a tool-tip popup window. If no settings are assigned to that button, "not assigned" or "N/A" will be displayed.

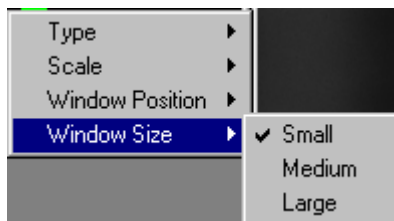
An image histogram is displayed automatically when you snap an image. You will see the image histogram, as shown here:



To adjust the histogram display, click the right mouse button on the preview image. The pop-out menus allow you to adjust the type, size, scale and position of the histogram, as shown here:



The *Scale* menu item allows you to select the scale for the histogram display. The available options are **Linear**, **Semi-Log on X axis**, or **Semi-Log on Y axis**. You may select only one type of scale at a time.



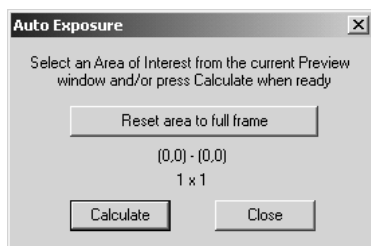
The *Window Size* menu allows you to increase or decrease the size of the histogram window. There are only three possible window sizes: small, medium and large.

### Acquire: The Digital Capture Dialog

You can adjust the boundaries of the histogram range by dragging the white bars in the image histogram window. The selected range will be displayed in the *Live Preview: Status* window.

**Auto-Set:** Clicking this button will start the process of setting the camera to produce a usable image. First, the driver will be reset to the factory defaults. Then, an auto-exposure will be done to get a good exposure time. If you are using a color camera, an auto-white balance will be performed to set the color balance correctly.

**Auto-Exp Area:** Clicking this button allows you to set an AOI for a live preview. If a live preview is not active, clicking this button will start one. You will see the following dialog:



**Reset Area to full frame:** Clicking this button resets the AOI equal to the full frame of the live preview.

**Calculate:** Click this button to calculate the best auto-exposure time for the current acquisition settings.

**Close:** Click this button to close the **Auto-Exposure** dialog and return to the *Basic Capture* dialog.

**Calculate Auto-Exp:** Click this button to calculate the best auto-exposure time for the current acquisition settings.

**Exp.Pvw/Exp.Acq:** These controls adjust the exposure times for the preview and actual acquisition in minutes, seconds, milliseconds, and nanoseconds. Click the **Lock icon** to make the exposure times equal for both preview and acquisition.

**Adj Exp for Binning:** Check this box to automatically adjust the exposure times and the zoom factor to keep the image size and brightness consistent whenever the binning value is changed. Both the preview and the acquire exposure times will be adjusted whenever a binning value or preview exposure valued changes. Note that the zoom factor adjustment may be limited by the number of zoom factors available for your camera driver. The **Auto-Adjust** will try to maintain image brightness but the results may not be perfect

*Acquire: The Digital Capture Dialog*

depending on your specific camera. Enabling this feature disables the **Lock Exposure Times** feature.

**Saturation Warning:** Use the **Saturation Warning** option to visualize saturated regions in the image preview. Saturated regions are pixels that are pure white. When this option is selected, all pure white pixels are displayed in red and all pure black pixels are displayed in blue.

**Preview full frame:** Checking this box resets the preview area to the maximum supported by your camera

**Workspace Preview:** Check **Workspace Preview** to display integrated live previewing in the image workspace window.

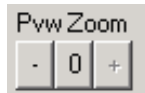
**Keep Preview Image:** Check this box to capture the last image displayed in the workspace window. If this option is unchecked, no image will be retained.

**Best Fit Display Range:** Checking the box automatically adjusts the display range of the captured images to the best fit.

**External Trigger:** Click this check box to toggle the use of an external trigger to snap an image. If you want to use the external trigger, you must first click this check box on, then click the **Snap** button, then press the external trigger when you are ready to capture the image. After clicking **Snap**, control of the computer is totally controlled by the external trigger; no further action can be taken until the trigger is activated.

**Select Device:** Choose your capture device from this drop-down list.

**Preview Zoom:** Indicate the degree to which you want your image increased or decreased by clicking the buttons shown below. Within the limits of your equipment and the refresh time you have set, the preview image will display current information. A smaller preview size may provide better response time than a full-size image.

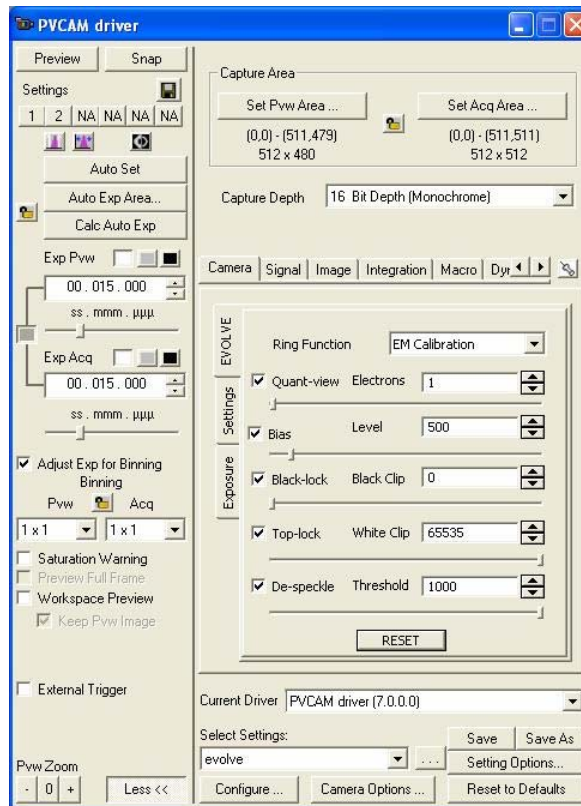


These options are enabled if your driver supports **Zoom** or the passthrough type is software controlled; otherwise, they will not appear. Clicking zero restores **Zoom** to its original settings.

**More/Less:** Clicking this button shows or hides the additional information on the right side of the dialog, as shown here (note that

## Acquire: The Digital Capture Dialog

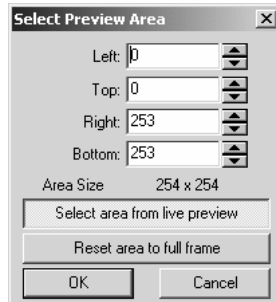
the tabbed areas are described in the *Digital Capture Tab Pages* section of this chapter):



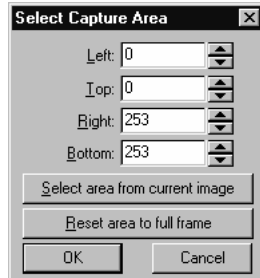
**Pvw Resolution/Acq Resolution:** These drop-down list boxes allow you to select different preview and acquire resolutions. If your driver does not support this feature, you will not see these selections.

**Capture Area:** The buttons in this area allow you to set an AOI for both preview and acquisition.

**Set Preview Area :** Clicking this button allows you to define the AOI for image preview. You will see the following dialog box:



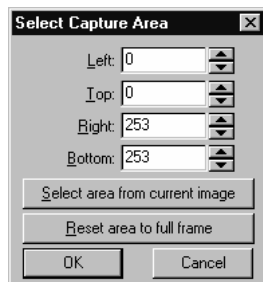
**Select Area from live preview:** If you do not have an active image, clicking this button will bring up a live preview and allow you to select an area of interest on the preview window.



**Select area from current image:** If you have an active image, clicking this button allows you to select an area of interest on the current image.

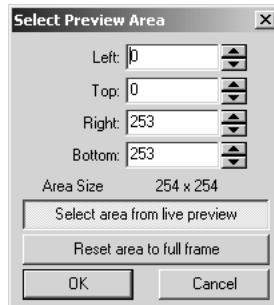
**Reset area to full frame:** If you have an active area in a preview already selected, clicking this button resets the capture area to the entire image enclosed within the frame, i.e. erasing your preview area.

**Set Acquisition Area:** Clicking this button allows you to define the AOI for image capture. You will see the following dialog box:



*Acquire: The Digital Capture Dialog*

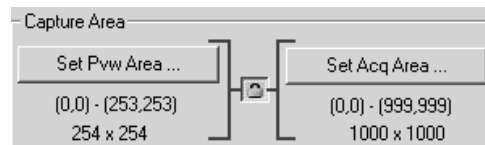
**Select area from current image:** If you have an active image, clicking this button allows you to select an area of interest on the current image.



**Select Area from live preview:** If you do not have an active image, clicking this button will bring up a live preview and allow you to select an area of interest on the preview window.

**Reset area to full frame:** If you have an active image in a preview already selected, clicking this button resets the capture area to the entire image enclosed within the frame, i.e. erasing your preview area.

Click the **Lock icon** to make the AOI settings equal for both preview and acquisition, as shown here:



**Capture Depth:** Select the color or monochrome acquisition depth from the drop down list box.

---

**Note:** The information that appears in the tabbed area of the dialog is described under the *Capture Tab Pages* section.

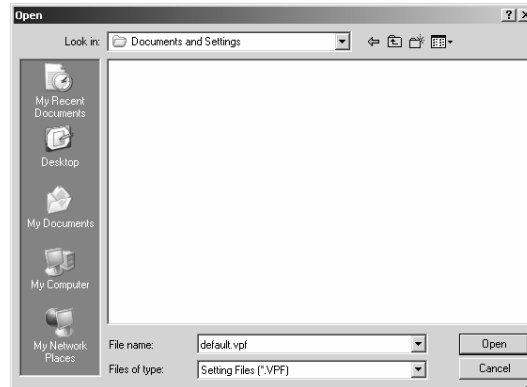
---

**Current Driver:** Select the appropriate driver from the drop-down list box.

**Settings File:** If you do not load a different file, the settings file for your driver will be default.vpf.

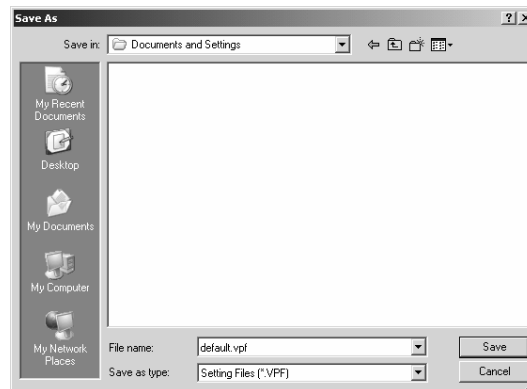
**Load Settings:** The settings file stores all the parameters for capturing images that you have defined on the different pages. Clicking this button allows you to load settings that you have previously saved. You will see the **Open** dialog box:

## Acquire: The Digital Capture Dialog



Please refer to the section about *Open and Save Dialog Boxes* earlier in this manual.

**Save Settings As:** Clicking this button allows you to save the settings that you have defined for this driver. You will see the **Save As** dialog box:



Please refer to the section about *Open and Save Dialog Boxes* earlier in this manual.

**Setting Options:** This drop-down list contains the .vpf files currently available for your acquisition system. Clicking the ... button allows you to load new settings from the **Documents and Settings** folder.

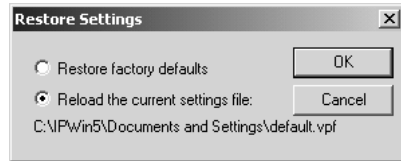
**Configure:** Click the **Configure** button to initialize settings for your hardware.

**Camera Options:** Click the **Camera Options** to set driver-specific options.

## Acquire: The Digital Capture Dialog

**Reset to Defaults:** Clicking this button returns all the settings to the factory defaults.

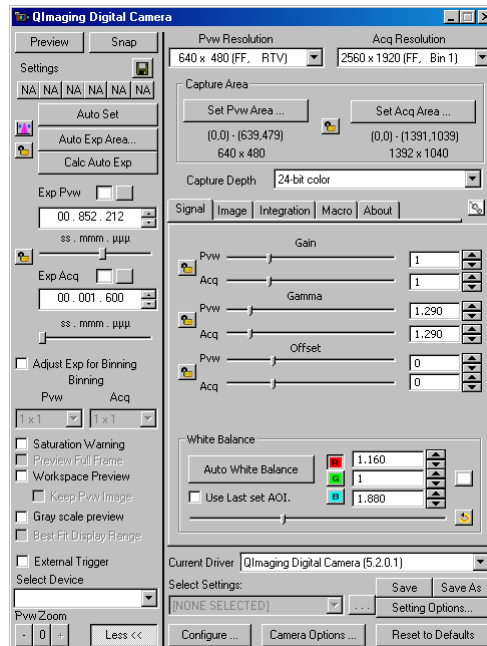
**Reload Settings:** Clicking this button will bring up the **Restore Settings** dialog box. You may then choose to restore the default (factory) settings or to reload the current settings file.



## Capture Tab Pages

The information that appears in the tabbed area depends on which tab you select. Clicking the pushpin icon will “freeze” the dialog at that particular tab.

## Signal Tab





*Acquire: The Digital Capture Dialog*

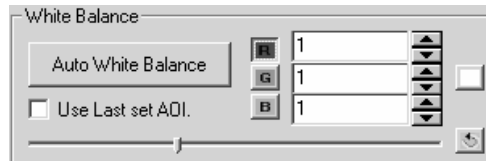
**Gain:** These sliders control the digital gain for both preview and acquisition. Preview and acquire may be adjusted individually or they may be locked together to set both simultaneously. Click the **Lock icon** to make the gain settings equal for both preview and acquisition.

**Gamma:** These sliders control the gamma for both preview and acquisition. Preview and acquire may be adjusted individually or they may be locked together to set both simultaneously. Click the **Lock icon** to make the gamma settings equal for both preview and acquisition.

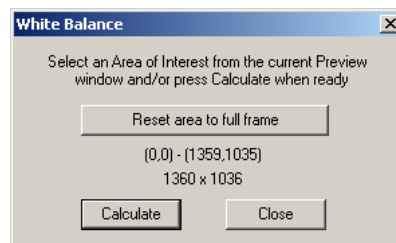
**Offset:** This feature allows you to adjust the vertical and horizontal offset of the capture device for both preview and acquisition. Preview and acquire may be adjusted individually or they may be locked together to set both simultaneously. Click the **Lock icon** to make the offset equal for both preview and acquisition.

**Custom Controls:** These controls are driver-specified and will depend on the driver you have selected. They may not appear if your driver does not have custom controls

**White Balance:** The **White Balance** controls allow you to adjust the white balance ratios for each of the color channels, either manually or automatically, as shown here:



**Auto White Balance:** Clicking this button will start a live preview, if one is not already active, and starts the process of calculating new color ratios, in order to establish a white balance based on the input image. You will see the **Auto White Balance** dialog, shown here:



*Acquire: The Digital Capture Dialog*

**Reset area to full frame:** If you have an active image in a preview already selected, clicking this button resets the capture area to the entire image enclosed within the frame, i.e. erasing your preview area.

**Calculate:** Click this button to calculate the white balance.

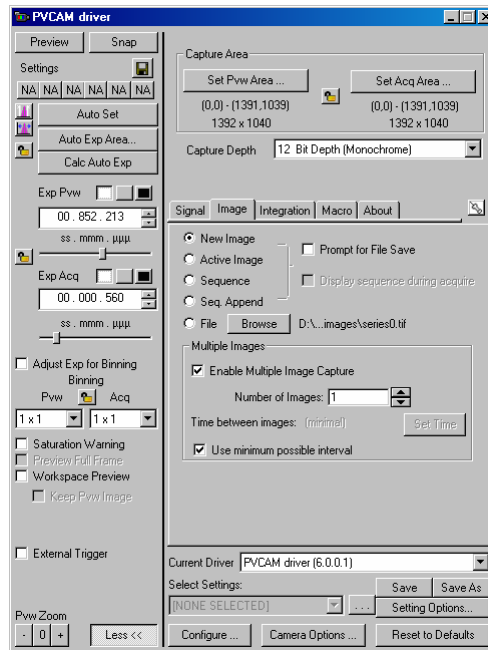
**Close:** Click **Close** to return to the **Acquire** page.

**Use Last Set AOI:** Clicking this button to use the AOI that you have most recently set, and will not bring up the **Auto-White Balance** dialog.

## Image Tab

The **Image** page contains the controls that determine how your image is captured, and where your images are stored. You can choose to use images in the open workspace, or images stored in a file. You may also select multiple image (multi-frame acquire).

Use the **Image** tabbed dialog if you want to automatically capture images directly from a camera or other video input device (e.g., VCR) on a timed basis. You may specify both the total number of images you want to capture and the frequency at which you want them acquired (*Timed Acquire*). For example, you could direct your application to capture an image every 20 minutes until 10 images had been accumulated.



**New Image:** Click this option button to create a new image when you click **Snap**.

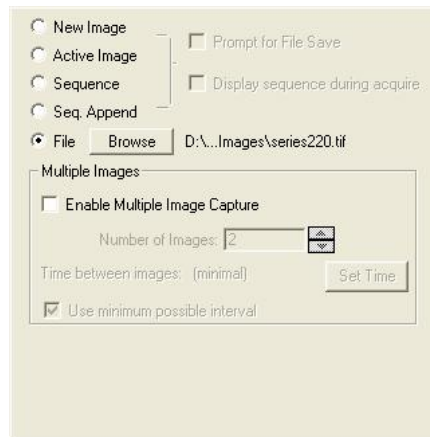
**Active Image:** Click this option button to replace the current image when you click **Snap**.

**Sequence:** Click this option button to store your images in a sequence file. (See the description of the *Sequencer* commands later in this chapter.)

*Acquire: The Digital Capture Dialog*

**Seq Append:** Check this box to append a newly captured sequence to a previously existing one.

**File:** Click this option button to store an image in a file when you click **Snap**.



**Prompt for File Save:** Checking this box will save the image file using the information specified in the **Select File Destination** dialog box, shown below. This checkbox is disabled when the **File** radio button is selected.

**Display Sequence During Acquire:** Checking this box will display each frame of a sequence as it is being captured, rather than capturing all the frames before displaying them. Checking this box will slow the sequence capture down, as each frame is displayed before the next one is captured.

**Browse:** Clicking this button sets the destination for the capture image.



**Path:** Enter the name of the drive and directory into which your captured images will be saved. Relative path names (i.e., “.” and “..”) can be used. You may either type the path information, or use the **Browse** button to select it from a standard **File** dialog box.

**File Name Prefix:** Enter a file name “prefix” for *Image-Pro* to use when generating files names for the accumulated images. This prefix will be combined with a sequence number to form a file name. For example, if a prefix of “PROC” was specified, and 11 images were to be acquired, they would be stored to files PROC00.TIF to PROC10.TIF (acquired images are always stored in TIFF format, so an extension of .TIF is automatically assigned).

You may type the prefix in this field, or you can specify it by selecting the name of an existing file using the **Browse** button; you may then modify it in this field to create a unique name.

If you select an existing file name, *Image-Pro* will issue a message giving you the choice of replacing the existing file or not.

**Start File Numbering:** Enter the sequence number to be appended to the file name “prefix” when the first image is stored. *Image-Pro* will automatically increment this number (by 1) when storing subsequent images.

**Number of Digits:** Enter the number of digits to use for the sequence number. This number determines the number of zeros to prepend to the sequence number when building the file name. For example, setting the number of digits to 3 will create a file name of **series000**, **series001**, **series002** etc.

**Browse:** Clicking this button displays the **Save As** dialog box. Please refer to the section about *Load and Save Dialog Boxes* in the first part of this manual.

Click **OK** to save your file destination information, or **Cancel** to return to the **Image** tab dialog.

**Multiple Images :** This group box contains the controls for acquiring several images at once.

**Enable Multiple Image Capture:** Check this box to turn on multiple image capture. Unchecking this box will turn off multiple image capture, and only single images will be captured.

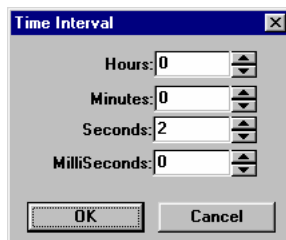
**Number of Images:** Enter the total number of images you want to accumulate during the timed-acquired session.

**Time Between Images:** Specify the interval, in Hours, Minutes, Seconds, and Hundredths of Seconds, at which you want to collect images. When accumulating images, *Image-Pro* will begin to

### Acquire: The Digital Capture Dialog

capture images at the specified interval after you press **Start** (and the next at  $2 * Interval$  after you press **Start**, and so forth).

**Set Time:** Clicking on this button displays the **Time Interval** dialog box:



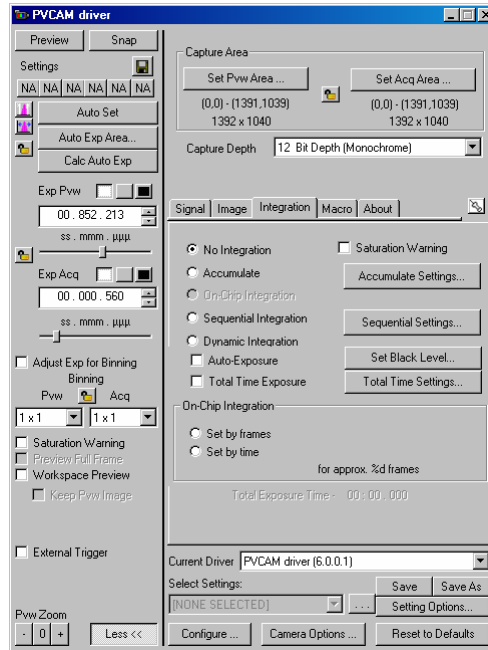
Enter the hours, minutes, seconds and milliseconds using the spin buttons in each box. Click OK to save your time interval, or Cancel to return to the **Image** page.

**Use Minimum Possible Interval:** Check this box to minimize, as much as possible, the time between images. When acquiring images in rapid succession, be aware that storing an image to disk takes a certain amount of time. For practical purposes, intervals less than 900 milliseconds do not work well (this assumes you have a fast machine -- the interval would be longer for slower systems). If your system is still processing an image when the interval for the next image arrives, *Image-Pro* will capture the next image as soon as it completes its processing of the current one. It will not simply skip the missed image and wait for the next interval to occur. Note that you may not be able to use the **Cancel** button while this feature is active.

### Integration Tab

When you select the Integration tab you will the options displayed below. Use the **Integration** page commands if you want to collect a number of frames and create a single image by averaging the corresponding pixel values of the collected images. This is usually done for noise-reduction purposes. You can also use **Integration** to *accumulate* frames (instead of averaging them), which might be done to boost intensity values under low-light conditions.

When you perform an **Integration** operation, your application accumulates the specified number of frames (i.e., adds the corresponding pixel values), then divides the result by the specified divisor. The resulting image is placed into a new, untitled image window within your application.



**No Integration:** This option disables the options on the Integration page. It should be selected if you are not doing integrated captures.

**Saturation Warning:** Use the **Saturation Warning** option to visualize saturated regions in the image preview. Saturated regions are pixels that are pure white. When this option is selected, all pure white pixels are displayed in red and all pure black pixels are displayed in blue.

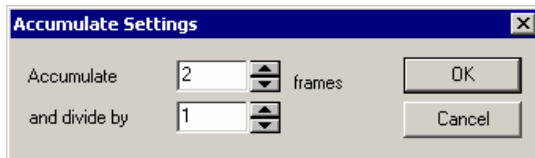
**Accumulate:** In this field, enter the number of frames you want to accumulate. If your hardware supports it, the specified number of frames will be collected and averaged by the hardware itself.

For noise-reduction purposes, an accumulation of 16 frames is usually sufficient. Very minimal quality gains are achieved by averaging more than this amount.

You must click this button to have your application generate averaged images.

**Accumulate Settings...:** This button is only available if the **Accumulate** option button is selected. When you click this button, the **Accumulate Settings** dialog box is displayed.

### Acquire: The Digital Capture Dialog

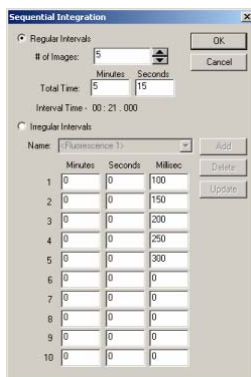


Select the number of frames to accumulate and a division factor and click on OK.

**On-Chip Integration:** Click this button if you are using the on-chip integration feature provided with some of the Media Cybernetics capture kits.

**Sequential Integration:** Select this option if you want to capture a sequence of integrated images, where each image represents a different exposure time. When this option is selected, you must define the number of images to be created and the sequence of increasing exposure times to be used through the dialog box accessed through the **Sequential Settings** button.

**Sequential Settings:** This button is only available if the **Sequential Integration** option button is selected. When you click this button, the **Sequential Integration** dialog box is displayed.



If you are using the sequential integration function, *Capture* will create a sequence file of images that represent integrated captures at increasing exposure times. The **Sequential Integration** dialog box allows you to define the number of images to be created and the exposure times to be used.

You can tell *Capture* to use either regularly-increasing exposure times or irregularly-increasing, user-defined exposure times.



**Regular Intervals:** Select this option button if you want *Capture* to create a series of images that represent integrated captures at regularly-increasing exposure times. When you select this option, you must specify the number of images you want created and the total exposure time. From these specifications, *Capture* calculates the rate of increase to use for the sequential exposures.

**Irregular Intervals:** Select this option button if you want *Capture* to create a series of images that represent integrated captures at irregularly-increasing exposure times. When you select this option, you can either use a predefined interval definition or specify a new one (see **Name** and “Defining a New Set of Irregular Interval” below)

**Name:** The **Name** pull down list box displays all of the default interval definitions and any that have been added by the user. If a pre-existing interval definition exists for your purposes, simply select it and the interval chart will be filled in with the appropriate values. To add the new set of interval definitions to the **Name** list box, type a new name into the **Name** list box and click on **Add**.

**Dynamic Integration:** This option allows you to create an integrated image that represents the accumulation of a series of image captures. If both the **Auto-Exposure** and **Total Time Exposure** checkboxes are left blank, this option lets you visualize the integration process onscreen, as images are being accumulated/integrated, and manually stop the process when the image appears as you want it.

**Auto-Exposure:** Check this checkbox if you are doing a dynamic integration and you want *Capture* to stop the capture operation (that is, stop accumulating images) when 1% of the image has reached saturation.

**Total Time Exposure:** Check this checkbox if you are doing a dynamic integration and you want the resulting image to represent the integration of a specific number of images over a specific amount of time. This has the advantage of allowing for time intervals to elapse between the images being integrated. The number of images and time frame are specified through the **Total Time Settings** button.

**Set Black Level...:** Use the dialog box that this button accesses to specify the pixel value of the darkest gray level that your camera is capable of transmitting. Pixel values in the image less than the value specified here will not be accumulated (that is, will be set to zero).

*Acquire: The Digital Capture Dialog*

**Total Time Settings:** If you have selected the **Total Time Exposure** option, click this button to access the **Dynamic Integration** dialog box where you can specify the total number of images to be integrated into a single image and span of time across which images should be acquired.

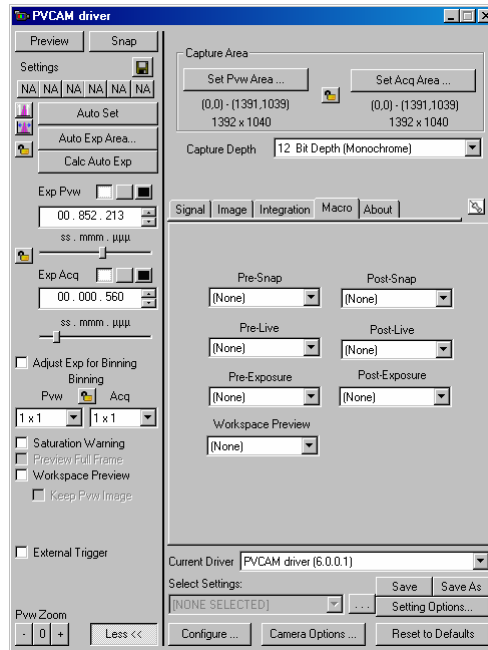
**On-Chip Integration:** Choose one of these selections to perform on-chip integration. During on-chip integration, the signal or charge will be accumulated on the CCD.

**Set by frames:** Click this button to set the integration time by enter a value for the number of frames.

**Set by time:** Click this button to set the integration time by entering a time value.

## Macro Tab

The **Macro** page contains any specialized routines that you have created to run before or after capturing an image.



**Pre-Snap Macro:** Enter the name of a macro you want *Image-Pro* to run before each snap. For example, you might want to sound a bell before each snap occurs. If the time required for your hardware to perform the Pre-Snap Macro and acquire the image exceeds the interval you have selected, *Image-Pro* will acquire images as rapidly as your hardware permits. Select **None** if you do not want a Pre-Snap Macro.

**Post-Snap Macro:** Enter the name of a macro you want *Image-Pro* to run after each snap. For example, you might want to load the image just saved, apply a filter, and resave the image. If the time required for your hardware to perform the Post-Snap Macro and acquire the image exceeds the interval you have selected, *Image-Pro* will acquire images as rapidly as your hardware permits. Select **None** if you do not want a Post-Snap Macro.

**Pre-Live Macro:** Enter the name of a macro you want *Image-Pro* to run before each live capture.

## Acquire: The Digital Capture Dialog

**Post-Live Macro:** Enter the name of a macro you want *Image-Pro* to run after each live capture.

**Pre-Exposure Macro:** Enter the name of a macro you want *Image-Pro* to run before each the exposure starts.

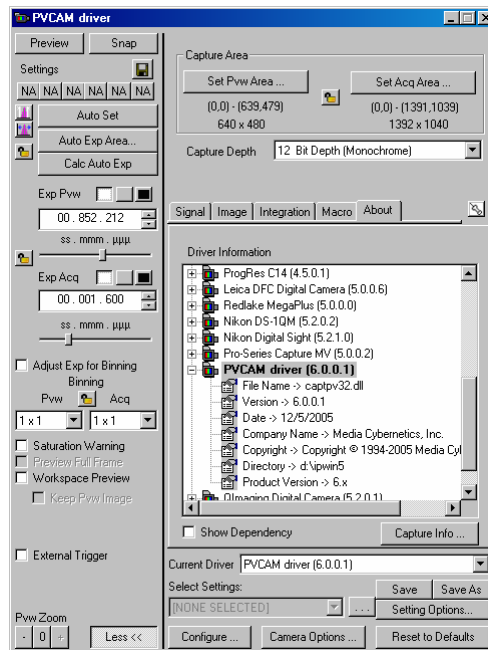
**Post-Exposure Macro:** Enter the name of a macro you want *Image-Pro* to run after each exposure is concluded.

If you have selected “minimum interval” from the **Image** page, the macros are played before the sequence, and after, but not during the capture.

**Workspace Preview:** Check **Workspace Preview** to display integrated live previewing.

## About Tab

The *About* tab contains information specific to your camera, driver, and capture module.



**Show Dependency:** Checking this box allows you to display the dependency information for the driver dll. This is useful information for update and technical services.

**Capture Info:** You can also save all the information shown above to a temporary file by pressing the **Capture Info** button. This will display the path and filename where this information will be saved and give the user the option to display the information in NOTEPAD if available. This is a sample window of the information being saved in the CaptureInfo.txt file.



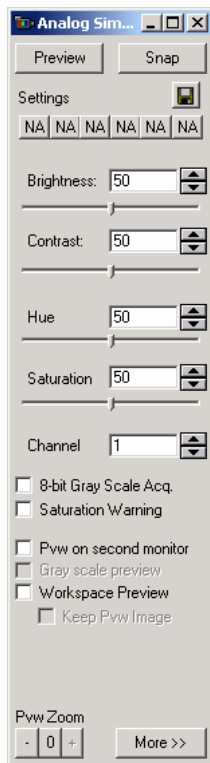
```
File Edit Format Help
CaptureInfo.txt - Notepad
Capture information
File Name -> MCCAPT32.DLL
Version -> 6.0.0.1
Date -> 12/3/2003
Company Name -> Media Cybernetics, Inc.
Copyright -> Copyright © 1993-2004 Media Cybernetics, Inc.
Directory -> d:\lpw\ins
Product Version -> 3.0
Analog Simulation (3.0.0.1)
File Name -> captads2.dll
Version -> 3.0.0.1
Date -> 11/25/2003
Company Name -> Media Cybernetics Inc.
Copyright -> Copyright © 1998-2003 Media Cybernetics, Inc.
Directory -> d:\lpw\ins
Product Version -> 3.x
CoolSnap-pro (4.1.1.1)
File Name -> captcs32.dll
Version -> 4.1.1.1
Date -> 9/28/2003
Company Name -> Media Cybernetics, Inc.
Copyright -> Copyright © 2000-2003 Media Cybernetics
Directory -> d:\lpw\ins
Product Version -> 4.x
Leica PC-500 (4.5.0.2)
File Name -> captcs00.dll
Version -> 4.5.0.2
Date -> 3/9/2003
Company Name -> Media Cybernetics
Copyright -> Copyright © 2002 Media Cybernetics
Directory -> d:\lpw\ins
Product Version -> 4.x only
Digital Simulation (5.0.0.2)
File Name -> captds2.dll
Version -> 5.0.0.2
Date -> 10/23/2003
Company Name -> Media Cybernetics, Inc.
Copyright -> Copyright © 1997-2003 Media Cybernetics, Inc.
Directory -> d:\lpw\ins
Product Version -> 3.x
Olympus SP70 (5.0.0.0)
File Name -> captsp70.dll
Version -> 5.0.0.0
Date -> 8/29/2003
Company Name -> Media Cybernetics Inc.
Copyright -> Copyright © 2003 Media Cybernetics Inc.
Directory -> d:\lpw\ins
Product Version -> 3.x
Olympus SP Camera (3.2.0.0)
File Name -> captspcam.dll
Version -> 3.2.0.0
Date -> 10/16/2004
Company Name -> Media Cybernetics Inc.
Copyright -> Copyright © 2004 Media Cybernetics Inc.
Directory -> d:\lpw\ins
Product Version -> 3.x
DirectShow (3.0.0.0)
File Name -> captds2.dll
Version -> 3.0.0.0
Date -> 10/7/2003
Company Name -> Media Cybernetics, L.P.
Copyright -> Copyright © 1999 Media Cybernetics
Directory -> d:\lpw\ins
Product Version -> 3.x or 4.0
Nikon DMX200P (5.0.0.1)
File Name -> captdm200p.dll
Version -> 5.0.0.1
Date -> 10/3/2003
Company Name -> Media Cybernetics Inc.
```

## The Analog Capture Dialog

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The analog capture dialog, formerly called the **minimal dialog**, has been enhanced and improved in *Image-Pro Plus v. 7.0*. The capture dialog has been revised so that it contains a selection of tabs, allowing you to have access to all the capture features. There is no “advanced” dialog available anymore. The *analog* capture dialog is described in the following section.

**Main dialog:** Clicking on this button presents a reduced form of the *Signal* dialog, as shown here:



**Preview/Stop:** Click **Preview** to preview the image before capturing it to a file. Click **Stop** to deactivate the live-video display.

**Snap:** Click this button to initiate the snap. The number of images, destination, and averaging are determined by the controls on the tabs in the **Advanced** dialog.

**Settings:** These buttons can be used to load your saved capture settings from *Image-Pro's Documents and Settings* folder quickly.

*Acquire: The Analog Capture Dialog*

**Brightness:** Use the spin buttons or the slider to decrease or increase the **Brightness** of the incoming live signal.

**Contrast:** Use the spin buttons or the slider to decrease or increase the **Contrast** of the incoming live signal.

**Hue:** Use the spin buttons or the slider to adjust the **Hue** of the captured image.

**Saturation:** Use the spin buttons or the slider to adjust the **Saturation** of the captured image.

**Channel:** Use the spin buttons or type in the value to select the input channel your camera cable is attached to: 0, 1, 2 or 3 (your hardware may have fewer channel choices than this - e.g. 0,1 or 0, 1, 2).

**8-bit gray scale acq:** Check this box if you are going to be capturing 8-bit gray scale images.

**Saturation Warning:** Use the **Saturation Warning** option to visualize saturated regions in the image preview. Saturated regions are pixels that are pure white. When this option is selected, all pure white pixels are displayed in red and all pure black pixels are displayed in blue.

**Preview on second monitor:** Click this check box to toggle the live-video display between dual-monitor (video conveyed to the second monitor) and software passthrough (video conveyed to the system monitor) mode. This check box will not appear if the board is configured for a single monitor.

**8-bit gray scale Preview:** Check this box if you are going to be capturing 8-bit gray scale images, and would like to see them before the actual capture.

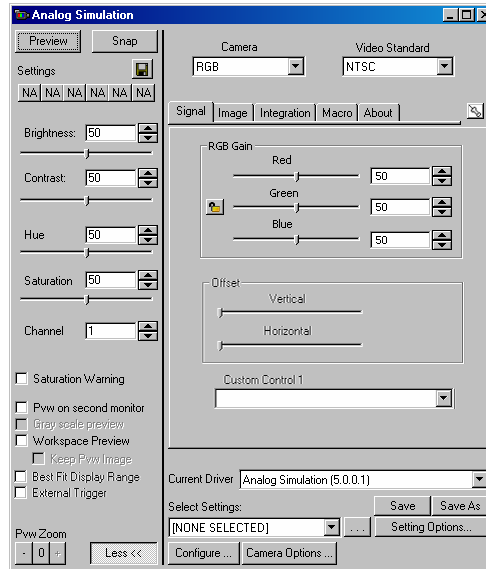
**Workspace Preview:** Check **Workspace Preview** to display integrated live previewing.

**Keep Preview Image:** Check this box to capture the last image displayed in the live preview window. If this option is unchecked, no image will be retained.

**Preview Zoom:** Indicate the degree to which you want your image increased or decreased. Within the limits of your equipment and the refresh time you have set, the preview image will display current information. A smaller preview size may provide better response time than a full-size image. These options are enabled if your driver supports **Zoom** or the passthrough type is software controlled; otherwise, they will not appear. Clicking zero restores **Zoom** to its original settings.

## Acquire: The Analog Capture Dialog

**More:** Click this button to hide or show the additional selections on the right side of the dialog, as shown here.



**Camera:** Choose your camera from the drop-down list. The current selections are composite, RGB, or s-video.

**Video Standard:** This group box displays the video standard that your frame grabber uses. Consult the documentation for your hardware if uncertain. This group box will not appear if *Image-Pro* can obtain the information from configuration files for the video input device or if your frame grabber only supports one type.

- **NTSC** (National Television System Committee): in general use in the United States..
- **PAL** (Phase Alternation Line): in general use in Europe.

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**Note:** The selections appearing in the tabbed area depend on the camera and driver selected. They are discussed under **Capture Tab Pages** later in this chapter.

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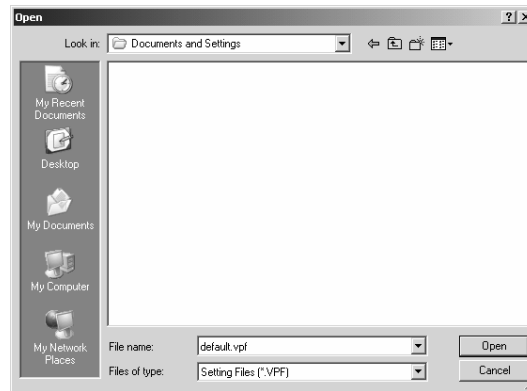
**Current Driver:** Select the appropriate driver from the drop-down list box.

**Select Settings:** This drop-down list contains the .vpf files currently available for your acquisition system. Clicking the ... button allows you to load new settings from the **Documents and Settings** folder.



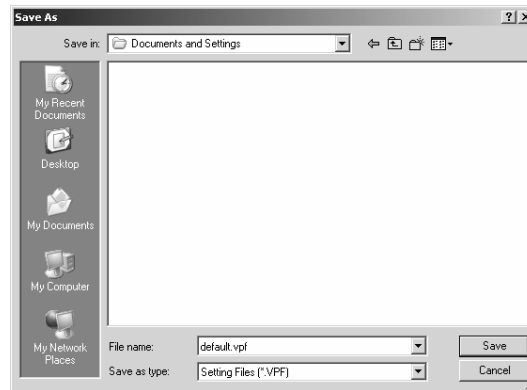
## Acquire: The Analog Capture Dialog

**Load Settings:** The settings file stores all the parameters for capturing images that you have defined on the different pages. Clicking this button allows you to load settings that you have previously saved. You will see the **Open** dialog box:



Please refer to the section about *Open and Save Dialog Boxes* earlier in this manual.

**Save Settings As:** Clicking this button allows you to save the settings that you have defined for this driver. You will see the **Save As** dialog box:



Please refer to the section about *Open and Save Dialog Boxes* earlier in this manual.

**Configure:** Click the **Configure** button to initialize settings for your hardware.

**Camera Options:** Click the **Camera Options** to set the options for your specific camera hardware.

*Acquire: The Analog Capture Dialog*

**Reset to Defaults:** Click this button to return your controls to their original or “factory” settings.

*Remember - the options that you see may vary with different types of capture board cameras.*

**Frame Offset:** This feature allows you to adjust the vertical and horizontal offset of the capture device.

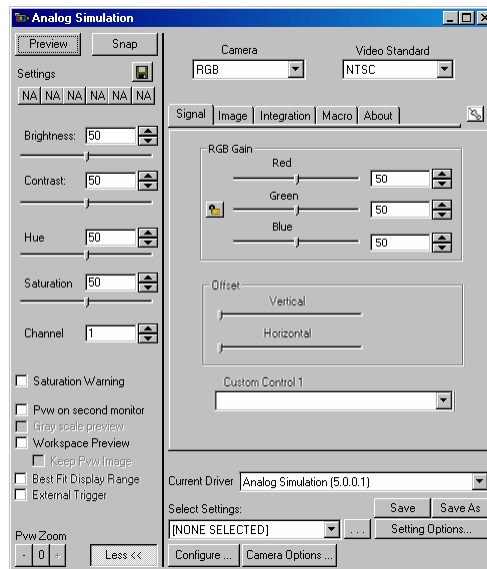
**Custom Controls:** These controls are driver-specific and will depend on the driver you have selected. They may not appear if your driver does not have custom controls.

## Analog Capture Tab Pages

The selections for an analog (non-digital) camera appear in the following section. Digital cameras are discussed at the beginning of this chapter.

### Signal Tab

The **Signal** tab is the first tab that appears. Click the push-pin icon to “freeze” the tab in place.



**RGB Gain:** Indicates the digital gain. Each channel may be adjusted individually or all three may be locked together to set the three channels simultaneously.

**Offset:** This feature allows you to adjust the vertical and horizontal offset of the capture device for both preview and acquisition. Preview and acquire may be adjusted individually or they may be locked together to set both simultaneously. Click the **Lock icon** to make the offset equal for both preview and acquisition.

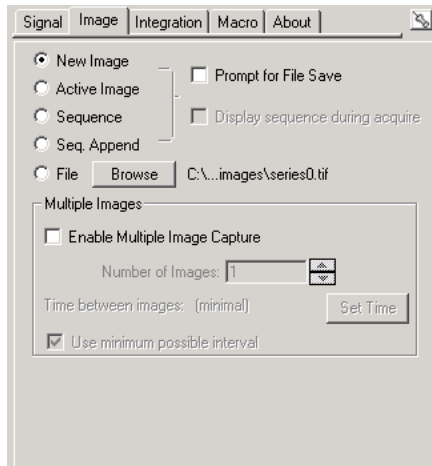
**Custom Controls:** These controls are driver-specified and will depend on the driver you have selected. They may not appear if your driver does not have custom controls

## Acquire: The Analog Capture Dialog

### Image Tab

The **Image** page contains the controls that determine how your image is captured, and where your images are stored. You can choose to use images in the open workspace, or images stored in a file. You may also select multiple image (multi-frame acquire).

Use the **Image** tabbed dialog if you want to automatically capture images directly from a camera or other video input device (e.g., VCR) on a timed basis. You may specify both the total number of images you want to capture and the frequency at which you want them acquired (*Timed Acquire*). For example, you could direct your application to capture an image every 20 minutes until 10 images had been accumulated.



**New Image:** Click this option button to create a new image when you click **Snap**.

**Active Image:** Click this option button to replace the current image when you click **Snap**.

**Sequence:** Click this option button to store your images in a sequence file. (See the description of the *Sequencer* commands later in this chapter.)

**Seq Append:** Check this box to append a newly captured sequence to a previously existing one.

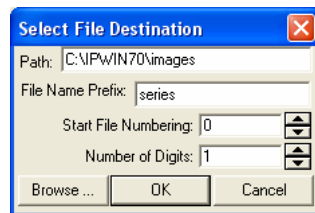
**File:** Click this option button to store an image in a file when you click **Snap**.

### *Acquire: The Analog Capture Dialog*

**Prompt for File Save:** Checking this box will display a standard File:Save dialog box after capturing an image.

**Display Sequence During Acquire:** Checking this box will display each frame of a sequence as it is being captured, rather than capturing all the frames before displaying them. Checking this box will slow the sequence capture down, as each frame is displayed before the next one is captured.

**Browse:** Clicking this button sets the destination for the capture image. You will see the following dialog box:



**Path:** Enter the name of the drive and directory into which your captured images will be saved. Relative path names (i.e., “.” and “..”) can be used. You may either type the path information, or use the **Browse** button to select it from a standard **File** dialog box.

**File Name Prefix:** Enter a file name “prefix” for *Image-Pro* to use when generating file names for the accumulated images. This prefix will be combined with a sequence number to form a file name. For example, if a prefix of “PROC” was specified, and 11 images were to be acquired, they would be stored to files PROC00.TIF to PROC10.TIF (acquired images are always stored in TIFF format, so an extension of .TIF is automatically assigned).

You may type the prefix in this field, or you can specify it by selecting the name of an existing file using the **Browse** button; you may then modify it in this field to create a unique name.

If you select an existing file name, *Image-Pro* will issue a message giving you the choice of replacing the existing file or not.

**Start File Numbering:** Enter the sequence number to be appended to the file name “prefix” when the first image is stored. *Image-Pro* will automatically increment this number (by 1) when storing subsequent images.

**Number of Digits:** Enter the number of digits to use for the sequence number. This number determines the number of zeros to prepend to the sequence number when building the file name. For

### Acquire: The Analog Capture Dialog

example, setting the number of digits to 3 will create a file name of **series000**, **series001**, **series002** etc.

**Browse:** Clicking this button displays the Save As dialog box.

Please refer to the section about Load and Save Dialog Boxes in the first part of this manual.

Click **OK** to save your file destination information, or **Cancel** to return to the **Image** tab dialog.

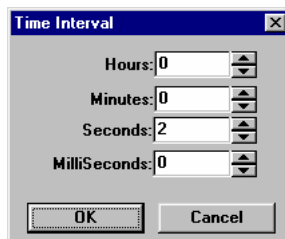
**Multiple Images :** This group box contains the controls for acquiring several images at once.

**Enable Multiple Image Capture:** Check this box to turn on multiple image capture. Unchecking this box will turn off multiple image capture, and only single images will be captured.

**Number of Images:** Enter the total number of images you want to accumulate during the timed-acquired session.

**Time Between Images:** Specify the interval, in Hours, Minutes, Seconds, and Hundredths of Seconds, at which you want to collect images. When accumulating images, *Image-Pro* will begin to capture images at the specified interval after you press **Start** (and the next at  $2 * Interval$  after you press **Start**, and so forth).

**Set Time:** Clicking on this button displays the **Time Interval** dialog box:



Enter the hours, minutes, seconds and milliseconds using the spin buttons in each box. Click OK to save your time interval, or Cancel to return to the **Image** page.

**Use Minimum Possible Interval:** Check this box to minimize, as much as possible, the time between images. When acquiring images in rapid succession, be aware that storing an image to disk takes a certain amount of time. For practical purposes, intervals less than 900 milliseconds do not work well (this assumes you have a fast machine -- the interval would be longer for slower systems). If your system is still processing an image when the interval for the next

### Acquire: The Analog Capture Dialog

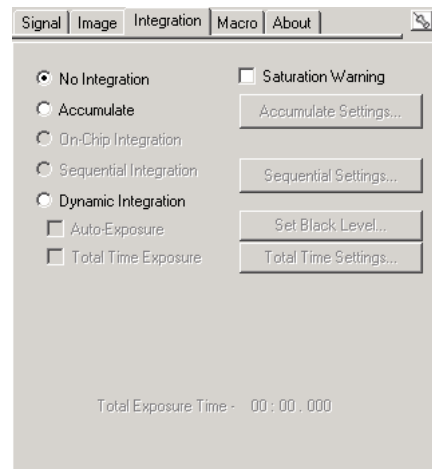
image arrives, *Image-Pro* will capture the next image as soon as it completes its processing of the current one. It will not simply skip the missed image and wait for the next interval to occur. Note that you may not be able to use the **Cancel** button while this feature is active.

### Integration Tab

Use the **Integration** page commands if you want to collect a number of frames and create a single image by averaging the corresponding pixel values of the collected images. This is usually done for noise-reduction purposes. You can also use **Integration** to *accumulate* frames (instead of averaging them), which might be done to boost intensity values under low-light conditions.

When you perform an **Integration** operation, your application accumulates the specified number of frames (i.e., adds the corresponding pixel values), then divides the result by the specified divisor. The resulting image is placed into a new, untitled image window within your application.

The **Integration** tab is shown here:



**No Integration:** This option disables the options on the Integration page. It should be selected if you are not doing integrated captures.

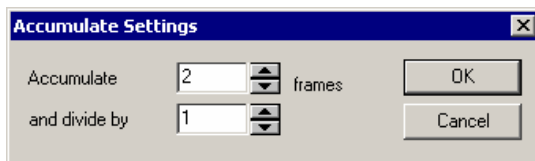
**Accumulate:** In this field, enter the number of frames you want to accumulate. If your hardware supports it, the specified number of frames will be collected and averaged by the hardware itself.

### Acquire: The Analog Capture Dialog

For noise-reduction purposes, an accumulation of 16 frames is usually sufficient. Very minimal quality gains are achieved by averaging more than this amount.

You must click this button to have your application generate averaged images.

**Accumulate Settings...:** This button is only available if the **Accumulate** option button is selected. When you click this button, the **Accumulate Settings** dialog box is displayed.

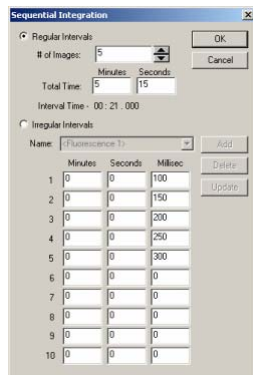


Select the number of frames to accumulate and a division factor and click on OK.

**On-Chip Integration:** Click this button if you are using the on-chip integration feature provided with some of the Media Cybernetics capture kits. The following additional controls will be available:

**Sequential Integration:** Select this option if you want to capture a sequence of integrated images, where each image represents a different exposure time. When this option is selected, you must define the number of images to be created and the sequence of increasing exposure times to be used through the dialog box accessed through the **Sequential Settings** button.

**Sequential Settings:** This button is only available if the **Sequential Integration** option button is selected. When you click this button, the **Sequential Integration** dialog box is displayed.





### *Acquire: The Analog Capture Dialog*

If you are using the sequential integration function, *Capture* will create a sequence file of images that represent integrated captures at increasing exposure times. The **Sequential Integration** dialog box allows you to define the number of images to be created and the exposure times to be used.

You can tell *Capture* to use either regularly-increasing exposure times or irregularly-increasing, user-defined exposure times.

**Regular Intervals:** Select this option button if you want *Capture* to create a series of images that represent integrated captures at regularly-increasing exposure times. When you select this option, you must specify the number of images you want created and the total exposure time. From these specifications, *Capture* calculates the rate of increase to use for the sequential exposures.

**Irregular Intervals:** Select this option button if you want *Capture* to create a series of images that represent integrated captures at irregularly-increasing exposure times. When you select this option, you can either use a predefined interval definition or specify a new one (see **Name** and “Defining a New Set of Irregular Interval” below)

**Name:** The **Name** pull down list box displays all of the default interval definitions and any that have been added by the user. If a pre-existing interval definition exists for your purposes, simply select it and the interval chart will be filled in with the appropriate values. To add the new set of interval definitions to the **Name** list box, type a new name into the **Name** list box and click on **Add**.

**Tip: Defining a New Set of Irregular Intervals**

You can create a sequence of up to 10 images with unique exposure times. To define a new sequence interval, simply select one of the predefined interval definitions from the **Name** list box and type over the specified exposure time values with the values you want to use.

**Note** - The values you enter in the interval chart for each image represent total exposure times, not exposure time increases from the previous image. Also, if you are creating less than ten images, enter zeros in the text boxes of the rows for images that you do not want created.

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To add the new set of interval definitions to the **Name** list box, type a new name into the **Name** list box and click on **Add**.

**Dynamic Integration:** This option allows you to create an integrated image that represents the accumulation of a series of image captures. If both the **Auto-Exposure** and **Total Time Exposure** checkboxes are left blank, this option lets you visualize the integration process onscreen, as images are being accumulated/integrated, and manually stop the process when the image appears as you want it.

**Auto-Exposure:** Check this checkbox if you are doing a dynamic integration and you want *Capture* to stop the capture operation (that is, stop accumulating images) when 1% of the image has reached saturation.

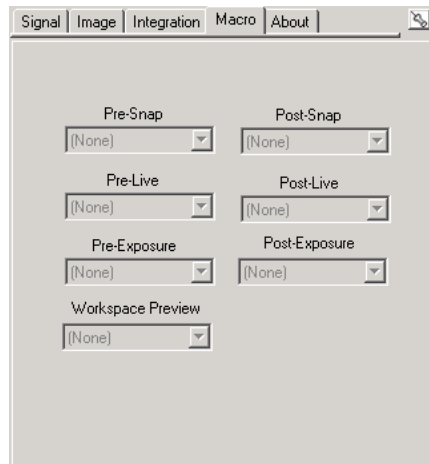
**Set Black Level...:** Use the dialog box that this button accesses to specify the pixel value of the darkest gray level that your camera is capable of transmitting. Pixel values in the image less than the value specified here will not be accumulated (that is, will be set to zero).

**Total Time Exposure:** Check this checkbox if you are doing a dynamic integration and you want the resulting image to represent the integration of a specific number of images over a specific amount of time. This has the advantage of allowing for time intervals to elapse between the images being integrated. The number of images and time frame are specified through the **Total Time Settings** button.

**Total Time Settings:** If you have selected the **Total Time Exposure** option, click this button to access the **Dynamic Integration** dialog box where you can specify the total number of images to be integrated into a single image and span of time across which images should be acquired.

## Macro Tab

The **Macro** page contains any specialized routines that you have created to run before or after capturing an image.



**Pre-Snap Macro:** Enter the name of a macro you want *Image-Pro* to run before each snap. For example, you might want to sound a bell before each snap occurs. If the time required for your hardware to perform the Pre-Snap Macro and acquire the image exceeds the interval you have selected, *Image-Pro* will acquire images as rapidly as your hardware permits. Select **None** if you do not want a Pre-Snap Macro.

**Post-Snap Macro:** Enter the name of a macro you want *Image-Pro* to run after each snap. For example, you might want to load the image just saved, apply a filter, and resave the image. If the time required for your hardware to perform the Post-Snap Macro and acquire the image exceeds the interval you have selected, *Image-Pro* will acquire images as rapidly as your hardware permits. Select **None** if you do not want a Post-Snap Macro.

**Pre-Live Macro:** Enter the name of a macro you want *Image-Pro* to run before each live capture.

**Post-Live Macro:** Enter the name of a macro you want *Image-Pro* to run after each live capture.

**Pre-Exposure Macro:** Enter the name of a macro you want *Image-Pro* to run before each the exposure starts.

## Acquire: The Analog Capture Dialog

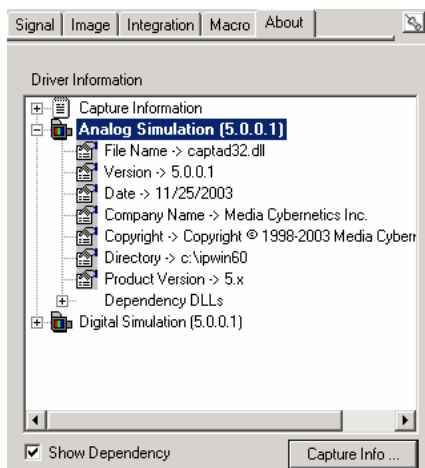
**Post-Exposure Macro:** Enter the name of a macro you want *Image-Pro* to run after each exposure is concluded.

If you have selected “minimum interval” from the **Image** page, the macros are played before the sequence, and after, but not during the capture.

**Workspace Preview:** Check **Workspace Preview** to display integrated live previewing.

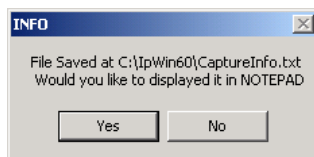
### About Tab

The **About** tab contains information specific to your camera, driver, and capture module, as shown here:



**Show Dependency:** Checking this box expands or contracts the information about your driver. The expanded version is shown above.

**Capture Info:** Click this button to display your capture information in text format. You will see the following dialog:

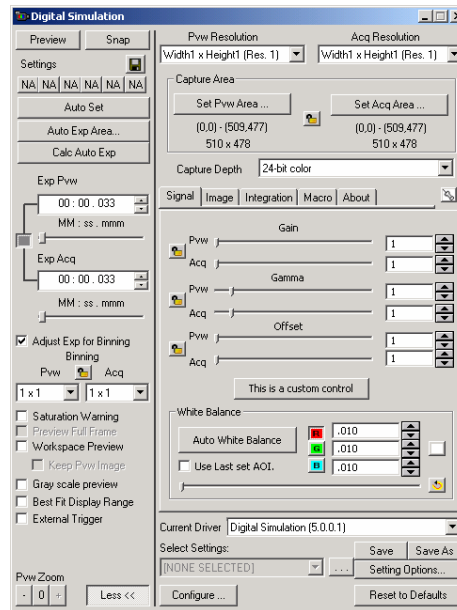


Click **Yes** or **No**.

## Capture Options

### MultiFrame/Timed

Selecting *MultiFrame/Timed* from the **Capture Options** menu displays the **Capture** dialog open to the *Signal* page.



**Gain:** These sliders control the digital gain for both preview and acquisition. Preview and acquire may be adjusted individually or they may be locked together to set both simultaneously. Click the **Lock icon** to make the gain settings equal for both preview and acquisition.

**Gamma:** These sliders control the gamma for both preview and acquisition. Preview and acquire may be adjusted individually or they may be locked together to set both simultaneously. Click the **Lock icon** to make the gamma settings equal for both preview and acquisition.

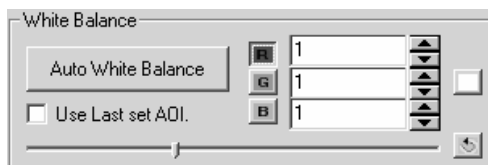
**Offset:** This feature allows you to adjust the vertical and horizontal offset of the capture device for both preview and acquisition. Preview and acquire may be adjusted individually or they may be

## Acquire: Capture Options

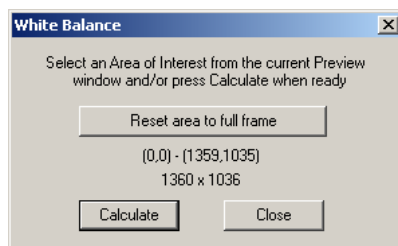
locked together to set both simultaneously. Click the **Lock icon** to make the offset equal for both preview and acquisition.

**Custom Controls:** These controls are driver-specified and will depend on the driver you have selected. They may not appear if your driver does not have custom controls

**White Balance:** The **White Balance** controls allow you to adjust the white balance ratios for each of the color channels, either manually or automatically, as shown here:



**Auto White Balance:** Clicking this button will start a live preview, if one is not already active, and starts the process of calculating new color ratios, in order to establish a white balance based on the input image. You will see the **Auto White Balance** dialog, shown here:



**Reset area to full frame:** If you have an active image in a preview already selected, clicking this button resets the capture area to the entire image enclosed within the frame, i.e. erasing your preview area.

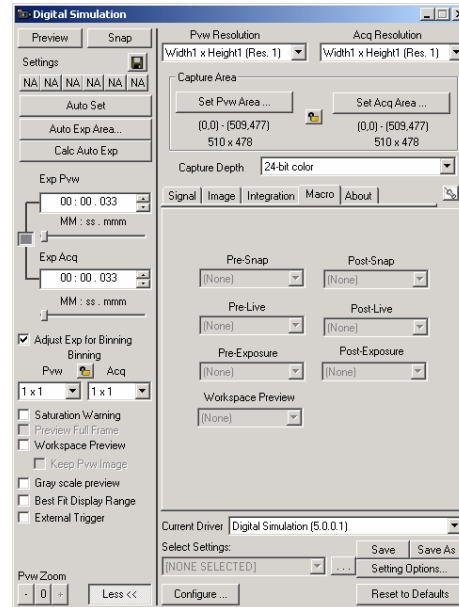
**Calculate:** Click this button to calculate the white balance.

**Close:** Click **Close** to return to the **Acquire** page.

**Use Last Set AOI:** Clicking this button to use the AOI that you have most recently set, and will not bring up the **Auto-White Balance** dialog.

## Video Average

When you select *Video Average* from the **Capture Options** menu, you will see the **Capture** dialog open to the **Macro** page.



**Pre-Snap Macro:** Enter the name of a macro you want *Image-Pro* to run before each snap. For example, you might want to sound a bell before each snap occurs. If the time required for your hardware to perform the Pre-Snap Macro and acquire the image exceeds the interval you have selected, *Image-Pro* will acquire images as rapidly as your hardware permits. Select **None** if you do not want a Pre-Snap Macro.

**Post-Snap Macro:** Enter the name of a macro you want *Image-Pro* to run after each snap. For example, you might want to load the image just saved, apply a filter, and resave the image. If the time required for your hardware to perform the Post-Snap Macro and acquire the image exceeds the interval you have selected, *Image-Pro* will acquire images as rapidly as your hardware permits. Select **None** if you do not want a Post-Snap Macro.

**Pre-Live Macro:** Enter the name of a macro you want *Image-Pro* to run before each live capture.

*Acquire:Capture Options*

**Post-Live Macro:** Enter the name of a macro you want *Image-Pro* to run after each live capture.

**Pre-Exposure Macro:** Enter the name of a macro you want *Image-Pro* to run before each the exposure starts.

**Post-Exposure Macro:** Enter the name of a macro you want *Image-Pro* to run after each exposure is concluded.

If you have selected “minimum interval” from the **Image** page, the macros are played before the sequence, and after, but not during the capture.

**Workspace Preview:** Check **Workspace Preview** to display integrated live previewing.



## **Video Print**

Use the *Video Print* command on systems configured with an auxiliary monitor (see dual monitor discussion) and a video printer. It is used to pass the active image or AOI from *Image-Pro's* application window back to the frame grabber, such that it is displayed on the auxiliary monitor and can be optionally printed.

If you have modified the image being printed (using a filter, for example, or drawing on the image), the modifications are reflected in the video print.

To print an image or AOI to your video printer, select the *Video Print* command. Your image (or AOI) will appear on the auxiliary monitor. From there you must perform whatever steps are required by your particular frame grabber and/or printer to initiate the print. Consult your device manufacturer's manuals for this procedure

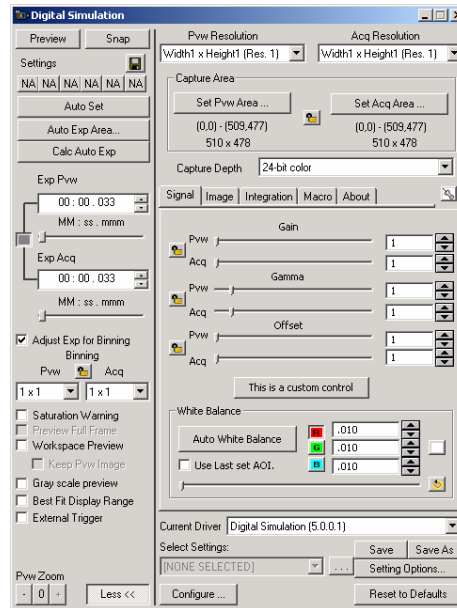
To close the **Acquire** dialog, click the Close button. When you exit, your setup options are recorded so that next time you select the *Acquire* command it will already be configured to these specifications.

If you minimize the **Acquire** window without clicking **Stop**, *Image-Pro* continues acquiring real-time video.

If you close the **Acquire** window without clicking **Stop**, the last image acquired remains on your second monitor (if you have one).

## Setup Acquire

When you choose *Setup Acquire* from the **Capture Options** menu, you will see the **Capture** dialog open to the **Signal** tab page. The **Signal** page contains information about the video signal that your analog camera uses. This page appears something like this:



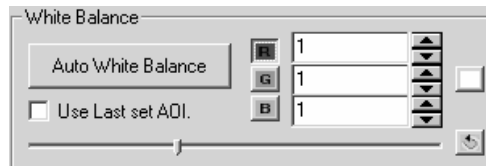
**Gain:** These sliders control the digital gain for both preview and acquisition. Preview and acquire may be adjusted individually or they may be locked together to set both simultaneously. Click the **Lock icon** to make the gain settings equal for both preview and acquisition.

**Gamma:** These sliders control the gamma for both preview and acquisition. Preview and acquire may be adjusted individually or they may be locked together to set both simultaneously. Click the **Lock icon** to make the gamma settings equal for both preview and acquisition.

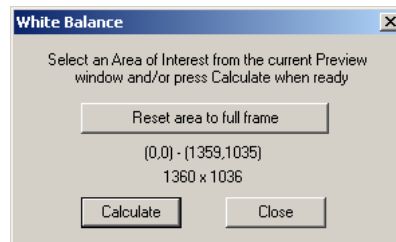
**Offset:** This feature allows you to adjust the vertical and horizontal offset of the capture device for both preview and acquisition. Preview and acquire may be adjusted individually or they may be locked together to set both simultaneously. Click the **Lock icon** to make the offset equal for both preview and acquisition.

**Custom Controls:** These controls are driver-specified and will depend on the driver you have selected. They may not appear if your driver does not have custom controls

**White Balance:** The **White Balance** controls allow you to adjust the white balance ratios for each of the color channels, either manually or automatically, as shown here:



**Auto White Balance:** Clicking this button will start a live preview, if one is not already active, and starts the process of calculating new color ratios, in order to establish a white balance based on the input image. You will see the **Auto White Balance** dialog, shown here:



**Reset area to full frame:** If you have an active image in a preview already selected, clicking this button resets the capture area to the entire image enclosed within the frame, i.e. erasing your preview area.

**Calculate:** Click this button to calculate the white balance.

**Close:** Click **Close** to return to the **Acquire** page.

**Use Last Set AOI:** Clicking this button to use the AOI that you have most recently set, and will not bring up the **Auto-White Balance** dialog.

## Scan

---

Use the *Scan* command to scan images into *Image-Pro*, using a TWAIN-compliant scanner. Use the *Select Scanner* command to select your TWAIN drive from those available on your system. The *Scan* command then initiates the scanning process directly.

The scanning process involves the following three basic steps:

- 1. Selecting your scanner** using the *Select Scanner* command. If you know that the scanner you plan to use has already been selected, this step is not required.
- 2. Initiating the scan** using the *Scan* command. When you select the *Scan* command, *Image-Pro* transfers control to the manufacturer's TWAIN interface for the scanner you are using.
- 3. Controlling the scan process.** See the manufacturer's user manual for operating instructions. When the scan is complete, your image will be displayed in a new window.

*Note - Before selecting the Scan command, be sure the current scanner matches the scanner connected to your system.*

The options available to you during the scan process depend upon the specific scanner you are using. Some or all of the following options may be available to you.

**DPI (Dots per inch):** This sets the resolution at which the scanner will acquire the image. Combined with the Margins setting, DPI will determine the size of the resultant scanned image. For example, a 3" x 4" area scanned at 100 dpi will result in an image that is 300 x 400 pixels in size. The available DPI settings will be dependent on the scanner.

*Note - After scanning an image, the spatial calibration will be set to reflect the DPI at which the image was scanned.*

**Gamma Correction:** Some scanners have built-in correction tables that will attempt to correct the image for various display and output devices. Experiment with this setting to determine which gives you the best results.

**Threshold:** This sets the cutoff point when scanning a tonal image (many levels of grays and/or colors) as a bilevel image

(two tones, black and white). Higher threshold settings will result in more areas of the image appearing white; lower settings will result in greater black areas. See **Halftone Type** below.

**Brightness:** This setting controls the brightness of the initial scanned image. This is similar to the brightness control on the **BCG Controls** and in the Color Map, but it is applied at the hardware level.

**Monochrome/Color:** This selection is available if you have a color scanner. Along with the Bit Depth setting, this option will determine the image class of the scanned image. See **Bits per Channel** below.

**Bits per Channel:** This determines the image class of the scanned image. For example, Monochrome at 1 bit per channel will result in a bilevel image; Monochrome at 8 bits will be a 256 gray scale image; Color at 8 bits will be a 24-bit true color image; Color at 1 bit (binary) will result in an 8-color Toolbar class image that looks similar to color newsprint when used with an appropriate halftone type (see below).

**Halftone Type:** If the **Bits per Channel** is set at binary (1), this option will be selectable in some scanners. The type of halftone patterns that are available will be determined by the scanner. Usually, the Threshold setting will affect the dot density of a halftone.

**Black/White Reverse:** This option will toggle negation of the image at scan time. The result is equivalent to performing a NOT Operation on an image.

**Margins:** This option allows you to scan just a portion of your image. This is useful for eliminating unwanted text, graphics or white space that surround the image you want to scan.

**Preview:** This option lets you view your image while you specify the margins of the area to scan.

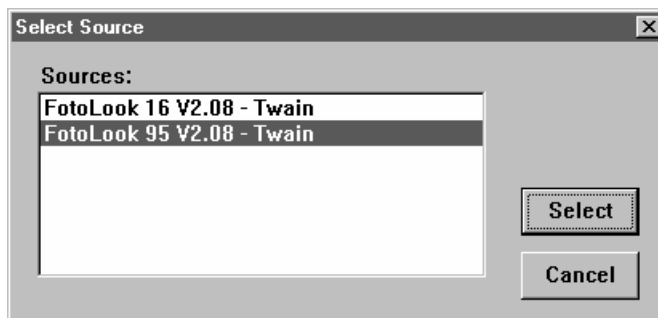
**Width/Height:** Note that the width and height of the image you are scanning do not necessarily reflect the width and height on your screen. Factors affecting screen size are the scanning resolution (dots per inch), the resolution at which Windows is operating (a lower resolution yields a larger image), and the physical size of your monitor.

Acquire:Select Scanner

## Select Scanner

---

Use the *Select Scanner* command to select or change the current TWAIN-compatible scanner. When you click the *Select Scanner* command, the **Select Source** dialog box appears.



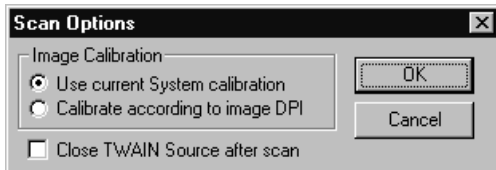
**Sources:** This list box presents all TWAIN drivers found on your system (*Image-Pro* searches the TWAIN folder located in the WINDOWS working folder for these drivers— e.g., \WINDOWS\TWAIN). Select the scanner you want to use. If your scanner is not listed under **Sources**, install it first, then select it.

**Select:** When you are happy with your choice, click the **Select** button. Control then passes to your scanner's TWAIN-compliant software.

## Scan Options

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Use the *Scan Options* command if you want to change the *Image-Pro Plus* scanning options. When you click the *Scan Options* command, you will see the **Scan Options** dialog box:



**Use Current System Calibration:** Click this option if you want to apply the current system calibration settings to newly-acquired images.

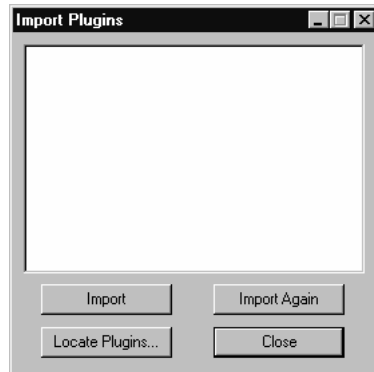
**Calibrate according to image DPI:** Click this option if you want to create a new calibration setting for the image, based on the number of dots per inch in the newly-acquired image. Calibration settings generated in this manner will be named `SCAN#DPI`, where # is the number of dots per inch, i.e. `SCAN300DPI`.

**Close TWAIN Source after scan:** Check this box if you want *Image-Pro Plus* to close the TWAIN source after acquiring the image. This is a step-saver if you normally close the TWAIN device after you scan an image.

## Import Plugins

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This option allows you to use third-party plug-in modules for importing images with *Image-Pro Plus*. When you select this command, you will see the **Import Plugins** dialog box:



**Import:** This button calls the selected import function.

**Import Again:** This button calls the same import function as selected previously.

**Locate Plugins:** Use this button to browse your system files for the third-party plug-ins.

**Close:** Closes the dialog box.



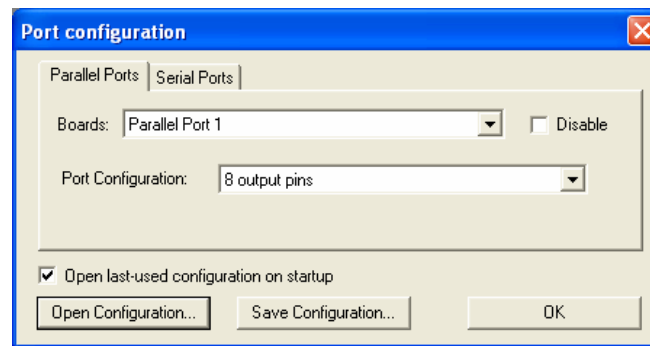
## Configure Ports

---

The *Configure Ports* dialog allows you to set up whichever I/O boards are installed in your computer, especially those aspects of the board that may change depending on the requirements of your experiment. This page allows you to control the configured outputs and read the configured inputs using a display of the current status of the ports. If you have IO-Pro installed on your computer, you can view the documentation by clicking on the product name.

### Parallel Ports

The *Parallel Ports* page displays the following dialog:



**Boards:** This drop-down list includes one entry for each parallel port that is detected.

**Disable:** A particular port can be disabled using the **Disable** check box, which may be necessary if it is used for a local printer or other hardware.

**Port Configuration:** There are 4 possible port configurations: a single 8-bit input port, a single 8-bit output port, 8 input pins, or 8 output pins.

**Open last-used configuration on startup:** This checkbox will cause the last configuration to be opened automatically when the configuration dialog is displayed or when any of the *Auto-Pro* functions are accessed.

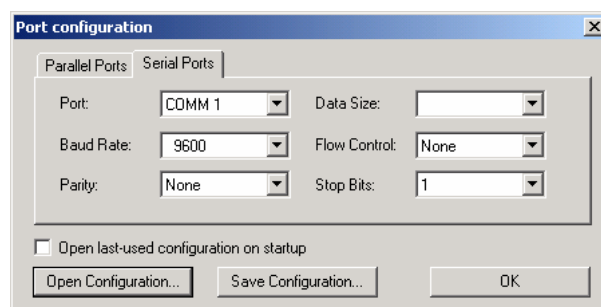
**Open Configuration:** Clicking this button will open a standard *File: Open* dialog that will allow the user to open a previously saved configuration.

*Acquire:Configure Ports*

**Save Configuration:** Clicking this button will open a **Save As** dialog to save the configuration. Opening a configuration successfully, saving a configuration, or pressing **OK** will all try to configure the ports according to the Port Configuration setting.

## Serial Ports

The *Serial Ports* page displays the following dialog box:



**Ports:** The Port drop-down list displays one entry for each serial port that is detected, with the result that the list may be discontinuous (e.g. COMM 1, COMM 2, and COMM 4). A particular port can be selected as the active port for configuration and I/O.

**Baud Rate:** Enter the baud rate for the selected port.

**Flow Control:** Enter the flow control for the selected port.

**Parity:** Indicate the correct parity for the selected port.

**Data Size:** Indicate the correct data size, which should be a value between 5 and 8.

**Stop bits:** Indicate the correct number of stop bits for the selected port.

**Open last-used configuration on startup:** This checkbox will cause the last configuration to be opened automatically when the configuration dialog is displayed or when any of the *Auto-Pro* functions are accessed.

**Open Configuration:** Clicking this button will open a standard *File: Open* dialog that will allow the user to open a previously saved configuration.

**Save Configuration:** Clicking this button will open a **Save As** dialog to save the configuration. Opening a configuration successfully,

### *Acquire:Configure Ports*

saving a configuration, or pressing **OK** will all try to configure the ports according to the Port Configuration setting.

**Auto-Apply Changes:** Check this box to automatically apply any changes to your settings form this page to the live preview window. Choosing this option disables the **Apply** button on the current (*Preview*) page and sends the commands directly to the camera as soon as the settings are changed.

**Auto-Adjust Binning:** Check this box to automatically adjust the exposure times and the zoom factor to keep the image size and brightness consistent whenever the binning value is changed. Both the preview and the acquire exposure times will be adjusted whenever a binning value or preview exposure valued changes. Note that the zoom factor adjustment may be limited by the number of zoom factors available for your camera driver. The **Auto-Adjust** will try to maintain image brightness but the results may not be perfect depending on your specific camera. Enabling this feature disables the **Lock Exposure Times** feature.

**Live Preview Context Window:** Clicking the right mouse button on any area in the preview window displays a context menu. This menu allows you to adjust the following options:

- Auto-Set
- Auto-Exposure
- Auto-White Balance
- Live Histogram
- Transparency

**Live Histogram:** Clicking the right mouse button in the **Live Histogram** window will display a context menu with different options for displaying a live histogram. Clicking the left mouse button in the **Live Histogram** window will toggle the channel selection for color cameras.

## **Scope-Pro and Stage-Pro**

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*Scope-Pro* (the *Image-Pro*<sup>®</sup> *Plus* Microscope Controller Module ) lets you use *Image-Pro Plus* to control and program the movement of your microscope. *Scope-Pro* also allows you to install and configure various components to control shutters, sliders, filter wheels, lamps, apertures, and other microscope hardware.

*Scope-Pro* incorporates the *Stage-Pro* functions, which allow you to control the movement of your trinocular microscope's motorized stage. It provides an environment in which your microscope can be completely controlled from the PC — virtually eliminating the need to put your eye to the ocular.

Moreover, repetitive stage movement can be automated by recording the motion in a macro. You can even use this capability to create a large, high-resolution image from a series of “tiles” collected from a single specimen. The *Stage-Pro* component, (which can be configured at the same time or anytime thereafter) permits you to control the motorized stage and Z focus controllers.

Repetitive movements for both microscope and stage can be automated by recording the motion in a macro.

If you configured an X/Y stage, ***Stage-Pro*** is installed and you will need to refer to the section titled *Working with Stage-Pro*.

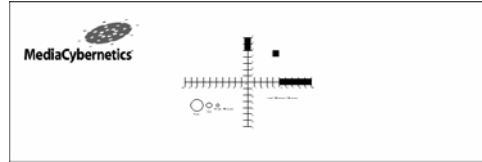
If you configured any non-stage hardware, or only configured a Z-focus component, ***Scope-Pro*** is installed and you should refer to the section titled *Working with Scope-Pro*.

You will want to review both sections if your configuration installed both programs.

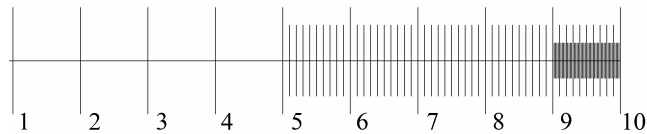
## **The Stage Micrometer**

### **Stage Micrometer Features**

The stage micrometer that came with your copy of *Scope-Pro* is pictured below. It has several regions of particular use in different applications. The biggest feature, the Media Cybernetics logo, is often useful for finding the correct focus, and for determining which is the top surface of the stage micrometer.

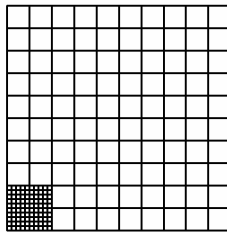


One arm of the central cross is pictured below. These arms are most useful for calibrating low magnification lenses (up to 20X), and have markings at 1 mm, 100  $\mu\text{m}$  and 20  $\mu\text{m}$ .



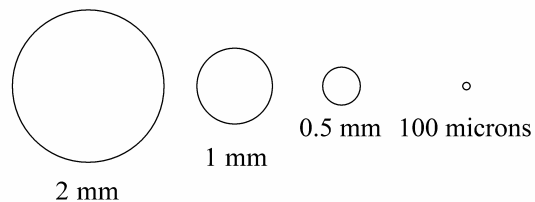
1 mm - 100 microns - 20 microns

The grid in the upper right quadrant of the Stage Micrometer is most useful for calibrating higher magnification lenses (40X and up), whose field of view will be completely filled by a portion of the grid. The grid is 1 mm square, with 100  $\mu\text{m}$  major divisions and 20  $\mu\text{m}$  divisions in the bottom left corner, as shown in the illustration on the next page.



The final graphic feature on the Stage Micrometer is a set of circles of varying sizes. Both the circles and the square can be used for evaluating geometric distortions in your image acquisition system.

Acquire:Configure Ports



### Using the Stage Micrometer

*The Stage Micrometer* is a calibration device, not a direct measuring device. It allows you to calibrate any measurements performed on captured images. Images are fundamentally a 2-dimensional array of intensities (pixels) – they do not inherently contain any spatial information. The Stage Micrometer allows you to relate pixels to real distances.

The *Spatial Calibration Wizard* (on the *Measure:Calibration* menu in *Image-Pro Plus*) will walk you through creating spatial calibrations for each of the available magnifications on your microscope. The basic procedure is to snap an image of some portion of the Stage Micrometer, and then measure the number of pixels along a line of known length, which in turn gives you a value of pixels/unit, where unit is your chosen spatial unit. You can repeat this procedure several times, if you wish, to get a mean value with an associated standard deviation.

## **The Scope-Pro Tab Dialogs**

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This section describes some of the possible configuration controls for each type of component. The actual controls that you will see will depend on the particular component installed.

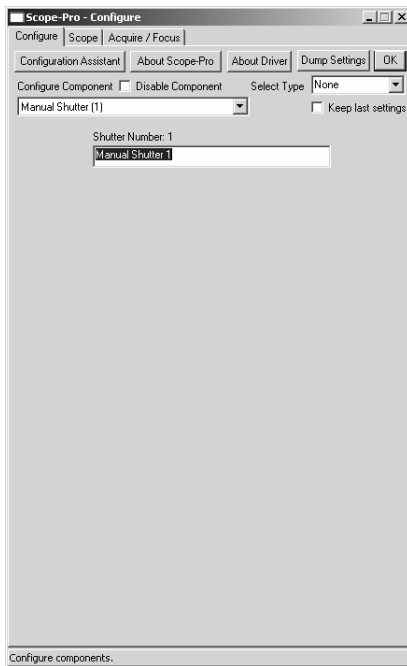
Remember if you configured a *single* Z-focus component, or if you configured some non-stage hardware, then you installed the **Scope-Pro** module. If you configured the X/Y stage component **or** the X/Y and Z stage components, then the **Stage-Pro** module was installed.

If you configured an X/Y stage component and a Z-stage component, and non-stage hardware, you have installed **both** *Stage-Pro* and *Scope-Pro*. You will have a choice of controlling the Z component from either *Stage-Pro* **or** *Scope-Pro*, but not both.

Acquire: The Scope-Pro Tab Dialogs

## The **Configure** Tab Page

The first time the *Scope-Pro* command is invoked, the **Scope-Pro Configure** tab page is presented, with none of the other tabs accessible.



The controls within the **Configure** tab page are described below.

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**Note:** If you are using the *Scope-Pro* configuration GUI, this page will look different. Please see the section at the end of this chapter, *GUI View of the Configure Tab page*.

---

**Configuration Assistant:** Click this button to use the *Configuration Assistant*. The *Configuration Assistant* is designed to help you set up the controllers that you selected when you installed *Scope-Pro*, and initialize the various components of each controller.

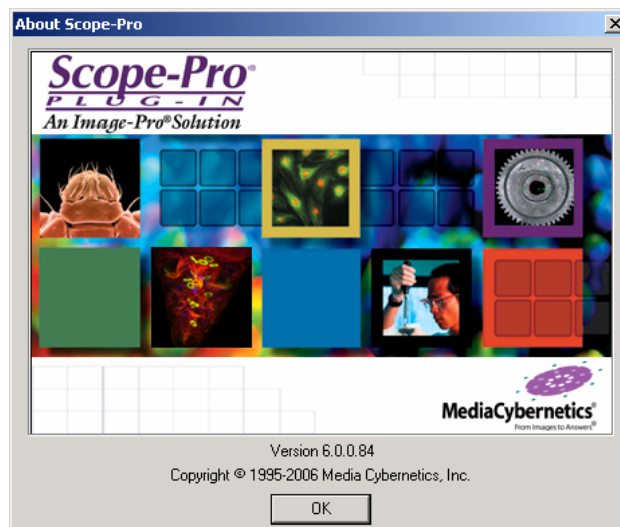


*Acquire: The Scope-Pro Tab Dialogs*



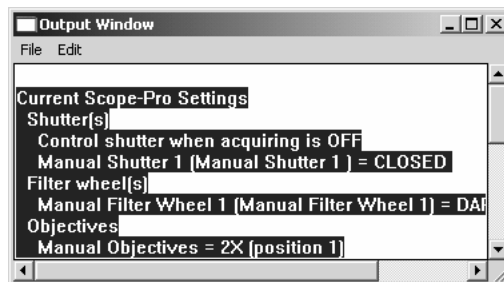
Follow the instructions on the screen to use the **Configuration Assistant**.

**About Scope-Pro:** Click this button to identify which version of *Scope-Pro* you have installed.



## Acquire: The Scope-Pro Tab Dialogs

**Dump Settings:** Click this button to send a text listing of the current *Scope-Pro* settings to the **Output Window**, as shown here:



**Disable Component:** Click this button to disable the component selected in the **Configure Component** list box. You may use this option to selectively disable components that will not configure because the corresponding hardware is either not present or not responding.

**Select Type:** This selection list allows you to use the classic *Scope-Pro Configure* and *Scope* pages, or to substitute a graphical user interface (GUI) for these two pages. The default, **None**, selects the classic view (shown above). To use the GUI view, select **Upright** or **Inverted**, depending on the type of microscope you will be using. Click **OK**, exit *Scope-Pro*, and restart *Scope-Pro* from the *Acquire* menu. The GUI view of the *Configure* page is described later in this section, and the GUI view of the *Scope* page is described at the end of the next section

**Keep last settings:** Check this box to retain the hardware settings from your last *Scope-Pro* session.

---

*Note:* Users are encouraged to use settings files to simplify the process of configuring *Scope-Pro*.

---

**Configure Component:** The **Configure Component** control will list all of the components that you configured in the *Scope-Pro* configuration utility.

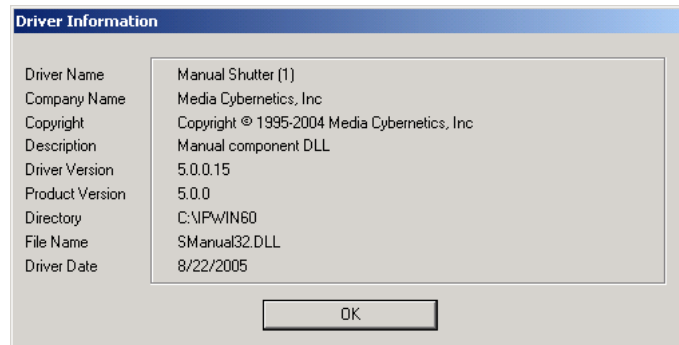
Select the specific component from the list. The lower portion of the *Configure* tab page will display whatever settings are required to configure the selected component. Once you select the appropriate component, the options on the *Configure* tab page

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window will change to reflect the selected component. The individual components are listed in more detail in the following sections.

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**About Driver:** Press this button to see the driver name, company name, copyright, driver and product version numbers, directory and filename of the driver, and the driver date, as shown here:

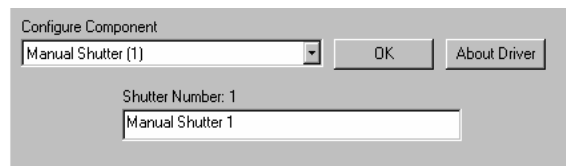


## Shutters

*Scope-Pro* supports up to 15 shutters.

Shutters will often not have any configurable parameters except for the user-definable name that is used to identify the shutter. (These are the names used to identify the shutter on the *Scope* tab page).

For instance, if you have two shutters, one in the transmitted light path and one in the reflected light path, you might want to name them "Transmitted Light" and "Reflected Light", respectively. A typical configuration page for specifying the user-defined name is illustrated here.



Component-specific configurable shutter parameters are possible where a controller supports several different shutters. In this case, the **Shutter** configuration may include radio buttons or a selection box to let you specify which shutter to control with the current component. Other possibilities include board IDs or bus addresses used to specify which shutter to control.

For details on component-specific shutter parameters for the components that you will be using, you will want to refer to the *Scope-Pro* device notes, which are provided in the **SCPNOTES.DOC** file .

---

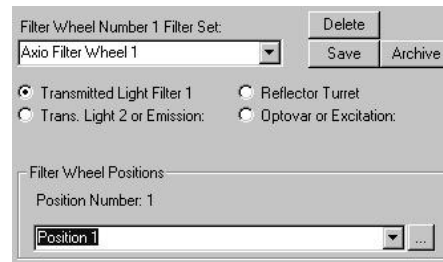
Note: The communications port settings for each driver will be attached to the first installed component which uses that driver. In many cases, that component is the shutter.

---

## Filter Wheels

*Scope-Pro* supports up to 15 filter wheels. These may include traditional filter wheels, reflector turrets, multiple wheel filter magazines, and neutral density filters. If more than 5 filter wheels are configured, the **Filter Wheel** group box on the *Scope* tab page will have a vertical scroll bar.

The typical controls supported by most filter wheel components are illustrated below:



**Filter Set configuration:** Select the filter set from the drop-down list.

Filters may be changed often according to the needs of the particular experiment or sample under observation. For this reason, *Scope-Pro* supports filter sets, or logical groupings of particular combinations of filters (in the example above, “Axio Filter Wheel 1”). These are selected using the user-defined name for the filter wheel on the *Scope* tab page described in the next section.

Initially, the default set simply identifies the positions of the wheel and is saved using the default filter wheel name (i.e., “Manual Filter Wheel 1”). It is also possible that there are component-specific filter wheel sets that were installed along with the component. All of these, along with any that you save, are listed in the **Filter Set** selection list for easy selection at any time.

As you first start out configuring a filter set, you will want to set the filter position names to correspond to the actual filters installed. Use the drop-down list to select each position.

If the controller has been initialized, either through the **Initialize** button or by pressing **OK** on the *Configure* tab page, the filter wheel will be set to the corresponding position. For this reason, it will be easiest to

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edit the filter set *after* initializing the filter wheel controller, when the effects of each filter can be easily seen.

Component-specific configurable filter wheel parameters are possible where a controller supports several different filter wheels and/or several different types of filtering mechanisms. In this case, the Filter Wheel configuration may include radio buttons (in the above example, radio buttons listed as the 4 types of filters) or a selection box to let you specify which filter wheel to control with the current component. Other possibilities include board IDs or bus addresses used to specify which filter wheel to control.

Once a position is selected, you can manually edit the current name for the filter wheel, or select a name from the current master list. For details on component-specific filter wheel parameters for the components that you will be using, you will want to refer to the *Scope-Pro* device notes, which are provided in the `SCPNOTES.DOC` file.

**Delete:** The **Delete** button is always available to delete the filter set corresponding to the current filter set name.

---

**Note:** The **Delete** button removes the filter set from your hard disk, but does not affect the position names in the current set or master list.

---


**Save:** As you change a filter wheel set, either by editing the set name or by editing the position names (see the “...” **button** below), the **Save** button will become available.

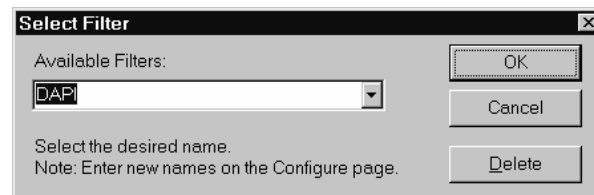
When selected, the current set of position names will be saved to a set under the current filter set name.

**Archive:** The **Archive** button marks the current filter set as read-only, so that it cannot be deleted or changed accidentally. **Archive** will also save your filter set, if necessary.

**Filter Wheel Positions:** This group box lists the filter wheel positions.

Once a position is selected, you can manually edit the current name for the position, or select a name from the current master list.

“...” **button** : The “...” **button** brings up a dialog with a selection list containing all the known filters, illustrated below:



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**Select Filter dialog box:** *Scope-Pro* is shipped with a list of commonly available filters, and any that you add by manually editing the position name on the **Configure** tab page will be added to that list.

**OK:** When you have selected the filter that you want, press the **OK** button to insert the name into the current filter wheel position.

If for any reason there are no names left in the master list, the selection list and **OK** button will be disabled until new filter names are added on the **Configure** tab page.

**Cancel:** Use **Cancel** at any time if you do not find the filter name you want.

**Delete:** Delete any unnecessary filter names by selecting the name in the selection list and then using the **Delete** button.

**Lens/Dye files:** Select one of the Lens or Dye files from the drop-down list.

### **Sliders**

*Scope-Pro* supports up to 2 slider components, which can be any sort of two-position control.

Sliders will often not have any configurable parameters except for the user-definable name that is used to identify the slider. These are the names used to identify the slider on the **Scope** tab page (described in the next section).

For instance, if you have two sliders, one that controls the light path and one that controls a condenser, you might want to name them “Light Path” and “Condenser”, respectively. A typical configuration page for specifying the user-defined name is illustrated below:



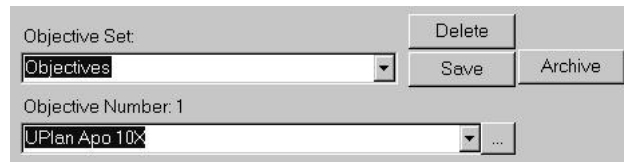
The image shows a small dialog box with a title bar that reads "Slider Number: 1". Below the title bar is a single-line text input field containing the text "Manual Slider 1". The dialog box has a simple, light-colored border.

Component-specific configurable slider parameters are possible where a controller supports several different sliders. In this case, the **Slider** configuration may include radio buttons or a selection box to let you specify which slider to control with the current component. Other possibilities include board IDs or bus addresses used to specify which slider to control.

For details on component-specific slider parameters for the components that you will be using, you will want to refer to the *Scope-Pro* device notes, which are provided in the **SCPNOTES.DOC** file.

## Objectives

Objectives will usually have the following set of controls:



**Objective Set configuration:** Select the objective set from the drop-down list. Objectives may be changed according to the needs of the particular experiment or sample under observation. For this reason, *Scope-Pro* supports objective sets, or logical groupings of particular combinations of objectives. These are selected using the user-defined name for the objective set on the *Scope* tab page described in the next section.

Initially, the default set simply identifies a set of usually available magnifications, and is saved using the default set name (i.e., “Objectives”). It is possible that the particular controller can inquire the installed objectives to set up the objective set, which would then be done when the **Initialize Microscope** button is selected. It is also possible that there are component-specific objective sets that were installed along with the component (in the above example, “UPlan Apo 10X”). All of these sets, along with any that you save, are listed in the **Objective Set** selection list for easy selection at any time.

As you first start out configuring an objective set, you will want to set the objective names to correspond to the actual objectives installed. Use the drop-down list to select each objective.

If the controller has been initialized, either through the **Initialize Microscope** button or by pressing **OK** on the *Configure* tab page, the objective turret will be set to the corresponding position. For this reason, it will be easiest to edit the objective set *after* initializing the objective controller, when the effects of each objective can be easily seen.

Once an objective is selected, you can manually edit the current name for the objective, or select a name from the current master list.

---

**Note:** The objective magnification may be used to determine the active range for auto-focus, when the auto-focus component supports this.

The objective magnification is determined by the objective name, in the following manner. Any number that is followed by an “X” (with or



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without intervening spaces) is considered to indicate the magnification.

For instance, all of the following would indicate a 10X objective: “10X”, “10 x”, “XYZ 10x”, and “100370-93 10X”. It would not be possible to determine the magnification from the following, however: “100”, “10 power”, or “FISH lens”.

Whenever an objective set is saved, or the configuration is approved with the **OK** button, Scope-Pro will check the objective names and warn if any do not clearly indicate the magnification. Names in parentheses (i.e., “(Not installed)” or “(none )”) will be ignored during this check and can be used for positions not bearing an objective.

---

For details on component-specific objective parameters for the components that you will be using, you will want to refer to the Scope-Pro device notes, which are provided in the SCPNOTES.DOC file.

**Delete:** The **Delete** button is always available to delete the objective set corresponding to the current objective set name.

---

**Note:** The **Delete** button removes the objective set from your hard disk, but does not affect the position names in the current set or master list.


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**Save:** As you change an objective set, either by editing the set name or by editing the objectives (see the “...” **button** below), the **Save** button will become available.

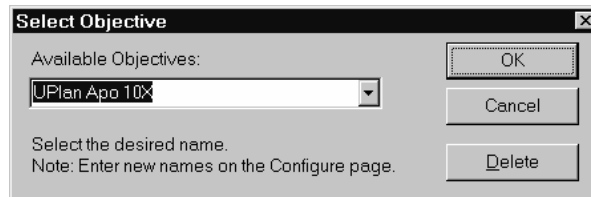
When selected, the current set of objectives will be saved to a set under the current objective set name.

**Archive:** The **Archive** button marks the current objective set as read-only, so that it cannot be deleted or changed accidentally. **Archive** will also save your objective set, if necessary.

**Objective number:** This selection box lists the objectives. Once an objective is selected, you can manually edit the current name for the objective, or select a name from the current master list.

**“...” button **: The “...” **button** brings up a dialog with a selection list containing all the known objectives:

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**Select Objective dialog box:** *Scope-Pro* is shipped with a list of commonly available objectives, and any that you add by manually editing the objective name on the *Configure* tab page will be added to that list.

**OK:** When you have selected the objective that you want, press the **OK** button to insert the name into the current objective.

If for any reason there are no names left in the master list, the selection list and **OK** button will be disabled until new objectives are added on the *Configure* tab page.

**Cancel:** Use **Cancel** at any time if you do not find the objective you want.

**Delete:** Delete any unnecessary objective by selecting the name in the selection list and then using the **Delete** button.

## Lamps

*Scope-Pro* will allow you to control the intensity of up to 2 lamps.

Lamps will often not have any configurable parameters except for the user-definable name that is used to identify the lamp. These are the names used to identify the lamp on the *Scope* tab page (described in the next section).

For instance, if you have two lamps, one that controls the transmitted light and one that controls a fluorescence excitation lamp, you might want to name them “Transmitted Light” and “Fluorescence”, respectively. A typical configuration page for specifying the user-defined name is illustrated:



Component-specific configurable lamp parameters are possible where a controller supports several different lamps or types of lamps. In this case, the **Lamp** configuration may include radio buttons or a selection box to let you specify which lamp to control with the current component. Other possibilities include board IDs or bus addresses used to specify which lamp to control, or the voltage range for the lamp.

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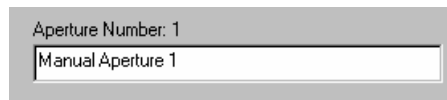
For details on component-specific lamp parameters for the components that you will be using, you will want to refer to the *Scope-Pro* device notes, which are provided in the **SCPNOTES.DOC** file.

### Apertures

*Scope-Pro* allows you to set the opening diameter of up to 4 apertures (which are also referred to as *irises* or *diaphragms*).

Apertures will often not have any configurable parameters except for the user-definable name that is used to identify the aperture. These are the names used to identify the lamp on the **Scope** tab page (described in the next section).

For instance, if you have two apertures, one that controls transmitted light and one that controls reflected light, you might want to name them “Transmitted Light” and “Reflected Light”, respectively. A typical configuration page for specifying the user-defined name is illustrated below:



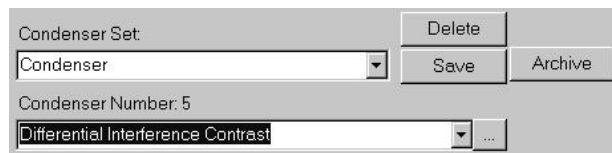
Aperture Number: 1  
Manual Aperture 1

Component-specific configurable aperture parameters are possible where a controller supports several different apertures or types of apertures. In this case, the **Aperture** configuration may include radio buttons or a selection box to let you specify which aperture to control with the current component. Other possibilities include board IDs or bus addresses used to specify which aperture to control.

For details on component-specific aperture parameters for the components that you will be using, you will want to refer to the *Scope-Pro* device notes, which are provided in the **SCPNOTES.DOC** file in the *Image-Pro* directory.

### Condensers

*Scope-Pro* supports controlling a single condenser component, which will usually have the following set of controls:



Condenser Set: Condenser [Delete] [Save] [Archive]  
Condenser Number: 5  
Differential Interference Contrast [...]

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**Condenser Set configuration:** Select the condenser set from the drop-down list. The condenser component may be changed according to the needs of the particular experiment or sample under observation, and a particular condenser component will usually have a fixed set of positions. These are selected using the user-defined positions for the condenser on the *Scope* tab page described in the next section .

Initially, the default set simply identifies a set of default positions, and is saved using the default set name (i.e., “Manual Condenser”). It is possible that the particular controller can inquire the installed condenser set, which would then be done when the **Initialize Microscope** button is selected. It is also possible that there are component-specific condenser sets that were installed along with the component. All of these sets, along with any that you save, are listed in the **Condenser Set** selection list for easy selection at any time.

As you first start out configuring a condenser set, you will want to set the names to correspond to the actual condensers installed. Use the drop-down list to select each condenser.

If the controller has been initialized, either through the **Initialize** button or by pressing **OK** on the *Configure* tab page, the condenser will be set to the corresponding position. For this reason, it will be easiest to edit the condenser set *after* initializing the condenser controller, when the effects of each condenser can be easily seen.

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Once a position is selected, you can manually edit the current name for the condenser, or select a name from the current master list. For details on component-specific condenser parameters for the components that you will be using, you will want to refer to the *Scope-Pro* device notes, which are provided in the `SCPNOTES.DOC` file.

**Delete:** The **Delete** button is always available to delete the condenser set corresponding to the current condenser set name.

---

**Note:** The **Delete** button removes the condenser set from your hard disk, but does not affect the position names in the current set or master list.


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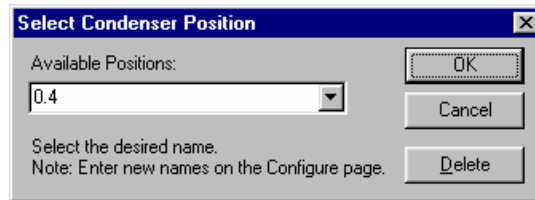
**Save:** As you change a condenser set, either by editing the set name or by editing the condenser positions (see the “...” button below), the **Save** button will become available.

When selected, the current set of position names will be saved to a set under the current condenser set name.

**Archive:** The **Archive** button marks the current condenser set as read-only, so that it cannot be deleted or changed accidentally. **Archive** will also save your condenser set, if necessary.

**Condenser Positions:** This group box lists the condenser positions. Once a position is selected, you can manually edit the current name for the condenser, or select a name from the current master list.

**“...” button** : The “...” button brings up a dialog with a selection list containing all the known condenser positions, illustrated below:



**Select Condenser Position dialog box:** *Scope-Pro* is shipped with a list of commonly available condensers, and any that you add by manually editing the position name on the *Configure* tab page will be added to that list.

**OK:** When you have selected the condenser position that you want, press the **OK** button to insert the name into the current condenser position.

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If for any reason there are no positions left in the master list, the selection list and **OK** button will be disabled until new condenser names are added on the **Configure** tab page.

**Cancel:** Use **Cancel** at any time if you do not find the condenser position you want.

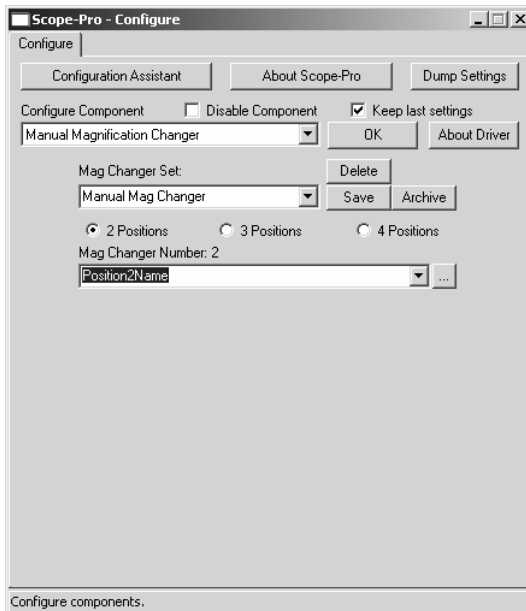
**Delete:** Delete any unnecessary condenser names by selecting the condenser position in the selection list and then using the **Delete** button.

### Focus

The *Scope-Pro* module includes a Z calibration capability that can precisely calibrate your stage's Z travel in absolute units if you have a precision standard of known thickness. When you configure your focus settings, you will see the **Acquire/Focus** page of *Scope-Pro*. Please refer to that section, later in this chapter, for specific information about the focus settings.

### Mag Changer

Until this point, automated mag changers were considered by *Scope-Pro* to be a type of filter wheel. In *Scope-Pro 7.0*, a new **Mag Changer** group appears as an option on the **Configure** tab page, as shown here:



**Delete:** The **Delete** button is always available to delete the mag changer set corresponding to the current mag changer set name.

---

**Note:** The **Delete** button removes the mag changer set from your hard disk, but does not affect the position names in the current set or master list.

---

**Save:** As you change a mag changer set, either by editing the set name or by editing the condenser positions (see the “...” **button** below), the **Save** button will become available.


When selected, the current set of position names will be saved to a set under the current mag changer set name.

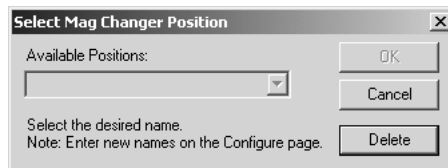
**Archive:** The **Archive** button marks the current mag changer set as read-only, so that it cannot be deleted or changed accidentally. **Archive** will also save your mag changer set, if necessary.

**2 Positions, 3 Positions or 4 Positions:** Click the radio button that indicates the correct number of positions for your mag changer.

**Charger Positions:** This group box lists the mag changer positions. Once a position is selected, you can manually edit the current name for the mag changer, or select a name from the current master list.

The position of the **Mag changer** is saved in a settings file. The power of the **mag changer** is stored in `ipscope.ini`. This number is used by the reference calibrations to adjust the X and Y pixels per unit.

**“...” button** : The “...” **button** brings up a dialog with a selection list containing all the known mag changer positions, illustrated below:



**OK:** When you have selected the mag changer position that you want, press the **OK** button to insert the name into the current mag changer position.

If for any reason there are no positions left in the master list, the selection list and **OK** button will be disabled until new mag changer names are added on the *Configure* tab page.

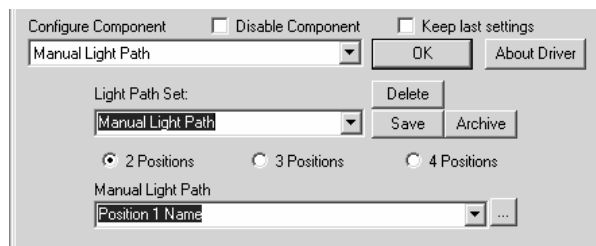
**Cancel:** Use **Cancel** at any time if you do not find the mag changer position you want.

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**Delete:** Delete any unnecessary mag changer names by selecting the mag changer position in the selection list and then using the **Delete** button.

### Light Path

The **Light Path** component is also available as part of the *Scope-Pro* configuration routine.



**Delete:** The **Delete** button is always available to delete the light path set corresponding to the current light path set name.

---

**Note:** The **Delete** button removes the light path set from your hard disk, but does not affect the position names in the current set or master list.

---


**Save:** As you change a light path set, either by editing the set name or by editing the Light Path positions (see the “...” button below), the **Save** button will become available.

When selected, the current set of position names will be saved to a set under the current **Light Path** set name.

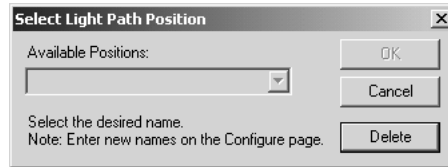
**Archive:** The **Archive** button marks the current light path set as read-only, so that it cannot be deleted or changed accidentally. **Archive** will also save your light path set, if necessary.

**2 Positions, 3 Positions or 4 Positions:** Click the radio button that indicates the correct number of positions for your light path.

**Light Path Positions:** This group box lists the light path positions. Once a position is selected, you can manually edit the current name for the Light Path, or select a name from the current master list. The position of the **Light Path** is saved in a settings file.

**“...” button** : The “...” button brings up a dialog with a selection list containing all the known light path positions, illustrated below:





**OK:** When you have selected the Light Path position that you want, press the **OK** button to insert the name into the current light path position.

If for any reason there are no positions left in the master list, the selection list and **OK** button will be disabled until new light path names are added on the *Configure* tab page.

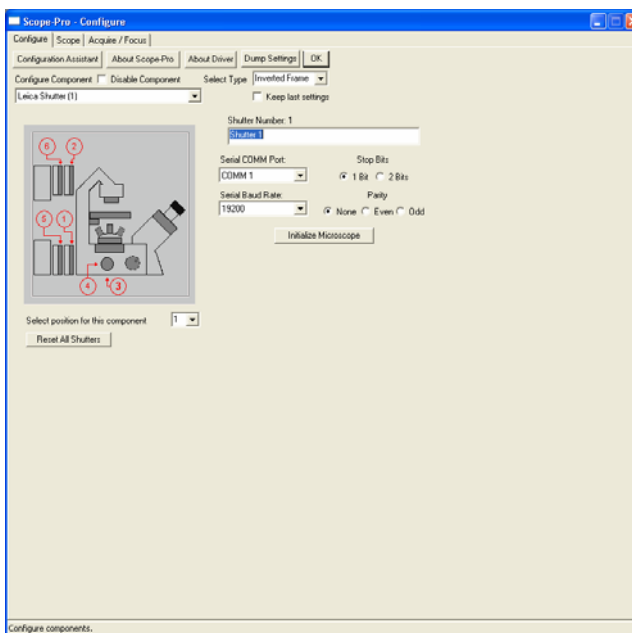
**Cancel:** Use **Cancel** at any time if you do not find the light path position you want.

**Delete:** Delete any unnecessary light path names by selecting the light path position in the selection list and then using the **Delete** button.

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## GUI View of the Configure Tab Page

If you have chosen the new *Scope-Pro* GUI (graphical user interface) your view of the **Configure** tab page:



When you select a specific component from the **Configure Component** drop-down list box, the possible positions for that component are displayed on the diagram. In the example above, the manual shutter has 6 possible positions on the microscope.

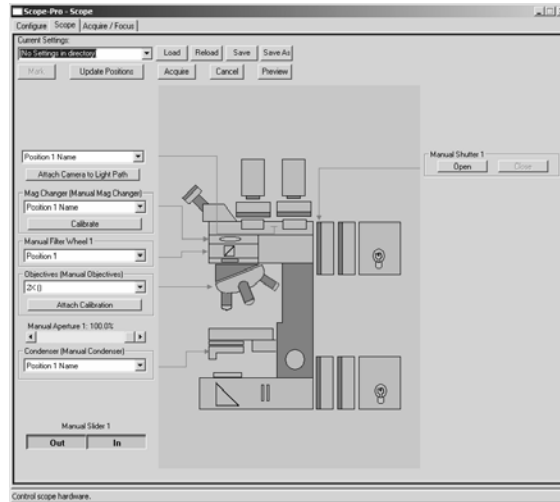
**Select position for this component:** Choose one of the numbered positions for the component (the manual shutter in this example) from the drop-down list box.

**Reset all shutters:** Clicking this button will reset the location of the manual shutter back to 0.

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Once you have chosen the positions for all of your components, you can switch to the **Scope** tab.

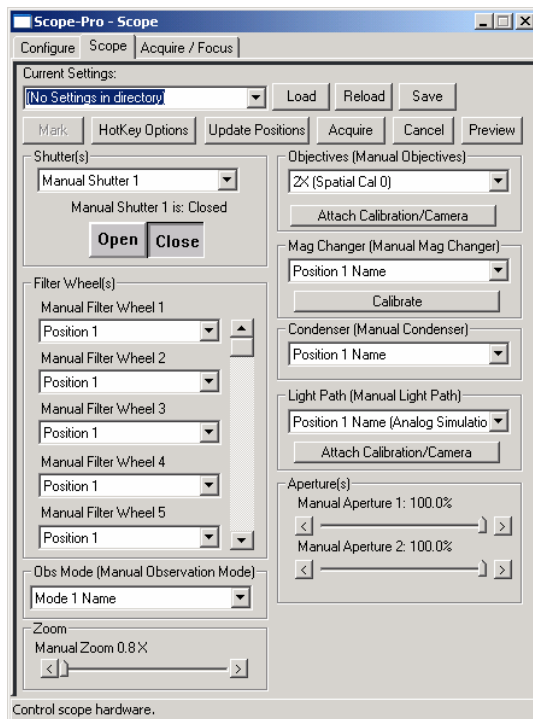
*Scope-Pro* will display the **Scope** tab dialog, with the diagram indicating the position of your components. The manual shutter location appears on the right side of the diagram, as shown here:



Other selected components are also displayed on the **Scope** tab page diagram. To change the position of any of them, return to the **Configure** tab page, and choose a component from the **Configure Component** drop-down list box. Then you can select a location for that component as you did for the manual shutter.

## The Scope Tab Page

The *Scope* tab page is the “control panel” from which you control the operation of your microscope equipment. You can use it to set the position of the different scope components. This tab page also contains special buttons that can be used to load predefined settings, acquire images and adjust hardware controls. The contents (and size) of this tab page will vary depending on the hardware that you have configured using the *Scope-Pro* setup program.



---

**Note:** If you are using the *Scope-Pro* configuration GUI, this page may look different. Please see the section entitled *GUI View of the Scope Tab Page*.

---

## Scope-Pro Settings

*Scope-Pro* maintains the current position of each component in what are referred to as “*Scope-Pro* settings”. The most recently used settings may be restored each time you start *Scope-Pro* in a new *Image-Pro* session by checking the **Keep Last Settings** box on the **Configure** tab. *Scope-Pro* settings are much more powerful than just restoring the last condition of your microscopy hardware.

The **Settings** controls give you the ability to manage any number of distinct *Scope-Pro* settings, each of which will result in a different microscopy setup for a different purpose. The **Settings** controls appear at the top of the **Scope** tab page (as well as *The Acquire/Focus Tab Page*):

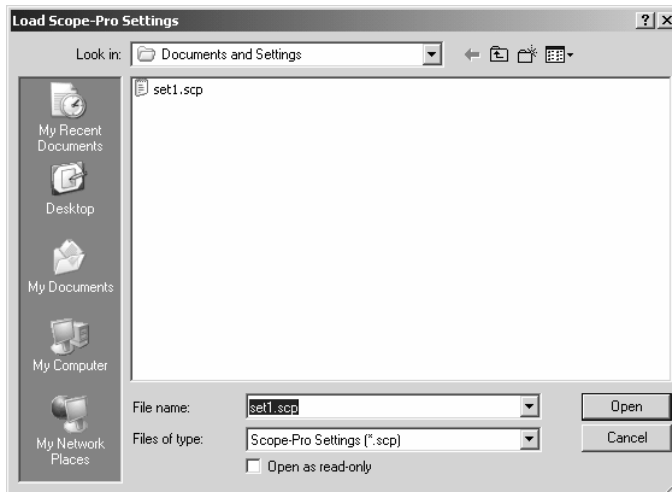


**Current Settings:** This box displays the name of your current *Scope-Pro* settings. In the example above, the current settings have been named *Set1* and can be restored at any time by picking *Set1* out of the **Current Settings** selection box.

This box will list all of the settings that are found in the current settings directory, which defaults to the application directory, but which can be set at any time using either the **Load** or **Save As** settings controls. A common application of *Scope-Pro* will be to save multiple settings that are very similar except for the filters used. Each of these related settings will be listed in the **Current Settings** selection box, and you can easily flip from one to the next.

**Load Settings:** Click this button to look for and load a settings file. You will see the following dialog box.

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Select the correct settings file, and click **Open** to load it, or **Cancel** to return to the *Scope-Pro* dialog. *Scope-Pro* settings files are identified by the extension **\*.scp** in the **Load Scope-Pro Settings** dialog box.

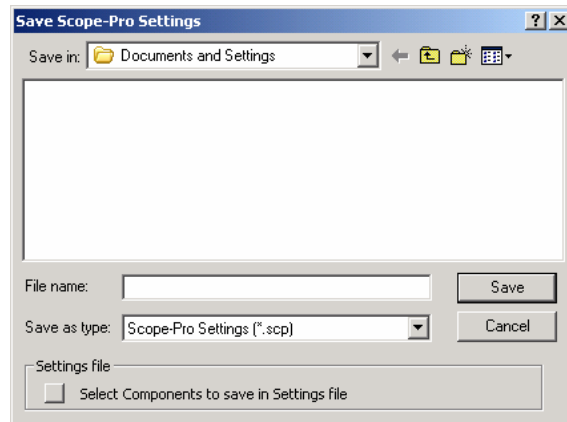
---

Tip: Although this dialog can be used simply to change settings (all of your current group of settings will be listed), this is most easily done with the **Current Settings** selection box. You will most often use the *Scope-Pro Settings* dialog to change the current Settings directory.

---

**Reload Settings:** Click this button to reload the current settings from disk. This button is only enabled if you have modified your settings, and there is a permanent copy on disk (after using the **Save** or **Save As** buttons).

**Save Settings:** Click this button to save your changes to the current settings file, as shown here. Select the correct settings file name and choose the appropriate component(s) to save. Click **Save**, or **Cancel** to return to the *Scope-Pro* dialog.

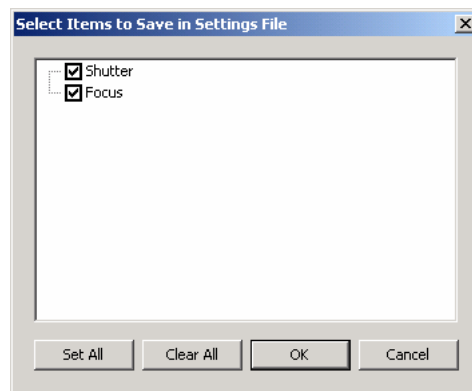


---

**Tip:** *Scope-Pro* remembers the current settings even after you have made changes to the original configuration. If you are fine-tuning your settings, you will want to use the **Save** button when they are correct.

---

**Select Components to Save in Settings File:** Checking this box displays the Select Settings dialog. Check the components that you wish to include in your settings file, and then click **OK**:



---

**Tip:** When multiple people are using a *Scope-Pro* installation, or even if the same person is doing several different types of microscopy, it will be helpful to use different locations for the different groups of *Scope-Pro* settings.

For instance, you might make “Fluorescence” and “Bright Field” directories under the application directory and store different settings in each according to their application. The first time that you run *Scope-Pro*, the program looks for *Scope-Pro* settings files in the host application’s “Documents and Settings” file (usually C:\IPWIN6\Documents and Settings ). If you load or save *Scope-Pro* settings files to or from a different directory, that directory will be used as the default *Scope-Pro* settings directory from that point on.

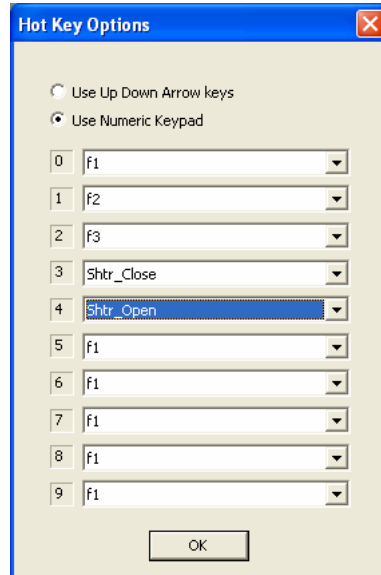
---

**Mark/Unmark:** You have the ability to “mark” some of the settings listed in the **Current Settings** selection box as a “set”. An asterisk appears next to the name of the marked settings. The **Up and Down Cursor Arrow** keys may be used to step up or down through the list of marked settings. The button reads **Mark** until the current settings are marked for inclusion in the set, and **Unmark** if the settings are already marked. Marked settings will appear as choices on the minimal dialog.

**Update Positions:** You may already be comfortable adjusting your microscope manually. The **Update Positions** button informs *Scope-Pro* that the hardware positions have been changed outside of *Scope-Pro*, either manually or by using controls on the microscope panel. *Scope-Pro* will inquire for the positions of each component supported by the controller, and update the current settings for that component.

**Hotkey Options:** Hotkey options allow you to assign individual *Scope-Pro* settings files to the numeric keypad on your computer. Clicking this button displays the following dialog:





Click **OK** when you have made your assignments.

**Acquire:** Clicking the **Acquire** button starts the process of capturing images with *Scope-Pro*.

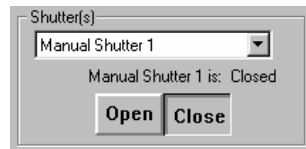
**Cancel:** Click this button to erase your changes and return to your original settings.

**Preview/Live Off:** Click this button to turn the **Live Preview** on or off.

### Shutters

The **Shutter** controls appear in the *Shutters* group under the settings group on the left side of the *Scope* tab page. Currently, *Scope-Pro* supports up to 15 shutters, and only 1 shutter may be controlled at a time. This group will be removed if there are no shutters configured.

The following will be the appearance when more than one shutter is configured:



---

**Note:** In every case where the examples show a name such as “Manual Shutter 1”, these names will be replaced by the current component names that you have set up on each component’s **Configuration** tab page.

---

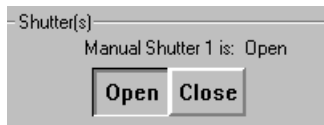
**Shutters:** The **Shutters** selection box displays the list of shutters that you have configured. Currently, *Scope-Pro* allows you to control only one shutter at a time. The **Shutters** selection box is used to select the active shutter, and will be removed if there is only one shutter configured.

---

**Note:** Under some configurations, a shutter may be an invalid choice for the current light path configuration. For instance, if selection of a particular reflector turret cube selects a reflected light or fluorescence light path, a shutter in the transmitted light path may be considered invalid by the shutter controller. Under these conditions, you may find that you cannot select the desired shutter until a light path is selected that is valid for that shutter.

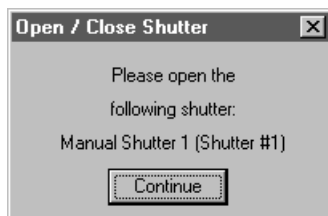
---

**Open/Close:** Clicking this button opens or closes the selected shutter. One of the buttons will depress, and the other button will pop up.



---

**Note:** If you are using the **Manual** component, you will see a dialog prompting you to open the selected shutter.



For all other hardware-based components, the controller will be instructed to open the associated shutter, and the status of the shutter will be updated.

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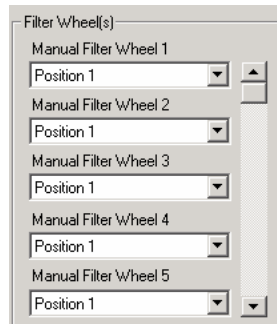
## Filter Wheels

The **Filter Wheel** controls appear in the *Filter Wheels* group on the left side of the *Scope* tab page. If more than 5 filter wheels are present, a scroll bar appears in the **Filter Wheel** group box. This group will be removed if there are no filter wheels configured.

---

**Note:** Various microscopy accessories may be categorized as **Filter Wheels** in addition to the obvious components. Some examples include: reflector or cube turrets, prism turrets positioned under the condenser, built-in neutral-density filter sets, etc. The Scope-Pro Setup program will generally list these by easily identifiable names, and will also describe them as “filter wheels”.

---



**Filter Wheel control selection box:** *Scope-Pro* will have two controls for each active filter wheel: the field giving the name of the current Filter Set, and the **Filter Wheel Control** selection box. You use the selection box to position the Filter Wheel at the desired position, selecting the position by the names in the currently configured Filter Set.

Refer to the section on configuring filter wheels earlier in this section for more information.

---

**Note:** Under some configurations, a filter wheel may be an invalid selection for the current light path configuration. For instance, if selection of a particular reflector turret cube selects a reflected light or fluorescence light path, a filter wheel in the transmitted light path may be considered invalid by the filter wheel. Under these conditions, you may find that the control selection box is disabled, and you cannot control the desired filter wheel until a light path is selected that is valid for the filter wheel.

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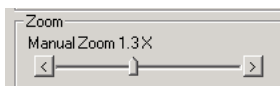
**Tip:** At least one of the supported automated microscopes exhibits the behavior outlined above. It will often be advisable to select a reflector turret position as the first step of determining a new setting.

The microscope may then make several related changes (i.e., aperture and lamp settings, or even objective selection), and Scope-Pro will reflect the changes to all of the affected components, including any components that have become invalid or have changed positions.

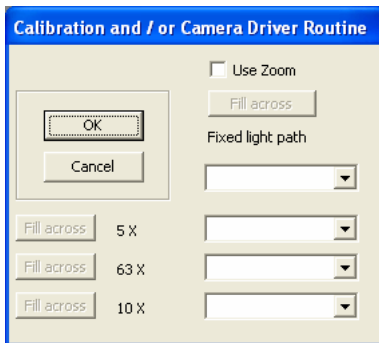
---

### Zoom

A **Zoom** control has been added to the Scope tab page to make it easier to control stereo microscopes, such as the Leica or Olympus models:



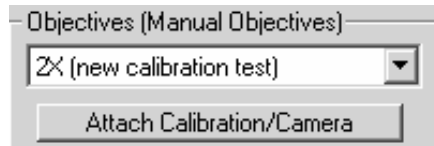
Changing the slider will change the position of the zoom hardware and modify the *Scope-Pro* multiplier that is sent to the *Image-Pro* reference / system calibration.



The **Use Zoom** checkbox turns this control on or off. If you do not have any zoom hardware, you will not see this dialog. Because zoom hardware can possibly be in one or more light paths, the objective, camera matrix will have to be expanded to handle these cases

## Objectives

The **Objective** controls appear in the *Objectives* group on the right side of the *Scope* tab page. The entire *Objectives* group will be removed if the **Objectives** control is not configured. The following will be the appearance if the objectives are configured:



**Objective selection box:** The **Objective Selection** box is used to select the desired objective, selecting the objective by the names in the currently configured **Objectives Set**.

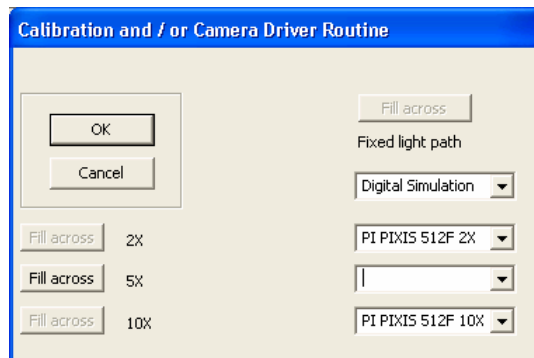
Refer to the section on configuring objectives earlier in this section for more information.

---

**Note:** Under some configurations, an objective may be an invalid choice for the current light path configuration. Under these conditions, you may find that the objective selection is returned to a valid selection, and you cannot select the desired objective until a light path is selected that is valid for the objective.

---

**Attach Calibration/Camera:** Clicking this button brings up a dialog of currently available *Image-Pro* calibrations, and indicates to which objectives they are attached.



This dialog associates spatial calibrations with the combination of a particular objective and a particular camera. If you do not have a **Light Path** component, you will only see one column of calibrations;

### *Acquire: The Scope-Pro Tab Dialogs*

otherwise, you will see one column of calibrations for each position of your **Light Path** component. By default, ALL of the drop downs in the dialog are empty, until you fill them. If you have unused positions on your objective turret, or your **Light Path** component has defined positions that cannot be used, you do not need to fill in those columns. But, every objective in every **Light Path** position that will be used MUST have both a capture driver and a calibration.

Before visiting this dialog, you should have defined at least one *reference calibration* for each camera and lens combination that you will use. For example, if you have two cameras, and 5 objectives, you should have at least 10 reference calibrations defined. The *Spatial Calibration Wizard* can help you define these reference calibrations. Please see your *Image-Pro* documentation for more information on reference calibrations, and how to create them. For each **Light Path** position that you will actually use, select the capture driver (and device name, if you have one driver controlling multiple cameras). Then select the appropriate reference calibration for that camera for each of the objectives. *Scope-Pro* will only accept reference calibrations in this dialog.

**Fill Across:** To repeat a position name or calibration selection across a row, click the **Fill Across** button. This button is only active for the row you are currently editing.

When you have selected capture drivers for each **Light Path** position, and reference calibrations for each objective (in all **Light Path** positions), click **OK**. This set of capture drivers and calibrations be stored as part of your *Scope-Pro* environment. Click **Cancel** to discard your changes and return to the *Scope* tab page.

If *Scope-Pro* has reference calibrations associated with the objectives, the *Image-Pro* system calibration will be updated when you change objectives. Also, if *Scope-Pro* has capture drivers associated with **Light Path** positions, your current capture driver will be changed when you select a new **Light Path** position.

---

**Note:** To take full advantage of *Scope-Pro*'s powerful new Calibration Management capability, *Scope-Pro* must have control of both your Objectives and your Light Path, even if these portions of your microscope are not motorized. You can achieve this by installing the Objectives and/or Light Path components of the Manual driver. If you are using the Manual driver, you will see a prompt dialog each time you change the position of a component, asking you to move the

component. Just make the necessary changes, if any, and click Continue.

---

**Tip:** Even if you do not have a motorized **Light Path** component, if you have multiple cameras that get swapped on and off of the microscope for different applications, or any other situation where you use multiple cameras with Scope-Pro, you may find it useful to install the **Manual Light Path** component, and assign drivers and calibrations to the various positions this component offers (up to four). This way, by simply selecting the correct **Light Path** position and objective, your system calibration (the calibration that will be applied to all newly captured images) will be updated for the current optical configuration.

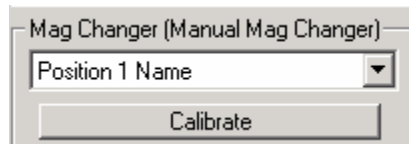
---

**Tip:** Many of the supported automated microscopes support an objective-related configuration of condensers, apertures, and/or lamp settings. It will often be advisable to select an objective as one of the first steps of determining a new setting. The microscope may then make several related changes (i.e. aperture, condenser, and/or lamp settings), and Scope-Pro will reflect the changes to all of the affected components, including any components that have become invalid or have changed positions.

---

### Mag Changer

Until this point, automated mag changers were considered by *Scope-Pro* to be a type of filter wheel. In *Scope-Pro 6.3*, a new **Mag changer** group appears under the **Objectives** group box, as shown here:



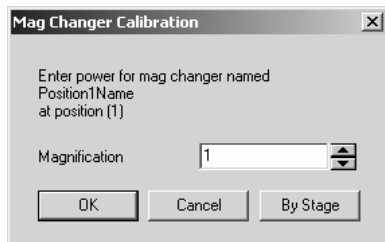
**Calibrate:** The **Calibrate** button allows you to enter a value for the mag changer or start a calibration routine, by clicking the **By Stage** button. At this point, the calibration routine is rather simple:

- Capture an image with the higher mag,

### Acquire: The Scope-Pro Tab Dialogs

- Click on two points on an object, switch to mag 1.0,
- Capture an image of the same object and click on the same two points.

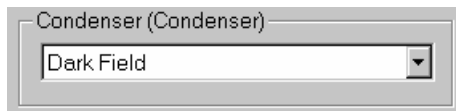
The **Mag Changer** value is then calculated from the two lines, as shown here:



The position of the **Mag changer** is saved in a settings file. The power of the mag changer is stored in **ipscope.ini**. This number is used by the reference calibrations to adjust the X and Y pixels per unit value.

### Condenser

The Condenser controls appear in the **Condenser Position** group on the right side of the **Scope** tab page. Currently, *Scope-Pro* supports a single condenser with a single control. The entire group will be removed if a condenser control is not configured.



**Condenser selection box:** Use the **Condenser Position** control box to position the condenser at the desired location. The name of the current condenser set will be used as the title of the condenser group.

Please refer to the section on configuring condensers earlier in this section for more information.

### Apertures

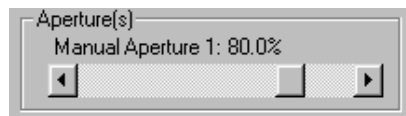
The **Aperture** controls appear in the **Apertures** group on the right side of the **Scope** tab page. Currently, *Scope-Pro* supports up to 2 apertures, which are also referred to in various manufacturers' literature as *irises*



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or *diaphragms*. This group will be removed if there are no apertures configured.

This is the appearance of this control with a single aperture configured:



**Aperture scroll bar:** The **Aperture** scroll bar is used to select the aperture opening, which is described as a percentage of the possible maximum opening.

---

**Note:** The **Aperture** scroll bar will only allow selection of openings that are supported by the Aperture component. This may mean that the indicated opening increases or decreases in discrete steps.

The **Aperture** scroll bar controls the aperture directly whenever you page the scroll bar left or right (by clicking in the area to the left or right of the scroll bar's "thumb") or increment it left or right using the arrow buttons. If you click on the "thumb" and drag the scroll bar, the displayed aperture opening will be updated, but the actual aperture setting will not be updated until you release the mouse button.

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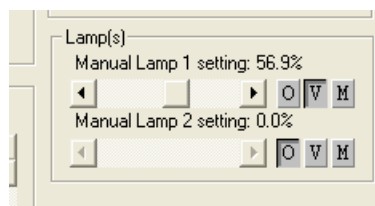
Refer to the section on configuring apertures earlier in this section for more information.

## Lamps

The **Lamp** controls appear in the *Lamps* group on the right side of the *Scope* tab page. Currently, *Scope-Pro* supports up to 2 lamps, which can be **continuously variable lamps** or those with only **on/off control**. This group will be removed if there are no lamps configured.

### 1) Continuously variable lamps

The following controls will appear for both lamps that are configured as continuously variable:



**Lamp Intensity Scroll Bar:** *Scope-Pro* will have two controls for most lamps:

- The field giving the name of the lamp component (in the example above, “Manual Lamp 1” and “Manual Lamp 2”), and
- The **Lamp Intensity** scroll bar which is used to select the lamp’s intensity.

The **Lamp Intensity** scroll bar controls the lamp directly whenever you move the scroll bar left or right (by clicking in the area to the left or right of the scroll bar’s “thumb”) or increment it left or right using the arrow buttons. If you click on the “thumb” and drag the scroll bar, the displayed intensity setting will be updated, but the actual lamp setting will not be updated until you release the mouse button.

For most lamps, the intensity will be described in lamp voltage, which is specific to the microscope or other lamp controller. You will want to refer to the user’s manual for the controller to determine the effects of various voltage settings.

In addition, the lamp control has three buttons: **Off**, **Variable**, or **Maximum**. Switching to **variable** will put you back to the last variable value before you switched to **Off** or **Max**.

---

**Note:** The **Lamp Intensity** scroll bar will only allow selection of settings that are supported by from the lamp component. This may mean that the range of the scroll bar may not extend all the way to 0V, for instance, or that the indicated voltage increases or decreases in discrete steps.

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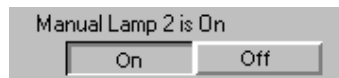
**Tip:** Many lamps will specify particular voltage settings to achieve particular color temperatures. You can use the **Lamp Intensity** scroll bar to select the required voltage precisely.

---

For more information on lamps, see the section on configuring lamps earlier in this section .

## 2) On/Off control lamps

For lamps that support only on/off control, the following controls appear:



**On:** Clicking this button will turn the associated lamp on, at which time the **On** button will depress, and the **Off** button will pop up.

**Off:** Clicking this button will turn the associated lamp off, at which time the **On** button will depress, and the **Off** button will pop up.

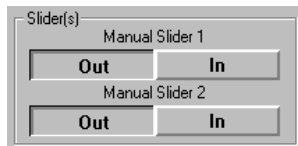
For more information on lamps, see the section on configuring lamps earlier in this section .

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### Sliders

The **Slider** controls appear in the *Sliders* group on the right side of the **Scope** tab page. Currently, *Scope-Pro* supports up to 2 sliders, which are any kind of 2-position control (i.e., a condenser in/out control, or a filter that can be inserted into the light path or removed). This group will be removed if there are no sliders configured.

The following will be the appearance with both sliders configured:



**In:** Clicking this button will move the associated slider to the “In” position, at which time the **In** button will depress, and the **Out** button will pop up.

**Out:** Clicking this button will move the associated slider to the “Out” position, at which time the **Out** button will depress, and the **In** button will pop up.

---

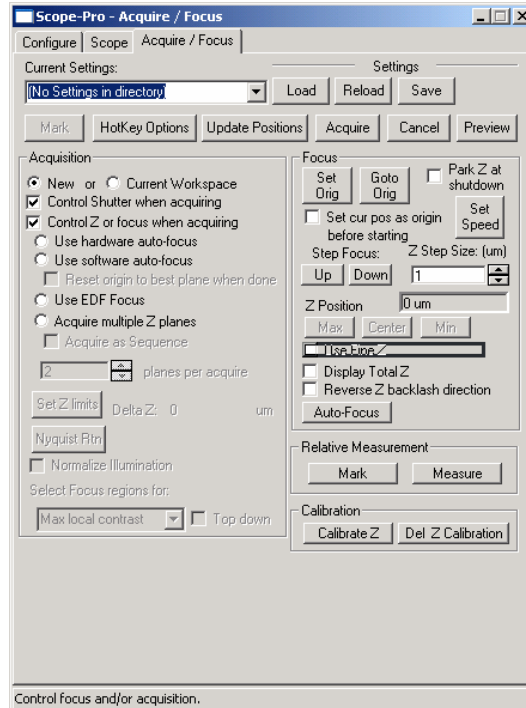
Note: The slider component determines the names of the two positions, which are used as the labels on the corresponding buttons. For example, several “light path” slider components support positions called “ocular” and “camera” for all slider components. *Pressing a button will move the component to the related position.*

---

Refer to the section on configuring sliders earlier in this section for more information.

## The Acquire/Focus Tab Page

The *Acquire/Focus* tab page contains the information required by *Scope-Pro* to acquire images and set the focus correctly:



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**Note:** Many of the controls illustrated here will only be available if *Scope-Pro* has been set up for Z Focus control. This includes all the options below the **Control Z or focus when acquiring** selection, including the Focus, Relative Measurement, and Z-Calibration groups.

---

---

*Note:* When Acquisition settings are available in *Scope-Pro* (**Acquire/Focus** tab page) and the same settings exist in *Image-Pro Plus*' *Capture* dialog box (displayed when invoking the **Multiframe/Timed Acquire** command from *Image-Pro Plus*'s *Acquire* menu), then *Scope-Pro*'s settings will be in effect and override *Image-Pro Plus*.

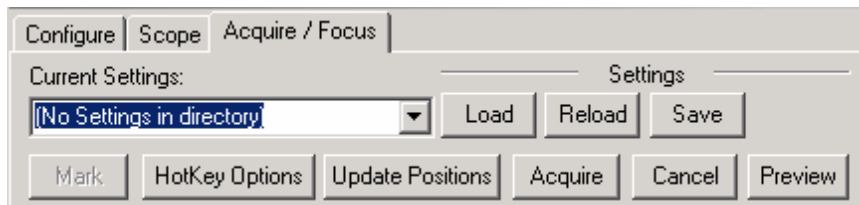
For example, if **Current Workspace** is checked in *Scope-Pro* and **New Workspace** is checked in *Image-Pro Plus*, the **Current Workspace** option will be used for capture from *Scope-Pro*.

---

*Scope-Pro* maintains the current position of each component in what are referred to as “*Scope-Pro* settings”. The most recently used settings are always restored whenever *Scope-Pro* is invoked from the *Image-Pro* menu. *Scope-Pro* settings are much more powerful than just restoring the last condition of your microscopy hardware.

### Current Settings group box

The **Settings** group controls give you the ability to manage any number of distinct *Scope-Pro* settings, each of which will result in a different microscopy setup for a different purpose. The **Settings** controls appear at the top of the **Acquire/Focus** tab page (as well as *The Scope Tab Page*):



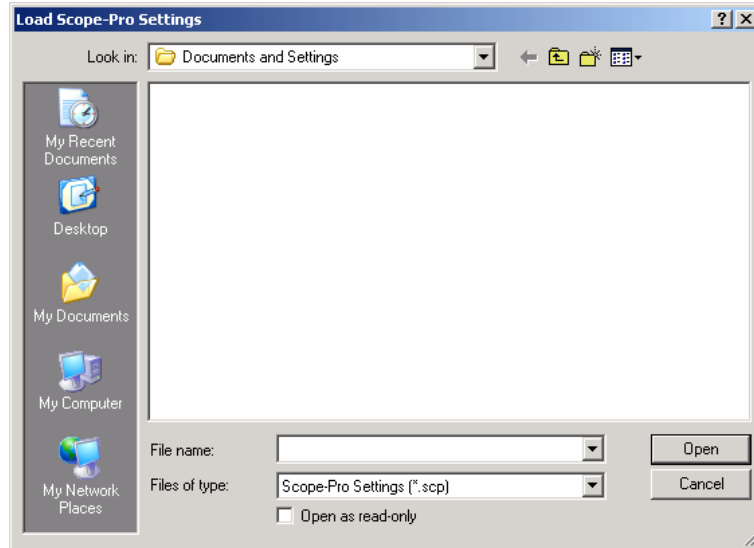
**Current Settings:** This box displays the name of your current *Scope-Pro* settings. In the example above, the current settings have been named “Set1” and can be restored at any time by picking *Set1* out of the **Current Settings** selection box.

This box will list all of the settings that are found in the current settings directory, which defaults to the application directory, but which can be set at any time using either the **Load** or **Save As** settings controls. A common application of *Scope-Pro* will be to save multiple settings that are very similar except for the filters used. Each of these related

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settings will be listed in the **Current Settings** selection box, and you can easily flip from one to the next.

**Load Settings:** Click this button to look for and load a settings file. You will see the following dialog box.



Select the correct settings file, and click **Open** to load it, or **Cancel** to return to the *Scope-Pro* dialog. *Scope-Pro* settings files are identified by the extension **\*.scp** in the **Load Scope-Pro Settings** dialog box.

---

**Tip:** Although this dialog can be used simply to change settings (all of your current group of settings will be listed), this is most easily done with the **Current Settings** selection box. You will most often use the **Load Scope-Pro Settings** dialog to change the current **Settings** directory.

---

**Open as Read-Only:** Check this box to open and load a *Scope-Pro* settings file that you will not be able to modify.

**Reload Settings:** Click this button to reload the current settings from disk. This button is only enabled if you have modified your settings, and there is a permanent copy on disk (after using the **Save** or **Save As** buttons).

**Save Settings:** Click this button to save your changes to the current settings file

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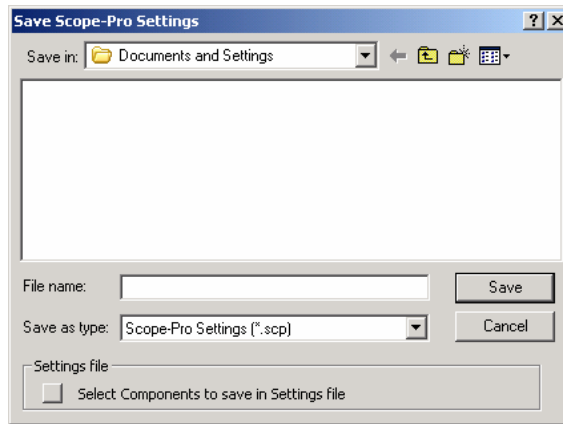
**Update Settings:** Click this button to update the current settings on disk. This button is only enabled if you have modified your settings.

---

**Tip:** *Scope-Pro* remembers the current settings even after you have made changes to the original configuration. If you are fine-tuning your settings, you will want to use the **Save** button when they are correct.

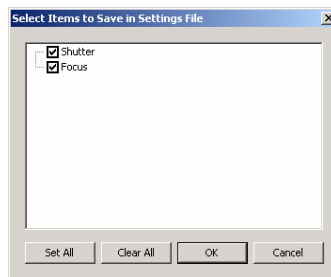
---

**Save As Settings:** Click this button to look for and save a settings file. You will see the following dialog box.



Select the correct settings file and choose the appropriate component(s) to save. Click **Save**, or **Cancel** to return to the *Scope-Pro* dialog.

**Select Components to Save in Settings File:** Checking this box displays the **Select Settings** dialog. Check the components that you wish to include in your settings file, and then click **OK**:



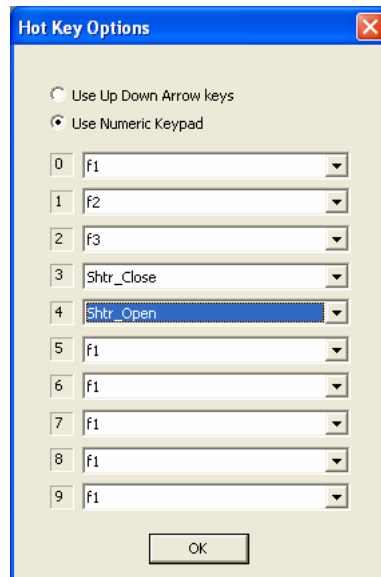


**Tip:** When multiple people are using a *Scope-Pro* installation, or even if the same person is doing several different types of microscopy, it will be helpful to use different locations for the different groups of *Scope-Pro* settings.

For instance, you might make “Fluorescence” and “Bright Field” directories under the application directory and store different settings in each according to their application. The first time that you run *Scope-Pro*, the program looks for *Scope-Pro* settings files in the host application’s “Documents and Settings” file (usually C:\IPWIN7\Documents and Settings). If you load or save *Scope-Pro* settings files to or from a different directory, that directory will be used as the default *Scope-Pro* settings directory from that point on.

**Mark/Unmark:** You have the ability to “mark” some of the settings listed in the **Current Settings** selection box as a “set”. An asterisk appears next to the name of the marked settings. The **Up and Down Cursor Arrow** keys may be used to step up or down through the list of marked settings. The button reads **Mark** until the current settings are marked for inclusion in the set, and **Unmark** if the settings are already marked.

**Hotkey Options:** Hotkey options allow you to assign individual *Scope-Pro* settings files to the numeric keypad on your computer. Clicking this button displays the following dialog:



Click **OK** when you have made your assignments.

## Acquire: The Scope-Pro Tab Dialogs

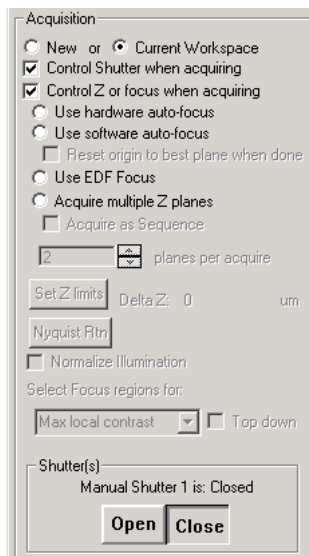
**Update Positions:** You may already be comfortable adjusting your microscope manually. The **Update Positions** button informs *Scope-Pro* that the hardware positions have been changed outside of *Scope-Pro*, either manually or by using controls on the microscope panel. *Scope-Pro* will inquire for the positions of each component supported by the controller, and update the current settings for that component.

**Acquire:** Clicking the **Acquire** button starts the process of capturing images with *Scope-Pro*.

**Preview/Live Off:** Click this button to turn the *Live Preview* on or off.

### Acquisition group box

The **Acquisition** group settings affect the way in which images are captured if you use the **Acquire** button from the *Scope* or *The Acquire/Focus Tab Pages*, or from the minimal dialog:



**New/Current Workspace :** Click the appropriate radio button to indicate if you want to place the images that will be acquired into a new workspace, or the one currently open.

---

**Note:** Selecting **New** or **Current Workspace** in *Scope-Pro* will **override** any *Image-Pro Plus* information set on the **Image**, **Integration**, and **Macros** tab of the **Capture** dialog box of *Image-Pro Plus* (**Acquire** menu).

---

**Control Shutter when acquiring:** Check this box to have *Scope-Pro* open the shutter before acquisition and close it afterward. This control will be removed if no shutters are configured.

**Control Z or focus when acquiring:** Various Z-plane and auto-focus options are available. This control, and all the controls below it in the **Acquisition** group box, will be removed if the Z Focus control is not installed.

**Use hardware auto-focus:** Check this box to activate the auto-focus before starting the acquire process. This control will be removed if the **Z Focus** control is not installed, or the focus controller does not support auto-focus.

---

**Tip:** There are a number of acquisition routines that will move the stage in the Z-axis while acquiring (i.e., extended depth of field, software auto-focus, and Z stacks). All of these routines will return the stage to the Z origin when they finish. It is therefore important that the Z origin be set correctly.

The following is the recommended procedure when using **all multiple Z routines**:

1. Focus (manually or using the **Up/Down** buttons)
2. Set Z origin
3. Set Z travel limits

This will assure that the stage returns to an in-focus position at the completion of Z acquisitions, that the "park" or "home" position is "safe" and in focus, and that the Z travel limits have a consistent origin so they can be used repeatedly.

---

**Use software auto-focus:** The *Scope-Pro* software returns the plane with the most pixels in focus (e.g., the "best" image selected). The best-focused plane is determined by the EDF module, using the criteria specified by the **Select Focus Regions for Control** dialog. This control will be removed if the Z Focus control is not installed.

**Reset origin to best plane when done:** Checking this box tells *Scope-Pro* to move the Z focus to the plane with the most pixels in focus when the software auto-focus is complete.

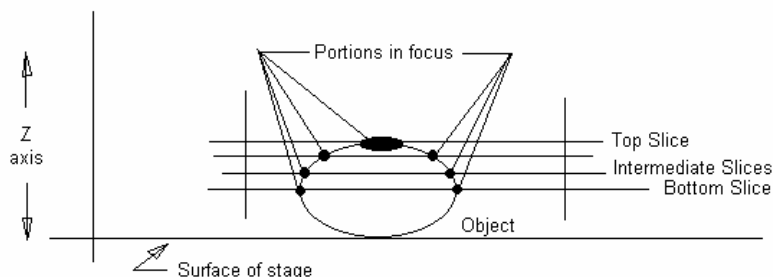
**Use EDF focus:** The **Use EDF focus** option combines pixels from the various source plane images using the areas that are most in focus (i.e., constructs a composite image from the portion of each "slice" that was in focus). Areas most in focus are determined according to the criteria

### Acquire: The Scope-Pro Tab Dialogs

selected by the **Select focus regions** for section (described below). This control and the remaining related controls will be removed if Z Focus control is not installed.

**Acquire Multiple Z Planes:** This function will cause *Scope-Pro* to snap a number of images at varying Z plane positions. This is the only one of the *Acquisition* options that can result in more than one image (i.e., a multi-frame sequence) being captured per *Scope-Pro Acquire*. There is a limit of 999 Z planes per acquisition.

The diagram below shows the Z-axis with the varying “slices” for the **Extended Depth of Field** capture option.



**Acquire as Sequence:** Check this box to acquire Z stack images as a sequence. Note that **Acquire as Sequence** is only supported when **Acquire multiple Z-planes** (above) is selected.

**Planes per acquire:** Indicate the number of plans in the Z stack to acquire for each sequence. Use the spin buttons or enter a number in the list box.

**Set Z travel limits:** Click this button to set the upper and lower limits for Z travel.

**Nyquist Rtn:** Click this button to use the Nyquist routine to calculate the optimum size of Z-stack slices to use for further processing.

**Delta Z:** Reports the corresponding Z distance between the top slice and bottom slice that will be captured.

**Normalize Illumination:** Use this option when the illumination or intensity of emitted light will change in the time that it takes to acquire all of the planes.

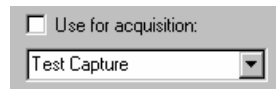
An example would be a sample that uses a fluorescent dye that degrades with exposure to light. The first captured plane will have a

higher intensity than the last plane captured. In this case all captured planes will be normalized before being processed into the final image.

**Select Focus Regions for:** There are four options to select what area is “in-focus”. Choose one from the drop-down list box.

- **Maximum Local Contrast:** This is the original method used in previous releases of *Scope-Pro*. It considers areas with high/maximum contrast to be in focus.
- **Max. Intensity:** This method is used for samples that are more intense than the background such as light emitting fluorescent dyes.
- **Min. Intensity:** This method is used for regions that are darker than the surrounding area to be in focus.
- **Maximum Depth Contrast:** This method calculates the average intensity of each pixel through the planes, and considers the pixel whose intensity is the most different from the average to be in focus.
- **Top Down:** Check this box to tell *Scope-Pro* to begin at the plane at the top of the stack for multi-plane/EDF acquisition. If this box is not checked, the acquisition will begin with the plane at the bottom of the stack.

**Use for acquisition:** This control is available only if capture extensions are installed. This option allows you to implement special acquisition functions (e.g., RGB composite capture from a B/W camera) to *Scope-Pro*. Installed capture extensions will appear in the selection box.



Select which capture extension to use and then check the **Use for acquisition** checkbox to use the specified capture extension for image acquisition.

### Focus controls group box

The **Focus** group controls are used to control the Z Focus position, and will be removed if Z Focus is not installed:



**Set Origin:** Click this button to set the origin of the Z-axis. The origin of an axis determines the motor position that will be displayed as position “0” for that axis. The Z travel limits are also relative to the current Z origin, so changing the origin can also change the absolute position of the travel limits (the delta z will not change).

**Go to Origin:** Click this button to move the stage to the position of origin.

**Park Z at shutdown:** Click this button to return the stage to the current Z origin on the Z-axis. If **Park Z at Shutdown** is selected, the stage will return to the Z origin every time you exit *Image-Pro*.

Since *Scope-Pro* will set the Z origin to the current Z position at start-up, this will result in consistent Z positioning and travel limits from session to session.

---

**Note:** For this feature to work reliably, you must not adjust the focus of the microscope in any way while *Image-Pro* and *Scope-Pro* are closed.

---

**Set current position as origin before starting acquire:** Select one of these checkboxes to use the current XY or Z location as the center starting position when the *Acquire* process begins. The Z option is available if *Scope-Pro* has a Z-focus motor. When this option is selected, the **Set Z Origin** button is disabled.

**Step Focus:** The **Step Focus** buttons are used to move the stage in single steps on the Z-axis (i.e., perpendicular or “up” and “down” to the slide).

**Up/Down:** Click the **Up** or **Down** button to move the stage one “step” “closer to” or “farther away” from the lens. A “step” is defined as the length (unit) currently specified in the **Z Step size field** (see below).

**Z Step size:** A logical Z-step is the amount of movement produced by a single click of the **Up** or **Down** buttons (see above). To specify a step size, enter a positive value representing the length of a single step in millimeters (mm). Once a step size has been specified, it becomes the default and will be retained from session to session.

**Z Position:** This indicates the current Z position. It is updated when you change the focus up or down.

- **Use Fine Z:** *Scope-Pro* allows you to select both **Coarse** and **Fine X, Y or Z** components. You may install one without the other.
- **Display Total Z:** If this box is checked, the values displayed will be the sum of any axes with both fine and course hardware. If this box is not checked, all position values will be displayed from the current position axis.
- **Reverse Z backlash direction:** For backlash correction for the Z-axis, your consistent direction must be **UP** to move to the desired position. This “swaps” or reverses the direction of the Z-axis for the up and down movements.

**Auto-Focus:** Click this button to auto-focus immediately. This button will be removed if Z Focus control is not installed, or if the Z Focus controller does not support auto-focus.

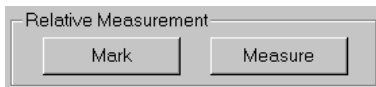
- **Auto-Focus for Low-Power lens:** This control indicates that *Scope-Pro* should use an auto-focus range appropriate for a low-power lens (less than or equal to 10X). This control will be removed if *Scope-Pro* objective control is installed (allowing *Scope-Pro* to determine the magnification itself), if Z Focus control is not installed, or if the Z Focus controller does not support auto-focus.
- **Auto-Focus for Medium-Power:** This control indicates that *Scope-Pro* should use an auto-focus range appropriate for a medium-power lens (greater than 10X but less than 50X). This control will be removed if *Scope-Pro* objective control is installed (allowing *Scope-Pro* to determine the magnification itself), if Z Focus control is not installed, or if the Z Focus controller does not support auto-focus.
- **Auto-Focus for High-Power:** This control indicates that *Scope-Pro* should use an auto-focus range appropriate for a high -

*Acquire: The Scope-Pro Tab Dialogs*

power lens (greater than or equal to 50X). This control will be removed if *Scope-Pro* objective control is installed (allowing *Scope-Pro* to determine the magnification itself), if Z Focus control is not installed, or if the Z Focus controller does not support auto-focus.

**Relative Measurement group box**

**Relative Measurement** is analogous to marking a position (the “reference point”) and then measuring the distance between that reference point and another position.



**Mark:** Click this button to mark the reference point.

**Measure:** This button measures the distance from the current position to the reference point.

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**Note:** These measurements are displayed in the **Output Window** on the Macro menu.

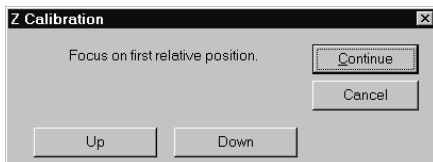
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**Z Calibration group box**

The **Z Calibration** group box sets up the calibration for the Z-axis.

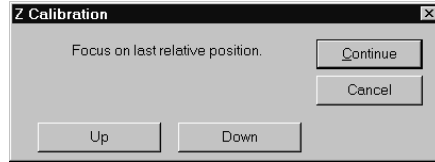


**Calibrate Z:** Press this button to calibrate the Z-axis. The following message box will appear.

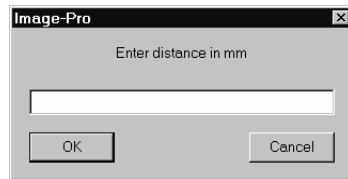


Click on the **Up** or **Down** buttons, or focus using the joystick on the top reference point (e.g., the top of the depth gauge or cover slip) and then press **Continue**.





Repeat the procedure to focus on the bottom reference point.



Enter the Z distance between the two reference points in millimeters (mm).

**Delete Z Calib:** Press this button to delete the current Z calibration.

---

*Notes on Z calibration:*

*Scope-Pro* has an internal ratio of motor pulses to vertical movement that is calculated from the step value on **The Configure Tab** Page. This value is used in all up and down stage movements. Because of variations in microscope frames, this ratio may not be as accurate as you require. The ratio has a correction multiplier (defaulted to 1.0) that can be used to modify the amount of vertical movement per motor pulse.

The Z calibration routine is what sets this correction factor. Basically, the routine works by prompting the user to move the stage to two different positions, entering a measured distance between the two positions, doing the math, and then setting the correction factor. The measurement that the user enters can be physically measured with a dial indicator mounted on the stage or by focusing on the top and bottom of something of known thickness, such as a 1 mm thick glass slide.

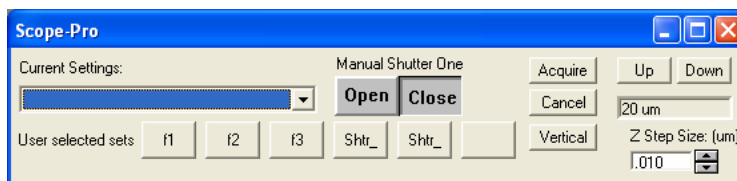
The most accurate way to do this is to stack two clean slides on top of each other with a small offset on the long side. First, focus on the top edge of the top slide, then move the stage and focus on the top edge of the bottom slide.

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## The Minimal Dialog

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The *Scope-Pro* tabbed dialog is large to accommodate the many controls that are needed to configure all of the components and determine different operating settings. Once these are set up for the stages of a particular experiment, work can be done from the *Scope-Pro* minimal dialog. *Scope-Pro* will remember from session to session whether you are using the tab dialog or the minimal dialog, illustrated below:



The **Minimal** dialog is displayed when you select the **Minimize** box from the main tabbed dialog. You can return to the tabbed dialog at any time by selecting the **Maximize** box on the **Minimal** dialog. The **Minimal** dialog can further be minimized to an icon on the taskbar when you select the minimize box.

The **Minimal** dialog displays the following controls from the main **Scope** tab page: the **Current Settings** selection box, buttons to load saved settings, control over the current shutter (if any), control over the focus and step size, and the **Acquire** button. The **Use Selected Sets** buttons indicated the marked sets (**Mark/Unmark**) sets from the normal view.

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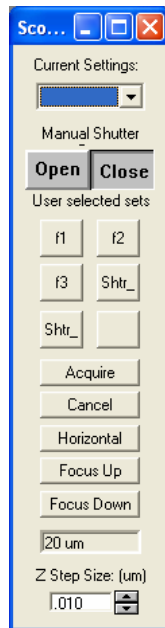
**Tip:** A typical usage of the Minimal dialog would be to use the main Scope tab page to set up and save various settings, and then do multiple acquires using those settings.

As an example, suppose you want to acquire a color image using a black-and-white capture system. Use the Scope tab page to set up three settings files ("Red", "Green", and "Blue"), set the filter wheel to the appropriate filter positions (with all other settings the same, or with only the filter wheel selected in the Save As dialog), and with the appropriate selections in the Acquire settings.

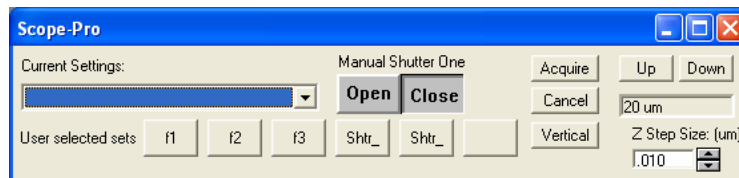
---

For instance, we would probably want Control the shutter option checked if we have a shutter configured. Using the Minimize box on the Scope-Pro tab dialog, we bring up the Minimal dialog. Using the Current Settings box, pick the “Red” settings, and click Acquire. Then pick “Green”, Acquire, and “Blue”, **Acquire**. We now have our three color channel images ready for compositing, so we select **Process, Color Channel, Merge**, select the three workspaces, and composite them into a true-color result.

The **Vertical** button allows you to change the orientation of the minimal dialog. Clicking this button moves the minimal dialog to the upper left hand corner of the *Image-Pro* workspace, and displays it in a vertical format, as shown here:



Clicking the **Horizontal** button returns the minimal dialog to its original orientation:



## The Stage-Pro Tab Dialogs

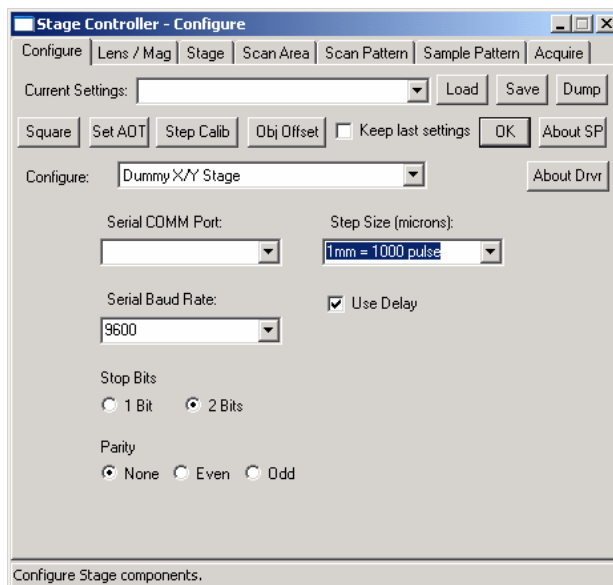
---

This section describes each of the *Stage-Pro* tab dialog pages in detail.

Remember if you configured the X/Y stage component **or** the X/Y and Z stage components, then *Stage-Pro* was installed. If you configured a single Z-focus component, then you installed *Scope-Pro* and need to refer to the previous section, *The Scope-Pro Tab Dialogs*.

### The Configure Tab Page

The first time the “*Stage-Pro*” command is invoked, the *Stage-Pro Configure* tab page is presented, as shown here:



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**Note:** When you select the **Stage-Pro** command for the first time you will be directed to the **Configure** dialog. It will not be automatically invoked again — selecting the **Stage-Pro** command from that point forward will invoke the last tab page used, unless **Stage-Pro** experiences problems communicating with your stage.

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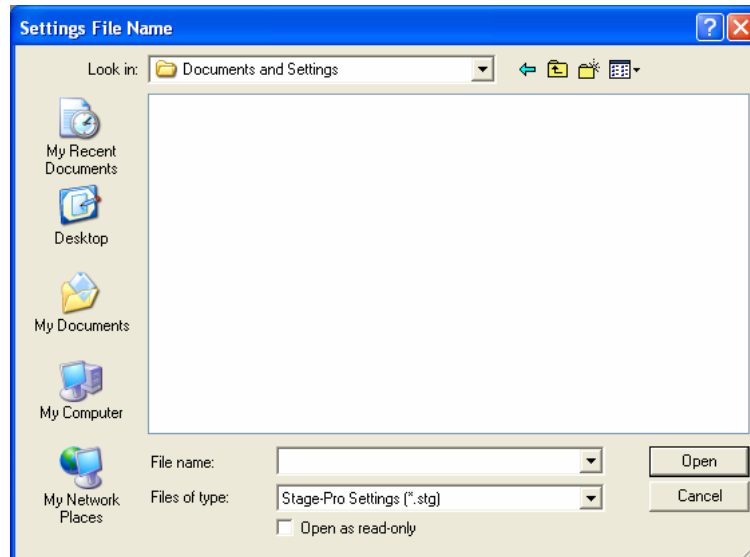
This tab page displays the operating characteristics (such as the step size) and the capabilities of your particular motorized stage. This information must be verified before the stage can be used successfully with *Image-Pro*. Once you have verified the information, the stage is configured and you can click **OK**.

## Acquire: The Stage-Pro Tab Dialogs

The controls on the *Configure* tab page are described below.

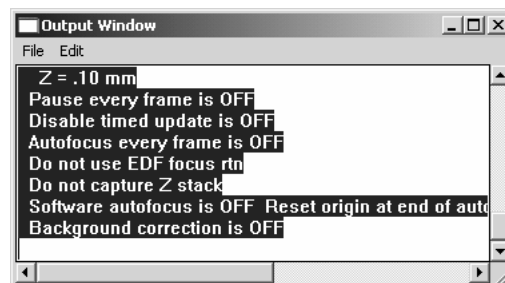
**Current Settings:** Select the settings file that you want to use from the drop-down list. These are previously saved files created in *Stage-Pro*.

**Load:** Click this button to load previously saved settings files. Settings files contain information about the scan area, patterns, and levels of the various installed components, and are identified by the extension **\*.stg** in the **Settings File Name** dialog box.



**Save:** Click this button to save the current settings in the **Settings File Name** dialog box shown above. *Stage-Pro* settings are identified by the extension **\*.stg**.

**Dump:** Clicking this button sends the details of your *Stage-Pro* settings file to the output window, as shown here:



## Acquire: The Stage-Pro Tab Dialogs

**Square:** This function is used to square the stage movement with the video camera. The most obvious sign that the stage is not aligned with the camera is that after you calibrate a lens the aspect ratio will be wrong

**Set AOT (Area of Travel):** Click this button to display the **Area of Travel** dialog that you saw when first starting up *Stage-Pro*. This dialog allows you to redefine the **Area of Travel**, which may be necessary if you have used the joystick to set the area of travel by corners, and then changed to a new slide. You will see the following dialog:

**Area of Travel**

Determine New Area of Travel

Use joystick to set Area of Travel by corners  
(Origin of Scan Area will be set to center of Area of Travel. Stage will then move to Origin of Scan Area.)

Use physical limits of stage  
(Stage will move to all end switches. Origin of Scan Area will be set to center of Area of Travel. Stage will then move to Origin of Scan Area.)

Use current position as center of Area of Travel  
Limits of Area of Travel will be set to +/-  
[ ] mm in X and [ ] mm in Y  
from current stage position.  
(Origin of Scan Area will be set to current stage position. Stage will not move.)

Previously Defined Area of Travel

Use joystick to move to origin of existing Area of Travel  
(Stage will then move to origin of last Scan Area)

Use current (parked) position as origin of existing Area of Travel. (Stage will then move to origin of last Scan Area)

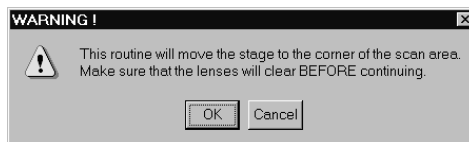
Z Reference Position

Use Current Z position as Reference Position.

Prompt for Reference Position

OK Cancel

This dialog affects the travel area of the stage (i.e., the physical limits of the stage movement). Follow any warnings that appear, such as the one shown below.



The **Area of Travel** dialog will be modified as above. If the “Arbitrarily defined limits” button is checked the “mm in X” and “mm in Y” edit boxes will be enabled. If the edit boxes contain numbers the “OK” button will be enabled. When you choose this option the current stage position will be used as the origin of the scan area and the center of the travel area. The extents of the travel area will be “mm in X” and “mm in Y” on both sides of the current position. No checking will be performed. We will assume that the user gave us good information and

that there is room to move in that area (please see *Appendix C* for more information).

**Z Reference Position:** Click the radio button that indicates how you want to set the Z position, if your stage is so equipped.

**Step Calib:** Click this button to launch the **Step Calibration** routine. You will see the following dialog:



Step calibration accounts for any small errors that may have occurred in the manufacturing of your stage. This is not to say that your stage is in any way defective, or out of specification. Rather, we have found that even the best stages, made with the tightest tolerances have some variation from stage to stage. This process calibrates the movement of the stage using the current spatial calibration for the current lens. So, it is **ABSOLUTELY** critical that you have an accurate spatial calibration for the lens you are using. Please see the *Image-Pro* manual for details on generating the reference calibrations used by *Scope-Pro* and *Stage-Pro*.

If you have measured the **Step Calibration** modifier by some other means, you can simply type that value into the **Step Mod** edit box and click the **Accept** button. If you do not know what the step modifier should be, choose one of the two radio buttons to tell *Stage-Pro* whether it will be looking at dark objects such as a speck of dust or bright objects such as a pin hole in a dark film. Then click **Step Calibration Routine**.

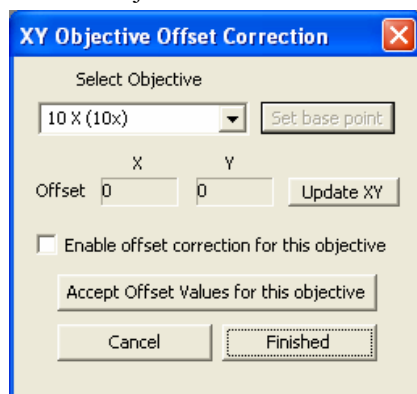
You will be prompted to place a small object in the top, left corner of the live preview. When you click Continue, you will be prompted to draw an AOI over the entire object - you cannot use the tip of a line since any objects that touch the edge of the AOI will be ignored. When you click OK, *Stage-Pro* will automatically identify the center of the object in your AOI and then restart the live preview and prompt you to move the object to the bottom, right corner of the live preview. When you click OK, you will be prompted to reposition the AOI over the

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object and click Continue. Based on the two object centers obtained from this process and the distance between the two locations reported by the stage, Stage-Pro will calculate a Step Modifier that serves to make a 100 um movement of the stage exactly equal in size to a 100 um object in the image.

**Obj Offset:** The offset correction routine attempts to move the XY position so that you keep the same view through all objectives.

1. To perform offset correction, click this button. You will see the XY Objective Offset Correction dialog:



2. When this dialog opens, it starts the workspace preview. A crosshair marker will appear over the image in the preview window.
3. Use the stage to move an identifiable object under the center of the crosshairs.
4. Click **Accept Offset Values for this Objective** when you are satisfied with the positioning.
5. Click the **Set Base Point** button to use this objective as the reference point for the other objectives.
6. Repeat steps 2-5 for the other objectives.
7. Click **Finished** when done.

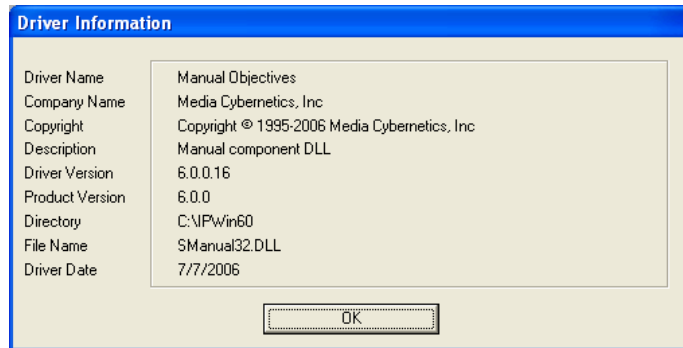


*Acquire: The Stage-Pro Tab Dialogs*

**About SP:** Click this button to display the software version information.



**About Dvr:** Click this button to see the driver name, company name, copyright, driver and product version numbers, directory and filename of the driver, and the driver date.



**Keep Last Settings:** Check this box to use the settings from your last *Scope-Pro* session.

**OK:** Press this button when you are finished modifying your configuration settings.

**Configure:** In the **Configure** list box, select the specific component you wish to configure.

Once you select the appropriate component, the options on the **Configure** tab page window will change to reflect the selected component. The components are listed in more detail in the sections following.

**Serial COMM Port:** In this field, select the serial port to which your motorized stage is connected (e.g., COMM 1, COMM 2, etc.). The default setting is **COMM 1**, but depending on your setup, you may

### Acquire: The Stage-Pro Tab Dialogs

need to select a different COMM port or a USB port (e.g., COMM 2, USB1).

**Step Size:** In this field the distance appears, in microns, by which the *stage* is moved with a single step of its X/Y or Z stepper motors. Many times the motorized-stage manufacturer expresses this distance as “steps per millimeter.” When this is the case, divide 1000 by the number of steps per millimeter, and enter the result in this field.

For example, if your stage is capable of 10,000 steps/mm, your step size will be 0.1, because:

$$1,000 / 10,000 = 0.1 \text{ micron}$$

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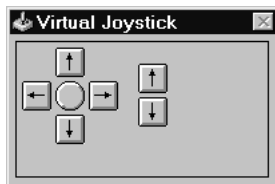
**Note:** The **Step Size** value should match the value indicated by the stage manufacturer. If you find that after properly squaring your stage and generating accurate spatial calibrations (see the discussion of the *Calibration Wizard* in your *Image-Pro Plus* documentation) that tiled images are not lining up properly, click the **Step Calib** button to have *Stage-Pro* measure the actual step size.

This stage **Step Size** field is **NOT the same** as the **Step** field that you set on the **Stage** page, although the smallest logical step size possible is determined by the stage step size that you set here.

---

**Has Auto-Focus:** Click this option *only* if your Z axis controller is equipped with a hardware auto-focus option. This option appears on the **Configure** tab page only if a focus module was installed.

**Has Joystick:** Click this option if your motor controller is equipped with a joystick controller. If your system is not equipped with a joystick, you can use the **Virtual Joystick** dialog (shown below) to move the stage. The last two buttons on the right will not be present if you do not have Z controls.



**Serial Baud Rate:** Enter the baud rate for your motor controller. This should default to the correct setting for your component and can be verified in your stage documentation

**Stop Bits:** Enter the number of stop bits. This should default to the correct setting for your component and can be verified in your stage documentation.

**Parity:** Enter the parity setting for your motor controller. This should default to the correct setting for your component and can be verified in your stage documentation.

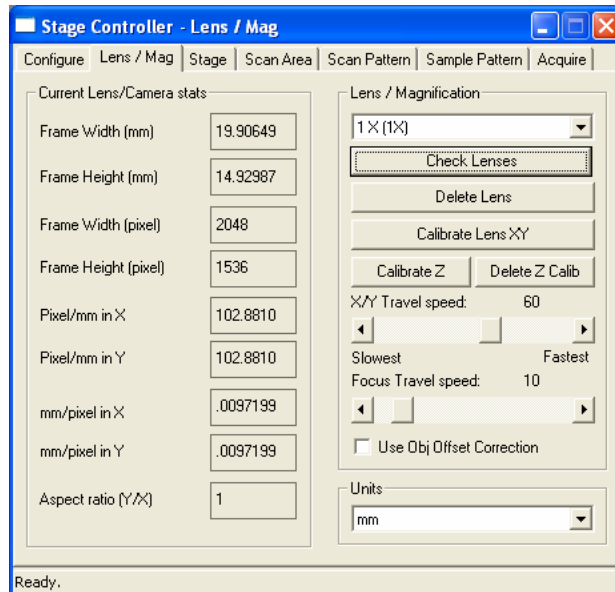
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**Note:** Your stage controller may have other configurable options not presented here. Please refer to the release notes and your hardware guides.

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## The Lens/Mag Tab Page

The *Lens/Mag (Magnification)* tab page shows the magnification, frame size, and other lens and camera options, and allows the user to select and calibrate installed lenses.



**Current Lens/Camera stats:** The following section describes the **Current Lens/Camera stats** group box options in detail.

**Frame Width (unit):** Width of the current frame in the selected units. You can set the units in the **Units** combo box at the bottom of this screen.

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**Frame Height (unit):** Height of the current frame in the selected units. You can set the units in the **Units** combo box at the bottom of this screen.

**Frame Width (pixels):** Width of the current frame in pixels.

**Frame Height (pixels):** Height of the current frame in pixels.

**Pixels per unit in X:** Number of pixels per unit on the X-axis.

**Pixels per unit in Y:** Number of pixels per unit on the Y-axis.

**Units per pixel X:** Number of units per pixel on the X-axis.

**Units per pixel Y:** Number of units per pixel on the Y-axis.

**Aspect ratio:** Aspect ratio X:Y.

**Lens Magnification:** The following section describes the **Lens/Magnification** group box options in detail.

**Magnification:** Use this option to select the lens used.

When you enter the lens tab page for the first time, the current magnification is blank. When you click on the down arrow, the list will pop down with a default list of **2X, 4X, 10X, 20X, 40X, and 100X**. The initial list will show them like this: **2X (with NO multiplier)**.

Once you modify the list, the multiplier tag is no longer used, if it is 0 or 1. Select a lens from the drop-down list. If the lens is calibrated, the list box will close and you will see the one you chose as the current lens.

If the lens is not calibrated (as will be the case on the first time) you will get a dialog box asking if you want to calibrate it. If you choose **Yes**, you will be taken through the calibration routine. If you choose **No**, the menu will go return back to the last calibrated lens (if there is one) or display a blank if no calibrated lenses are available.

---

**Note:** The list in the popup needs to match the hardware that you have on your microscope. If your scope has more/less/different lenses you need to use the **New** and **Delete Lens** buttons to make them match. You do not have to calibrate all the lenses at once. You do have to calibrate at least one lens before proceeding; and you must calibrate each lens before you can use that lens.

---

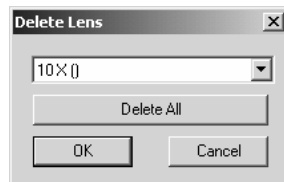
**New:** Pressing the **New** button will display a dialog box that enables you to add objectives if none are configured currently. Indicate the power of the lens you are adding, and the multiplier to be used. Click **OK** to set the new lens information.

### Acquire: The Stage-Pro Tab Dialogs

If *Scope-Pro* has control of the mag changer and objectives in your setup, then the **New Lens** button will not appear, and be replaced by the **Check Lenses** button.

**Check Lenses:** Clicking this button displays the list of lenses and associated calibrations that you have configured in *Scope-Pro*.

**Delete Lens:** Clicking **Delete Lens** will ask you to confirm that you want to delete the current lens settings. When you click this button, it brings up a list box of current lenses.



Click on the lens you want to delete, and the list box closes. Click **OK** to delete it or **Cancel** if you change your mind. Click **Delete All** to remove all of the current calibrated lenses. You will then have to redefine at least one lens, and calibrate it, in order to have access to the rest of *Stage-Pro*.

**Calibrate Lens (XY):** Press this button to calibrate the selected lens. You will see the **Objective Not Calibrated** dialog box:



---

**Note:** *If you have a motorized objective controlled by Scope-Pro, things will run more smoothly if you define your list of objectives and attach calibrations to these objectives in Scope-Pro. Then you may import the whole list into Stage-Pro using the Check Lenses button.*

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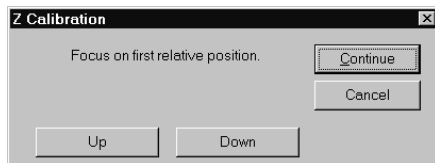
If you have a **Light Path** component in *Scope-Pro*, you must configure it, including attaching cameras to the ports and configuring the objectives, and attaching one calibrated lens to each camera, in order for *Stage-Pro* to handle the calibrations properly as *Scope-Pro* adjusts

*Acquire: The Stage-Pro Tab Dialogs*

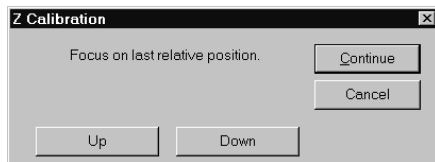
the configuration of your microscope. If you have a motorized light path but do not have a motorized objective (nose piece) the easiest way to work with *Stage-Pro* will be to install the manual objectives component.

Please refer to the discussion of calibrating lenses by stage movement that appears in Chapter 6.

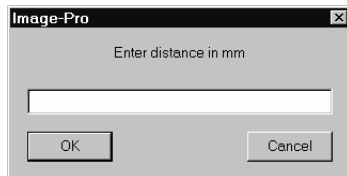
**Calibrate Z:** Press this button to calibrate the Z-axis. The following message box will appear.



Click on the **Up** or **Down** buttons, or focus using the joystick on the top reference point (e.g., the top of the depth gauge or cover slip) and then press **Continue**.



Repeat the procedure to focus on the bottom reference point.



Enter the Z distance between the two reference points in millimeters (mm).

**Delete Z Calib:** Press this button to delete the current Z calibration.

**Notes on Z calibration:**

*Stage-Pro* has an internal ratio of motor pulses to vertical movement that is calculated from the step value on the **Configuration Tab**. This value is used in all up and down stage movements. Because of variations in microscope frames, this ratio may not be as accurate as you require. The ratio has a correction multiplier (defaulted to 1.0) that can be used to modify the amount of vertical movement per motor pulse.

*The Z calibration routine is what sets this correction factor. Basically, the routine works by prompting the user to move the stage to two different positions, entering a measured distance between the two positions, doing the math, and then setting the correction factor. The measurement that the user enters can be physically measured with a dial indicator mounted on the stage or by focusing on the top and bottom of something of known thickness, such as a 1 mm thick glass slide.*

---

**X/Y Travel speed:** Use this scroll bar to set the relative speed for the stage movement. The speed you select is for a specific objective, where on a relative scale of 1-100, 1 is the slowest and 100 is the fastest.

**Focus Travel speed:** This scroll bar controls the travel speed of the focus, if the controller supports separate speed settings. The scroll bar may be disabled if your stage controller does not support adjustable travel speed for Z focus.

**Use Offset Correction:** Check this box to use the offset correction defined on the **Configure** tab page. This is only valid if you have an appropriate objective configured.

**Units:** Enter the calibration units: **mm**, **microns**, or **mils**.

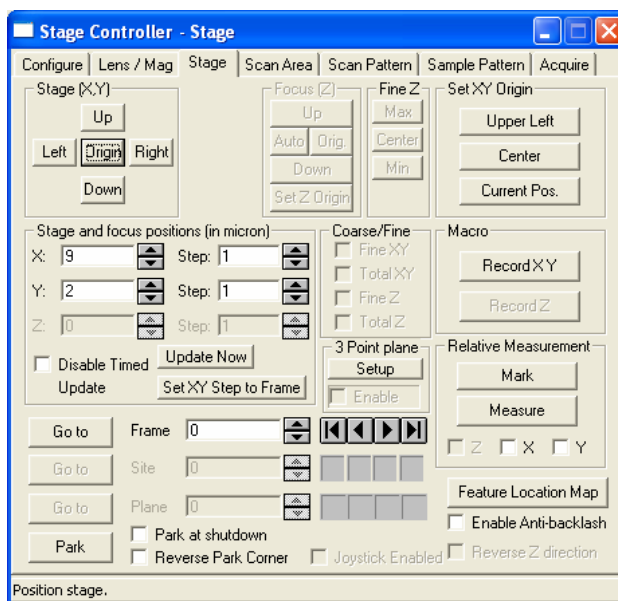
---

**Note:** The units you select here determine how positions and distances will be displayed throughout *Stage-Pro*.

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## The Stage Tab Page

The **Stage** tab allows you to control the movement of your microscope's stage and focus. You can use it to move the stage to a specific point on the X-, Y- or Z- axis, or to move the stage in single, user-definable steps relative to its current position. The **Stage** tab also contains special buttons that can be used to record stage motion in a macro, which lets you program a series of movements for later execution. Note that the appearance of this page may vary, depending on the sample patterns that you have selected, and the components that you have installed in *Scope-Pro*.



The buttons in the **Stage (X/Y)** and **Focus (Z)** group boxes are used to produce single-step movement. A single-click of an **Up**, **Down**, **Left** or **Right** button results in a single step in the specified direction of the stage or focus. The size of this step is adjustable, and is set in the **Stage and focus positions** group box.

You can also produce movement by entering an absolute X, Y, or Z position in the **Stage and focus positions** group box. A value (in the currently selected unit from the **Lens** tab; in this case, microns) entered in the **X**, **Y**, or **Z** field causes the stage or focus to move to the specified point.



### Acquire: The Stage-Pro Tab Dialogs

Movement is measured from the currently-designated “origin” on the respective axis, which is defined using the buttons in the **Set XY Origin** group box (upper right corner) or the **Set Z origin** button in the **Focus (Z)** group box (upper middle).

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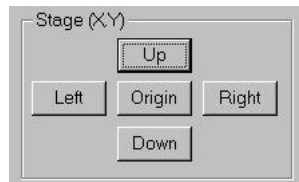
**Note:** The **Stage** dialog can be used in conjunction with your motorized stage’s manual controls (usually a joystick). When you move the stage or focus manually, the X, Y, and Z positions are monitored by Stage-Pro and are reflected in the **Stage and Focus Position (mm)** group box. This feature is especially useful in conjunction with the **Set XY Origin** and **Set Z Origin** buttons, described below.

---

The buttons in the **Macro** group box are used to record the current stage and focus positions in a macro. You can use these buttons to “teach” *Image-Pro Plus* a sequence of steps that can later be played back automatically.

The controls within the **Stage** tab page are described below.

#### Stage (X/Y) group box



**Stage (X/Y) :** The buttons in the **Stage (XY)** group box are used to move the stage in single steps on the XY-plane (i.e., the plane parallel to the slide).

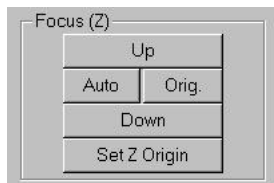
**Up/Down:** Click the **Up** or **Down** button to move the stage one “step” in the specified direction along the Y axis. A “step” is defined as the distance currently specified in the **Y Step** field of the **Stage and focus positions** group box (see below).

**Left/Right:** Click the **Left** or **Right** button to move the stage one step in the specified direction along the X-axis. A “step” is defined as the distance currently specified in the **X Step** field of the **Stage and focus positions** group box (see below).

**Origin:** The **Origin** button moves the stage to the “origin” of the X/Y plane. The X/Y origin is set using the buttons in the **Set XY Origin** group box (see below).

*Acquire: The Stage-Pro Tab Dialogs*

**Focus (Z) group box**



**Focus (Z):** The buttons in the Stage (Z) group box are used to move the stage in single steps on the Z-axis (i.e., perpendicular or “up and down” to the slide).

**Up/Down:** Click the Up or Down button to move the stage one “step” “closer to” or “farther away” from the lens. A “step” is defined as the distance currently specified in the Z Step field of the Stage and focus positions group box. See *Stage and focus positions*, below.

**Auto :** Click the Auto button to automatically establish the proper focus position. This button is only available if your stage controller has auto-focus capability.

When you click Auto, the stage controller’s auto-focus cycle is initiated and the hourglass cursor is displayed. You will receive control again when focus has been reached.

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**Note:** If *Stage-Pro* cannot verify the presence or absence of the auto-focus circuitry and auto-focus was inadvertently enabled during setup, this button may be erroneously active. Clicking **Auto** under these circumstances can cause the stage controller to stop responding.

If your controller is not equipped with auto-focus, be sure the “Enable Auto-Focus” option is disabled in the “Configure” dialog, described earlier in this section.

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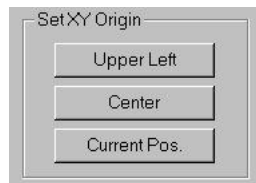
*Acquire: The Stage-Pro Tab Dialogs*

**Orig:** Click the **Orig.** button to move the stage to the “origin” of the Z-axis. The Z “origin” is usually set to be the Z position where the sample is most in focus and can be set using the **Set Z Origin** button.

**Set Z Origin:** Click this button to set the origin of the Z-axis. The origin of an axis determines the stage position that will be displayed as position “0” for that axis, in the **Stage** dialog box. The Z travel limits are also relative to the current Z origin, so changing the origin can also change the absolute position of the travel limits (the Delta Z will not change).

To set the XY origin, see the **Set XY Origin** group box below.

**Set XY Origin group box**



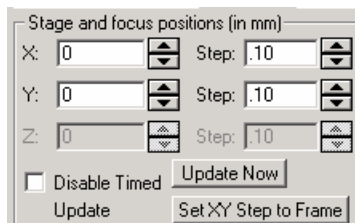
**Set XY Origin :** The buttons in this group box are used to set the origin of the X- and Y-axes. The origin of an axis determines the stage position that will be displayed as position “0” for that axis in the **Stage** dialog box.

**Upper Left:** Click this button to set the origin of the XY plane to the upper-left corner of the Area of Travel. This action also moves the stage so that the lens is directly above this point.

**Center:** Click this button to set the origin of the XY plane to the center of the area of travel. This action also moves the stage so that the lens is directly above this point.

**Current Position:** Click this button to establish the current X and Y positions as the origin of the X and Y-axes, respectively.

### Stage and focus positions group box



**Stage and focus positions (in units):** This group box contains the controls used to move the stage to a specific point. It also contains the fields used to define the size of a single step.

**X/Y/Z:** The **X**, **Y**, and **Z** fields on the left side of the group box report the current position, in the appropriate units (**millimeters**, **microns**, or **mils**) as selected on The **Lens/Mag** Tab Page.

*Stage-Pro* polls the hardware for these positions every half second, so any movement produced by the stage's control (typically, a joystick) is captured and reported here. These fields are initialized with the stage's current position when you go to the **Stage** tab page.

The **X**, **Y**, and **Z** fields are also used to move the stage to a specific position relative to the current origin. To specify a stage position, enter the value in the appropriate **X**, **Y**, or **Z** field. Fractional and negative values are allowed.

**Step:** The **Step** fields on the right side of the group box are used to specify the size of a "logical step" for a given axis. A logical step is the amount of movement produced by a single click of the **Up**, **Down**, **Left** or **Right** button in the **Stage X/Y** or **Focus (Z)** group boxes.

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**Note:** The "logical step" is a convenient item to make your work with *Stage-Pro* easier. It is **unrelated** to the stage step size that you set on The **Configure** Tab Page, though the smallest logical step size possible is determined by the stage step size.

## Acquire: The Stage-Pro Tab Dialogs

You can set the logical step size to convenient values for stepping the stage around the area of your slide that you are interested in. For instance, you will want to set a very small Z focus step size when using the **Up** and **Down** buttons to focus on a very flat sample; while you may want a larger step for a thicker or more uneven sample.

---

**Important:** It is very easy to set such a small logical step size that the stage movement is invisible to the naked eye. It is also easy to set a step size large enough to run the stage into your lens (or the optics under the stage).  
**Be cautious!**

**Disable Timed Update:** Check this box to prevent *Stage-Pro* from automatically refreshing the **X**, **Y**, and **Z** fields with the current physical location of the stage at regular intervals.

Ordinarily, you would not check this box, so that *Stage-Pro* can track the stage as you move it using the joystick and/or other controls on this tab page. However, on older, slower computers or with a slow stage controller, the position update is time-consuming and might make it difficult to use the controls on this tab page. You can check **Disable Timed Update** to prevent the timed updates.

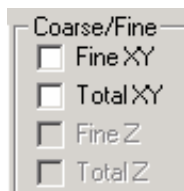
If you do select this option, the only way to get an update (without using one of the other controls on this page) will be to press the **Update Now** button.

**Update Now:** Click the **Update Now** button to refresh the **X**, **Y**, and **Z** fields with the current physical position of the stage. Under normal operating conditions this action is not necessary. However, if you have also checked **Disable Timed Update**, the position will be updated only when you move the stage using one of the other controls on this tab page. In this event, the stage positions can be obtained with a click of the **Update Now** button.

**Set XY Step to Frame:** The **Set XY Step to Frame** button sets the X and Y step size equal to one frame as captured by your current camera. This is the same value as from The *Lens/Mag* Tab Page.

### Coarse/Fine group box

The **Coarse/Fine** group box lets you control secondary X, Y, and Z axes.

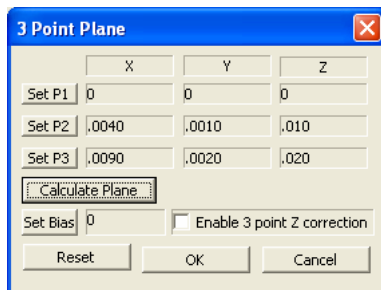


The check boxes enable you to select either coarse or fine X, Y, or Z. If the **Total n** is *not* checked, all position values will be displayed from the individual (current) axis. If **Total n** is checked, values displayed will be the sum of any axes that have both fine and coarse hardware. Fine versions will be separate components. A **Fine X, Y, or Z** can be installed without a coarse equivalent, but it will still be installed as a **Fine** component

### 3 Point Plane group box

The 3-point plane is the software version of the “continuous focus hardware”. It allows you to map three points on a slide, put them in focus, remember the Z positions, then make a linear map of where the focus would be across the rest of the slide and make that the default Z plane.

**Setup:** Clicking the **Setup** button opens the following dialog:



**Set P 1, 2, 3**, buttons: Move to position 1, 2 or 3, focus, and press “**Set Pn**” button. Set positions 2 and 3 will not be enabled until position 1 has been set.

**Calculate plane:** Clicking this button lets you map the 3 points that you have defined.

**Enable** check box turns the function off and on.

**Set Bias** button: After the plane has been defined you can define an offset or bias to be added to the Z position. Focus on an object, and press the button. The routine will calculate the Z position for the current XY position, read the current Z position, and use the difference as the bias.

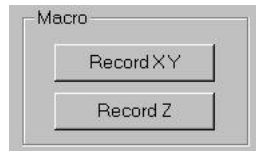
The **Set Bias** button will not be enabled until the plane has been defined.

**Reset** will clear all variables.

**OK** saves your **P** settings. It is not enabled until 3 points are defined.

**Cancel** returns you to the *Stage* tab dialog without saving your settings.

#### Macro group box

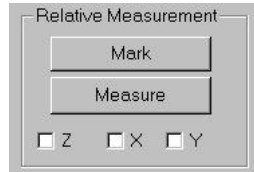


**Macro:** This group box is used to record the stage's current position in a macro so that, when the macro is played back, the stage is automatically moved to the same position. The buttons in this group box are not enabled unless a macro is being recorded.

**Record X/Y:** Click this button to record the current X/Y positions to the macro. This action causes an **IpStageXY( )** statement to be written to the macro, which, when played back, will automatically move the stage to the same position on the XY plane (assuming the XY origin is also the same during playback).

**Record Z:** Click this button to record the current Z position to the macro. This action causes an **IpStageZ( )** statement to be written to the macro, which, when played back, will automatically move the focus to this very same position (assuming the Z origin is also the same during playback).

### Relative Measurement group box



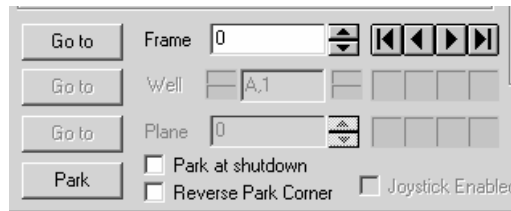
**Relative Measurement:** **Relative Measurement** is analogous to marking a position (the “reference point”) and then measuring the distance between that reference point and another position. The measurement will be displayed in the *Image-Pro Plus Output* window.

**Mark:** Click this button to mark the reference point.

**Measure:** This button measures the distance from the current stage position to the reference point.

**Z/X/Y:** Check the appropriate axis you want to measure. You can select one or more (any combination).

### Go to group box



**Go to:** Click the **Go to** button to move the stage to the position indicated in the frame or plane control.

**Frame:** This button has two functions.

1. When used with the **Go to** button, **Frame** indicates where to position the stage in the current scan area.

**Scan area** is the number of video frames in X and Y as defined on the **Scan Area** tab. This will respect all options set in those tabs. These include frames in X and Y, contiguous/non-contiguous, guard frame, horizontal/vertical, solid, and hollow. This control allows you to move through the scan area just as a *Stage-Pro* acquire would. For example for a 3x3 horizontal scan area the frame numbers would be as follows:

0	1	2
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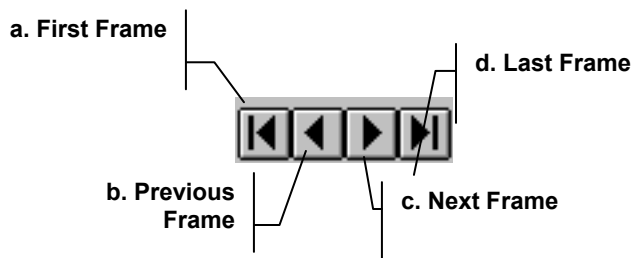


5	4	3	
6	7	8	

Frame 0 would be the currently defined origin.

2. When used with the navigation controls (below), the **Frame** button indicates the current location of the stage in the pattern. Pattern positions are numbered from 0 (zero) to  $n-1$  (one less than the number of frames ( $n$ ) in the scan area).

The picture below illustrates the **navigation controls** on the *Stage* tab page:



- a. Click this control to move the stage to the first position in the current pattern (position 0).
- b. Click this control to move to the previous position in the current pattern.
- c. Click this control to move to the next position in the current pattern.
- d. Click this control to move to the last position ( $n-1$ ) in the current pattern.

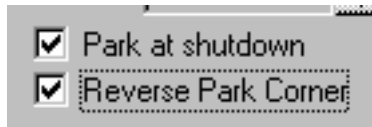
**Well (or Site):** This control will only be active if you defined an outer sample pattern and set the origin for the outer sample pattern. The **Well** control will let you move to the origin of a specific well or slide, inside the current active group, of the currently selected outer sample pattern. This position will become the current XY origin.

To go to a frame other than 0 in the well pattern, use the frame control. For more information, refer to the *Sample Pattern* tab section.

**Plane:** This control will only be active if *Stage-Pro* has a Z component installed and the **Set Z travel limits** option on the *Acquire* tab have been set. With this you can step through the defined Z planes. For more information, see the later section on the *Acquire* tab.

*Acquire: The Stage-Pro Tab Dialogs*

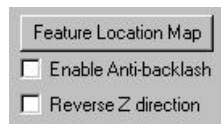
**Park/Park at Shutdown:** Click these controls to return the stage to the origin of the travel area. If **Park at Shutdown** is selected, the stage will return to this position every time you exit *Image-Pro*. Use of **Park at Shutdown** is strongly recommended if **Use current position as origin** will be used at initialization.



**Reverse Park Corner:** Checking this box indicates that you wish to change the “park” position of the stage. Usually, the stage parks at the most negative position. Checking this box will park the stage at the most positive position.

**Joystick enabled:** Click this control to re-enable your joystick if it was turned off. Note that most stages do not use this control.

**Feature Location Map group box**



**Feature Location Map:** **Feature Location Map** allows you to move the stage to a specific location by clicking on an image that has been acquired through *Stage-Pro*, and **not** through the *Image-Pro Capture* module.

When you press **Feature Location Map**, *Scope-Pro* is minimized, live preview is started, and you are presented with the following dialog:



**Go to object:** **Go to object** is the default mode. When you click on your image, the stage will move to a position where the object you clicked on will be in the center of the live preview window. This function will work with single frames, tiled images, and sequences in XY or Z. The image must have been acquired through *Stage-Pro*, and not through the *Image-Pro* capture module.

*Acquire: The Stage-Pro Tab Dialogs*

**Go to frame:** This option is most useful on a tiled image. It will move the stage to the frame position that the object you click on was captured in.

**Finished:** Press this button when you are done, and the dialog is dismissed, live preview is turned off, and you are returned to *Stage-Pro*.

**Enable Antibacklash:** This option will attempt to reduce gear lash (slop) by always moving the stage to a frame position from a positive direction. With this option turned on, moves in the positive direction will move as normal. Moves in negative direction will go past their destination, stop, and move to the destination from the positive direction.

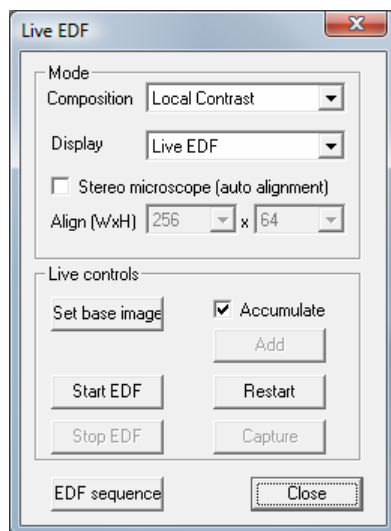
**Reverse Z direction:** For backlash correction for the Z-axis, your consistent direction must be **UP** to move to the desired position. This “swaps” or reverses the direction of the Z-axis for the up and down movements.

## Live EDF

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*Live Extended Depth of Field (EDF)* allows you to create real-time EDF images using a microscope with a manual focus control. It also supports stereo microscopes, where changing the focus position shifts the image.

To use *Live EDF*, go to the *Acquire* menu on the *Image-Pro Plus* toolbar. You will see the following dialog:



The *Mode* group contains the following options

**Composition:** The pull-down combo box contains composition options:

- **Local contrast:** Choose this option for real time EDF using the **Local Contrast** algorithm. This is most useful for high contrast brightfield images.
- **Maximum:** Choose this option for real-time EDF using the maximum composition mode for sample imaging on a dark background. For example, imaging of fluorescent samples.
- **Minimum:** Choose the minimum composition mode for transparent mode illumination of brightfield images
- **Difference:** Illustrates the difference between the base image and the current image (image with a grey background)

- **Abs Difference:** Shows the absolute difference between the base image and the current image. Background is black, but you can also activate Pseudo-color on gray images, so you that can see changing objects in color on a black background.

**Display:** This combo box lists the viewer options:

- **Live EDF:** Displays the live EDF image (when live EDF is running)
- **Dual View:** Displays a live preview and live EDF side by side
- **Live Preview:** Displays a **live** preview (EDF is running in background)
- **PIP EDF:** Displays the picture in picture - EDF in the corner
- **PIP Live:** Displays picture in picture - Live preview in the corner

**Stereo microscope:** Check this option to enable auto alignment for every acquired frame. The size of the alignment area can be selected in **Align (WxH)** controls. Note that a large alignment area may slow down live preview.

When stereo alignment is on in Live EDF, every added frame is aligned with the resulting image before calculating EDF. It allows you to create an EDF image using a stereo microscope that shifts the image in X direction while changing the focus position. The alignment is done using cross-correlation algorithm using the central area of the image. The size of the area is defined by **Width and Height** parameters. When the shifts between frames are not big and the central part of the image has enough texture, small values for **Width and Height** (default) produce good results. If the shifts are significant, the size of the alignment area should be increased (the size should be at least 2 times larger than the shift). Normally, stereo microscopes have shifts in X direction, so the width of the alignment area is bigger than the height. Increasing the size of the alignment area improves reliability of alignment but slows down the frame rate of the live preview.

The *Live controls* group contains the following options:

**Set base image:** Use this button to set the current image as the base, so it can be used with the Difference or Abs Difference composition modes to set reference image.

**Accumulate:** When the Accumulate option is on (checked), every new live image is added to the composition. This option is the preferred method for local contrast composition. In other modes, you can switch

### *Acquire:Live EDF*

this option off (unchecked) and add images to a composition manually by clicking the **Add** button.

**Add:** Use this button to add images to an image composition manually.

**Start EDF:** Click this button to start the **Live EDF** using the current camera settings. Note that the EDF will use the image selected with the Set base image button. You can reset the base image to the current image, and re-start **Live EDF** by clicking the **Restart** button.

**Restart:** Use this button to restart the **Live EDF** if you have stopped it.

**Stop EDF:** Click this button to halt the live composition. Note that it doesn't stop the live preview or reset the EDF. If you click Start EDF again, the old accumulated result will still be active. Use Restart button to start a new composition.

**Capture:** Click this button to capture the result of EDF, and stop **Live EDF**.

**EDF sequence:** This button is for test purposes only. Clicking it will create an EDF image from the active sequence. This button can be used to test various options on previously existing sequences.

**Close:** Clicking this button closes the dialog and stops live EDF.

### **Sample usage: Live EDF (Accumulate option: On)**

This mode is useful for creating EDF-ed images in real-time

1. Open Live EDF plug-in.
2. Set Options as:
  - Composition: Local Contrast for Reflective mode microscopes (or Minimum for Transparent mode microscopes)
  - Display: Dual View
  - Accumulate: Checked (on)
  - Stereo microscope: If you use a stereo microscope., check this for Auto-alignment.
3. Align (W x H): Set alignment size to define the size of the auto-alignment area in the center of the image. Depending on the maximum shift of stereo microscope the value can be set from 128 to 1024. Because shift of stereo microscopes is predominantly horizontal, Default setting of align area is 256 x 64.

### *Acquire:Live EDF*

4. Click the Preview button on the capture dialog to start the Live Preview.
5. Focus the microscope at the lowest position of the object
6. Click Stop Live Preview
7. Click Start EDF(or Restart) button to start live EDF
8. Move the microscope focus up slowly to the upper position of the object. Live image will be shown on the left side of the workspace, and the EDF image will be shown on the right side of the workspace.
9. Click **Capture** to stop and collect the EDF image.

### **Manual Image composition (Accumulate option: Off)**

This mode is useful for:

- Creating a live color composite using a color camera
- Creating a live brightfield/darkfield image composite
- Creating a live image with difference checking,
- or any other real-time image composition.

### **Creating a live color composite using a color camera**

1. Open the **Live EDF** plug-in from the *Acquire menu*.
2. Set Options as:
  - a. Composition: Maximum
  - b. Display: Dual View
  - c. Stereo micro scope: Off
  - d. Accumulate: Off
3. Click the **Preview** button on the capture dialog to start the Live Preview
4. Set the microscope and camera to get the first channel (color) of the object.
5. Click **Start EDF(or Restart)** button to start live color composite

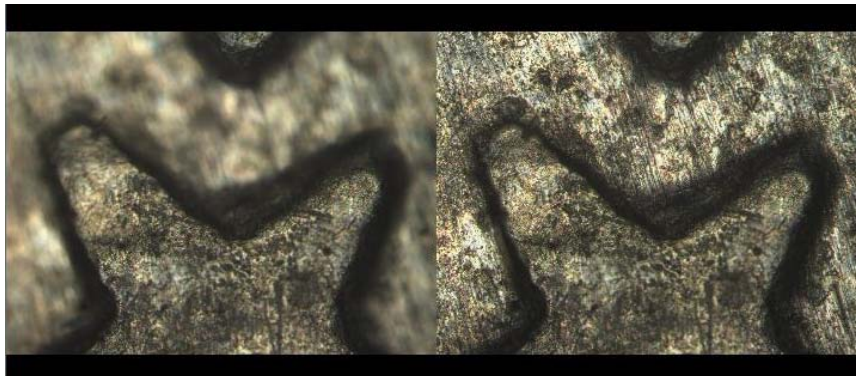
### *Acquire:Live EDF*

6. Click **Set base image** button to add first channel to the live color composite.
7. Click **Stop EDF** button to change the microscope and camera settings for next channel.
8. Click the **Preview** button on the capture dialog to start Live Preview again
9. Change the microscope and camera settings for the next channel
10. Click the **Stop EDF** button.
11. Click the **Start EDF** button
12. Click the **Add** button to add a second channel to the live color composite.

Repeat steps 4 through 10 to add additional channels

13. Click the **Capture** button to stop and to get the composite image.

A sample dual-viewed, real-time EDF composite image is shown here:



### **Additional Notes:**

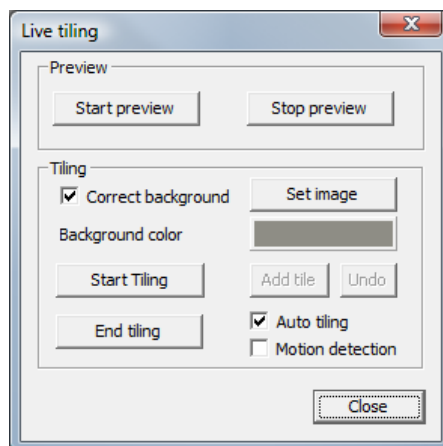
The success of both **Live EDF** and **Live Tiling** operations depend on optimizing the capture. Anything you can do to improve the preview frame rate in the workspace prior to starting the live operations will help. This includes using a faster camera, and setting the exposure time, binning, and ROI to increase the frame rate. Once the capture parameters are set, do not adjust them during the **Live EDF** or **Live Tiling** operations.



## Live Tiling

---

*Live Tiling* enables you to create of real-time large-area tiled images using microscopes with manually controlled stages. When you select *Live Tiling* from the *Image-Pro Plus Acquire menu*, you will see the following dialog:



The *Preview* area contains the controls to start and stop the live image preview in the workspace.

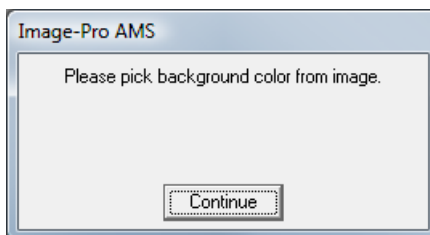
**Start preview:** Click this button to start the workspace preview

**Stop preview:** Click this button to stop the workspace preview.

The *Tiling* group contains the options for auto-tiling:

**Correct Background:** Use this option if the microscope has uneven illumination. Use an empty area on the slide as the background, move it under microscope, and click the Set image button.

**Background color:** Use this option for empty areas outside the tiles on output image. Clicking the color button will activate the color picking mode. The color picking dialog is shown here:



### *Acquire:Live Tiling*

Clicking on the image area with the desired color will set the selected color as the image background.

Move your microscope to the area of the specimen to tile, and click the Start tiling button. When you click Start Tiling, the current image will become the starting point of the tiled image. Yellow rectangles will show you the outline of the original frame when you move objects under microscope.

The tiling uses cross-correlation to find image shifts. Use objects with texture for tiling. Note that empty fields cannot be aligned correctly automatically. When you see that the yellow frame follows the image correctly, you can start tiling. Note that the maximum image shift between slices cannot exceed 25-40%.

The **Auto-tiling** option is the easiest way to perform the tiling process. With auto-tiling, the images will be added to the output automatically when the image shift exceeds 25% (when internal yellow rectangle touches image border). Move the stage slowly, and the images will be acquired and tiled automatically.

Motion blur is a common problem when capturing images of moving objects. This problem can be eliminated by activating the Motion detection option. Activating this option is recommended for long exposure times. When this option is active, a new tile in auto-tiling mode will be added only when the camera is still.

Use the following procedure to tile images in automatic mode:

1. To start tiling activate **Auto-tiling** and **Motion detection**
2. Move object by 30-35% from the original position (when the inner rectangle will touch image border) and stop the stage. The image will be automatically captured when the object will stay still for 2 frames. It will eliminate motion blur and improve the quality of tiled image.
3. Repeat step 2)(move-stop) until whole area is tiled.

The tiles can be also added manually using the **Add tile** button. If the tiled position is not correct, use the **Undo** button to remove the last tile and try another location. Note that if you use **Auto-tiling** you may need to switch this option off. Click **Undo**, then move the microscope to a location when correlation is stable, and activate the **Auto-tiling** option again.

Sometimes the focus knob needs adjustment during auto-tiling. By default the images are tiled with blending (which is appropriate for most cases). When you adjust the focus and add tiles again, the new

### *Acquire:Live Tiling*

image will be blended with old, de-focused image. To disable blending, hold down the <Ctrl> key while clicking the **Add tile** button. The new tile will overwrite the old, de-focused image (but in this case the tile edges might be visible).

When the tiling is done, click the **End tiling** button. It will crop the output and any blank areas will be filled by the background color.

#### **Additional Notes:**

**Recovery Instructions:** If the feature that you are trying to tile gets “lost”, The following procedure will help you to recover.

- Move the stage to the previous tile position (indicated by a rectangle on the output image).
- When the correlation between the live view and the search pattern on the output image is established (rectangles on the live image turn yellow and correctly follow stage movements), continue tiling while adding tiles manually or activating the Auto tiling option.

**Warning Message:** If you try to adjust your capture settings during the Live EDF or Live Tiling operation, you will see the following message:

- Please do not adjust the capture parameters during Live EDF or Tiling. If you need to change some capture options, stop any live operations, adjust the parameters, and then start a new live session.

#### **Sample Usage: Auto-tiling**

1. Click **Start preview**.
2. If you want to perform background correction, follow the steps below:
  - a. Check **Correct background**.
  - b. Move the stage to get background image.
  - c. Click **Set image**.
3. Move the stage to the start point of the object to be tiled.
4. Click the **color picker** button right next to the **Background color**.

### *Acquire:Live Tiling*

5. Move the cursor to the point of the selected color. Any blank areas will be filled with this color on the live preview image.
6. Click **Start tiling**.
7. Check **Auto-tiling**.
8. Move the object under microscope slowly, and the images should be tiled automatically as you move.
9. When the tiling is done, click the **End tiling** button. It will crop the output, and any blank area will be filled by the color you selected earlier.

#### *Note that:*

- a. You don't have to specify the output size and/or the starting position. The output image will be automatically expanded to include any new tiles, so you can start tiling from any point of the sample, and expand it to any direction (though the image must still have some texture).
- b. When you click **Start Tiling**, the current image becomes the starting point of the tiled image. Yellow rectangles will show you the outline of the original frame when you move objects under the microscope. The tiling uses cross-correlation to find the image shift. Use objects with texture for tiling. Also, note that empty fields cannot be aligned correctly.

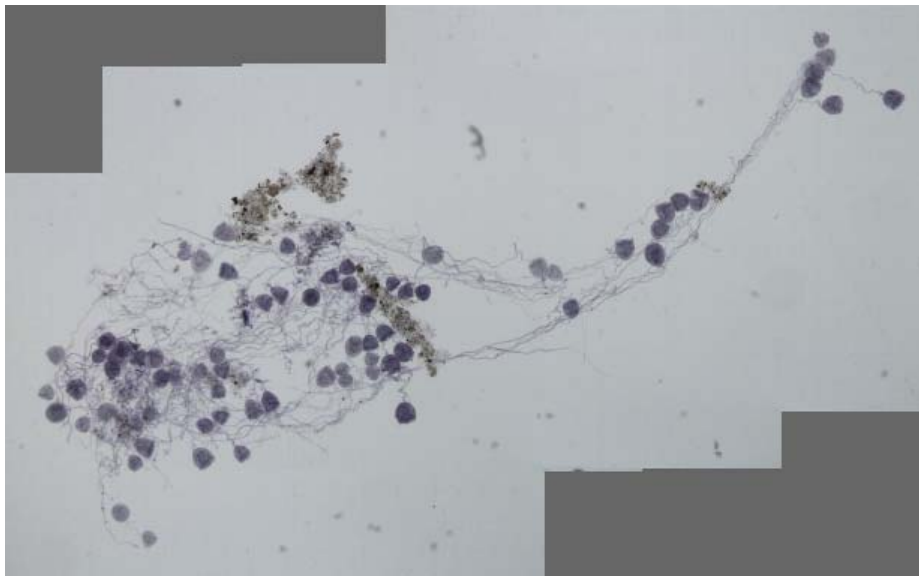
### **Sample Usage: Manual Tiling**

1. Click Start preview.
2. If you want to perform background correction, follow the steps below:
  - a. Check Correct background.
  - b. Move the stage to get background image.
  - c. Click Set image.
3. Move the stage to the start point of the object to be tiled.
4. Click the color picker button right next to the Background color.
5. Move the cursor to the point of the selected color. Any blank areas will be filled with this color on the live preview image.
6. Click **Start tiling**.

*Acquire:Live Tiling*

7. Move object under microscope slowly until the inner rectangle touches the image border and click “**Add tile**”.
8. Repeat step 7 until the whole image is tiled.
9. When the tiling is done, click the **End tiling** button. It will crop the output, and any blank area will be filled by the color you selected earlier..

**Sample tiled images:**



*Acquire:Live Tiling*

## **The Advanced Menu**

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The *Advanced* menu is where you'll find the *3D Surface Inspector*, *Advanced Acquisition (AFA)*, *SharpStack*, and *3D Constructor* features of *Image-Pro Plus 7.0*.

## Advanced Acquisition/AFA

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This module includes *Scope-Pro* as well as the advanced acquisition functions. It enables you to acquire many different types of image stacks or sequences.

### The AFA Window

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To start the *AFA* module and begin working with it, you must open *Image-Pro Plus* **first** and then the main *AFA* window.

Follow the steps below:

1. Turn on your computer.
2. Click **Start:Programs: Image Pro Plus 7.0:Image Pro Plus**. This will bring you to the *Image-Pro Plus* window.

---

**Note:** In order to use *AFA* to its fullest potential, *Scope-Pro* and *Capture* settings should be established **prior** to using *AFA*. Otherwise, the current default settings in *Scope-Pro* and *Capture* will be used.

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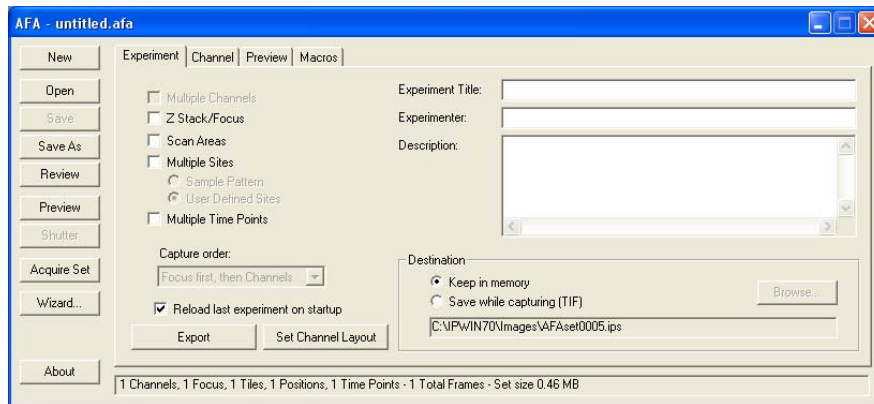
3. Start the *AFA* module by selecting the *AFA* option from the Advanced menu on *Image-Pro*'s menu bar.  
*Scope-Pro* and *Stage-Pro* will initialize if they have not been run already.
4. The following picture shows the *Image-Pro Plus* window and the *AFA* dialog box.



## The AFA Dialog Box

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When you start *AFA* for the *first* time, only some of the tabs are visible.



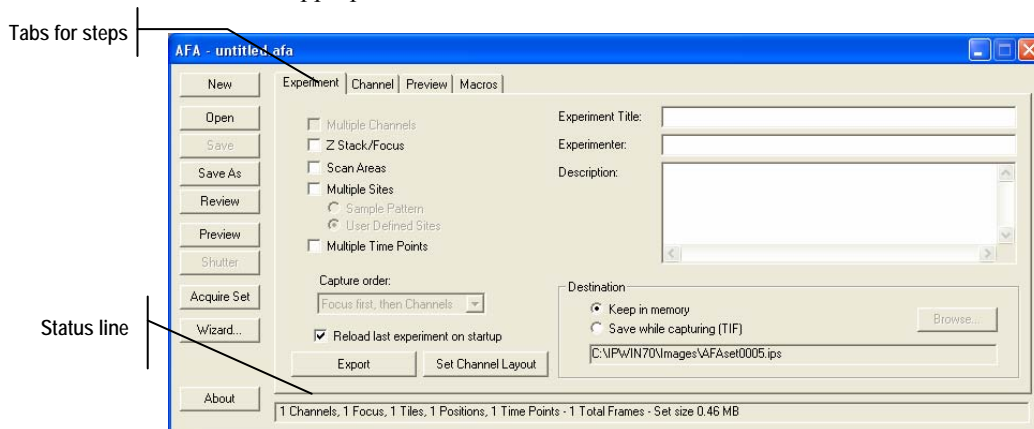
Additional tabs will appear once an *AFA* settings file has either been created (**New** button) or by loading (**Open** button) an existing *AFA* settings (\*.**AFA**) file, or by checking the various options on the *Experiment* tab.

## The AFA Tab Dialogs

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AFA's tab dialog structure looks like index cards arranged in a Rolodex® or card file. The names of the main steps of available procedures are printed on the tabs at the top of the screen, as shown in the next picture:

Clicking on any tab displays a page of functions and options for that step. You can easily switch back and forth between pages by clicking on the appropriate tab.



AFA is designed to make the imaging process easy to understand and use. The individual tabs are arranged in a logical progression from left to right, according to the steps used in a laboratory.

The names of tabs that are currently not available will not appear.

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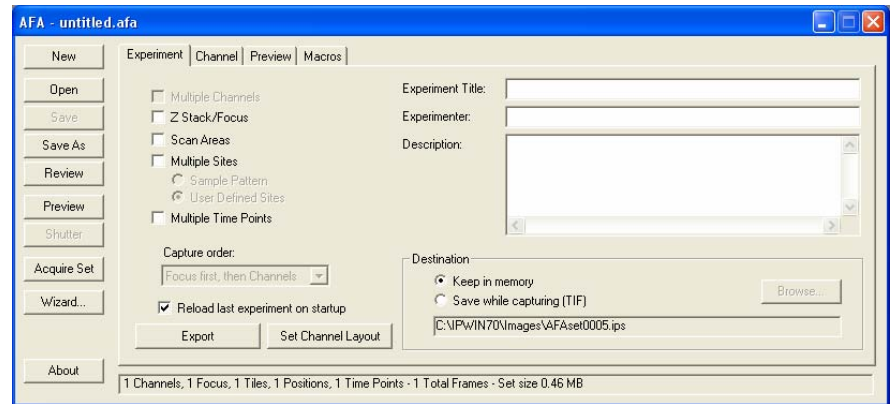
**Note:** AFA provides a status line indicating how many channels, focus positions, stage locations, tiles, and time points will be captured in a single acquisition. This will have an impact on the acquisition time and storage space required.

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## Description of the AFA Tab Dialog Boxes

The following shows each of the seven *AFA* tab dialog boxes, with a brief description of its functions.

### *Experiment tab dialog*



- Name and describe your experiment
- Choose your acquisition selections
- Determine whether to change Z position or channel (faster or more frequently)
- Name your channels and put them into the desired order
- Determine the destination of the captured images.

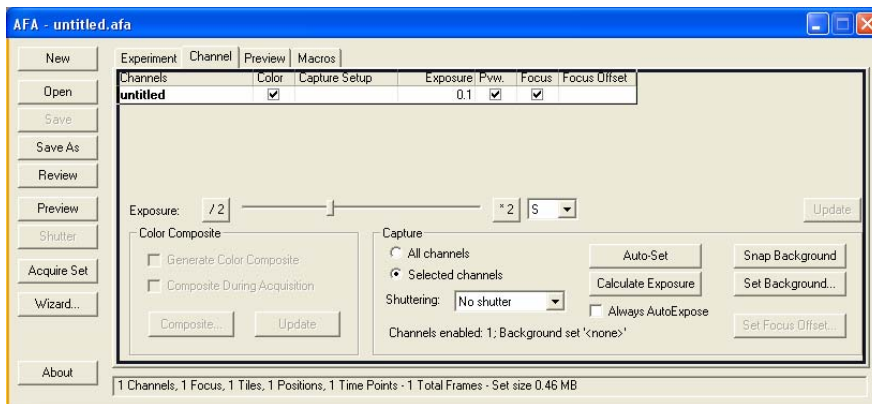
---

**Note:** AFA will estimate the captured set size and number of frames in order to help you decide if keeping the images in is memory dangerous or problematic. Saving your images to disk will delay the process slightly. However writing the images to disk as they are acquired has more predictable timing than using virtual memory to maintain them. Saving images to disk as they are captured minimizes data loss in the event of a power outage or other catastrophic event.

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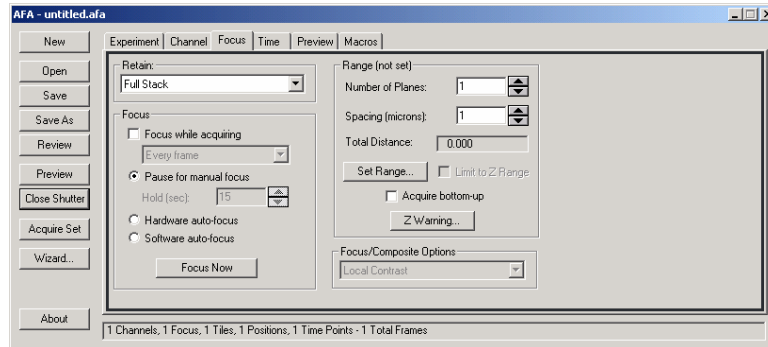
## Advanced Menu: The AFA Tab Dialogs

### Channel tab dialog



- Change detailed information about the different channels, including microscope and camera settings.
- Select or create microscope and camera settings
- Enter microscope characteristics for future reference
- Create color composites and select the color composite hue
- Choose how images will be acquired and add associated data (like wavelength, NA, etc.) that can be used in later processing such as deconvolution.
- Set the exposure times for the different channels
- Control the shutters
- View and adjust all exposure settings
- Select some or all of the channels for capture
- Enable/Disable auto-exposure
- Acquire and set the background for the images

**Focus tab dialog**



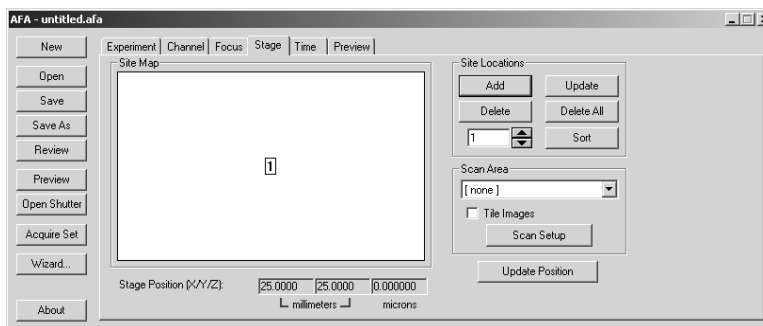
- Select the number of planes and type of image to acquire
- Select the focus settings
- Focus or autofocus the current sample
- Enable/disable autofocus.

## Advanced Menu: The AFA Tab Dialogs

### Stage tab dialog

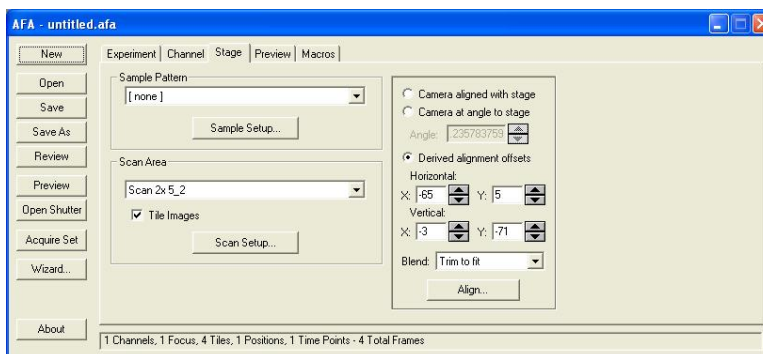
There are two possible forms for the **Stage** tab, depending on whether you have selected **Sample Pattern** or **User-Defined Positions** on the **Experiment tab**.

This version of the **Stage** tab appears if **User-Defined Position** was chosen in the **Experiment tab**.

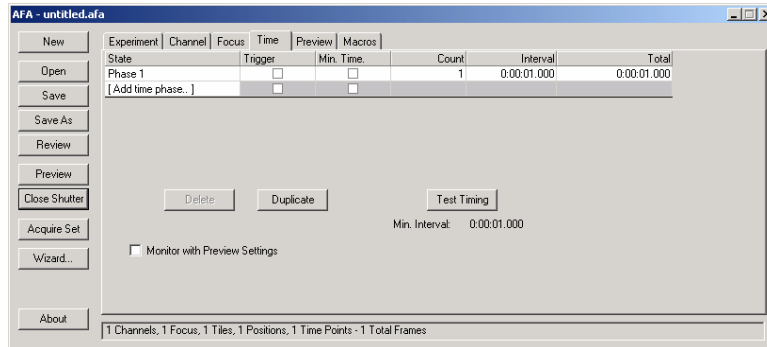


- Determine scanning /tiling area for wells or user-defined positions
- Control the X/Y/Z positions where images are acquired.

If you have chosen the **Sample Pattern** on the **Experiment tab**, the **Stage** tab will look like this:

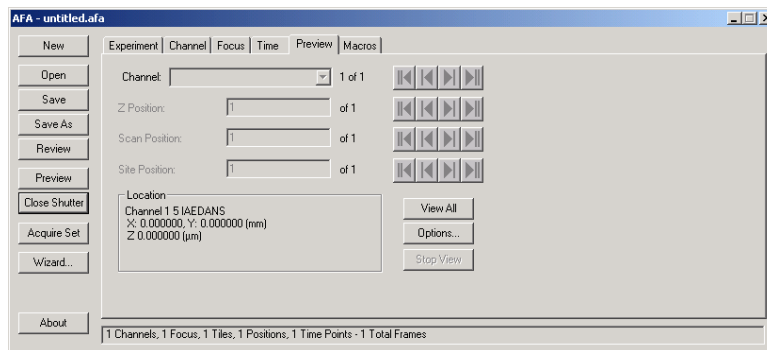


**Time tab**



- Set number and length of time points for time lapse acquisition
- Test how long an acquisition will take.

**Preview tab**



- Control the position of each dimension used for live preview
- Examine where you will be capturing

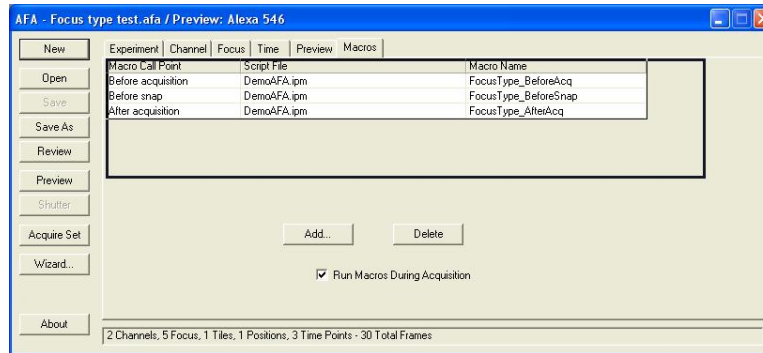
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**Note:** The *Preview* tab is disabled when the stage is moving.

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## Advanced Menu: The AFA Tab Dialogs

### Macros tab

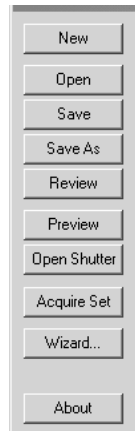


The new **Macros** tab aims to simplify the process of customizing AFA by giving you, the user, an entry point for adding a simple (often recordable) macro to the AFA loop at a defined point. This will eliminate the need for you to work through the multiple loops in Macro\_AFA, and figure out how to add your special piece of code.



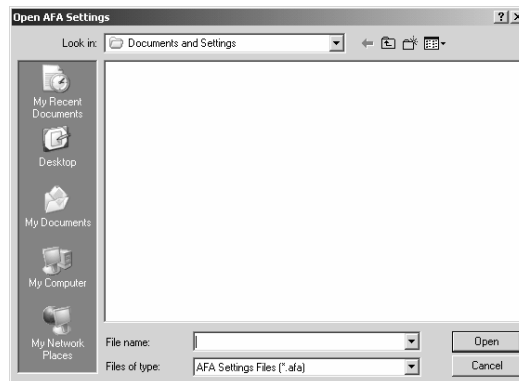
## AFA's Buttons and Minimal Dialog

These buttons are found left side of the *AFA* tab dialog box.



**New:** Use the **New** button to create a new settings (**.AFA**) file for *AFA*. The settings file stores all the parameters for the capture set that you will define on the various tabs. You **must** define the parameters in the other tab dialogs before a new settings file is functional in *AFA*.

**Open:** Click the **Open** button to load a settings file that you have previously saved. You will see the Open AFA Settings dialog box:



- **Look in:** Select the folder from which you want to select your settings file.
- **File name:** Select the name of the settings file (**.AFA**) to open. You can either type the name of the file (with its entire path, if it is

### Advanced Menu: The AFA Tab Dialogs

not in the current folder), or use the **Look in** and **Files of type** selections to obtain a list of file names from which you can select.

- **Files of type:** Select the format of the file you want to open. *AFA* lists the **.AFA** format for settings files as the default.

**Save:** Use the **Save** button to immediately store the changes made to the current settings file (**.AFA**) and to save the file to disk. This includes all the settings that you have defined on the different *AFA* tab dialogs.

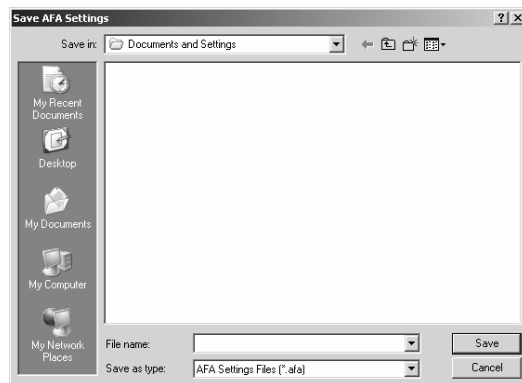
If you have loaded an *AFA* settings file or used *Save As* to save the existing settings to a file and if any setting from the *AFA* dialog tabs changes, the **Save** button becomes active. When you change a settings file (**.AFA**) or wish to switch or create another settings file, *AFA* prompts you to save the changes. The same action occurs when you exit *Image-Pro Plus* as well.

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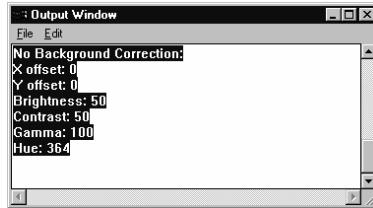
**Note:** *AFA* will estimate the captured set size and number of frames in order to help you decide if keeping the images in memory dangerous or problematic. Saving your images to disk will delay the process slightly. However writing the images to disk as they are acquired has more predictable timing than using virtual memory to maintain them. Saving images to disk as they are captured minimizes data loss in the event of a power outage or other catastrophic event.

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**Save As:** Click the **Save As** button to store the settings file to a new filename and/or directory.



**Review:** Click the **Review** button to see a text listing of all the **.AFA** settings in the current file for review or notes. You will see the **Output Window** displaying the current settings. You may also find that copying or printing this information is a useful way of documenting your experiments.



Refer to your *Image-Pro Plus Reference Guide* for more information on the **Output Window**.

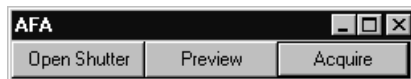
**Preview:** Click this button to start/stop the live preview, with the appropriate capture settings. *AFA*'s **Preview** is similar to the *Capture Preview* function (under the *Acquire* menu), with the additional functionality of setting the microscope configuration. Note that clicking the **Preview** button will move the microscope stage to the position shown on the *Preview* tab.

**Open/Close Shutter.** Click this button to open or close the shutter on the currently-configured camera or microscope.

**Acquire Set:** Click this button to start the advanced image acquisition process.

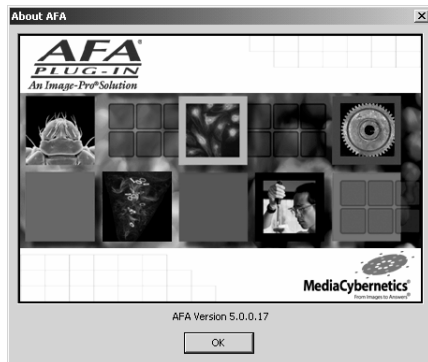
**Wizard:** Click this button to launch the *AFA Setup Wizard*, described earlier in this chapter.

**Minimize:** Use the Minimize button (in the upper right corner of the *AFA* dialog) to show the minimized version (reduced form) of the *AFA* dialog box.



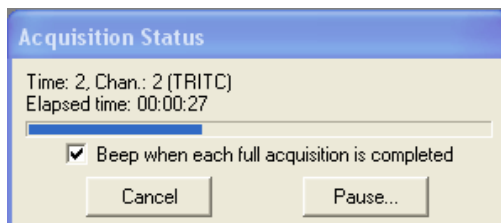
Advanced Menu: The AFA Tab Dialogs

- **About:** Click this button to see the AFA version information.



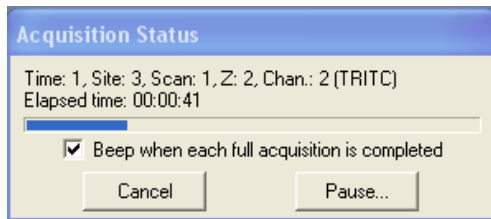
### The Acquisition Status Dialog

The **Acquisition Status** includes the name of the current channel, the current status of all active dimensions, and the elapsed time from the beginning of acquisition:



*Acquisition Status Dialog*

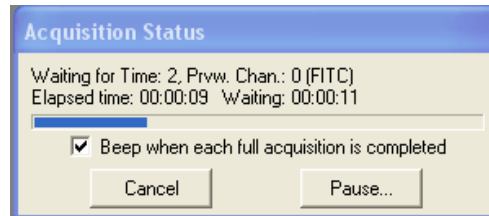
The **Acquisition Status** dialog indicates only the currently selected dimensions, so the illustration above represents the appearance for a multi-channel time lapse acquisition. Since there is always at least one channel, the channel information is shown regardless of whether the multiple channels option is selected on the **Experiment** page. When all dimensions are selected, the status display looks something like this:



*Acquisition Status dialog with all dimensions selected*

*Advanced Menu: The AFA Tab Dialogs*

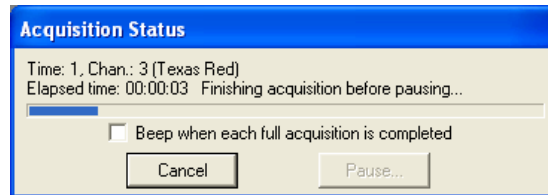
- The elapsed time indicator will update the elapsed time from the beginning of acquisition, and will update at least once a second.
- Between acquisitions, the status dialog is in “wait mode”:



*Acquisition Status dialog (waiting)*

When the **Beep when each full acquisition is completed** option is selected, *AFA* will beep at the end of a complete “acquisition set”, e.g. at the end of each time point.

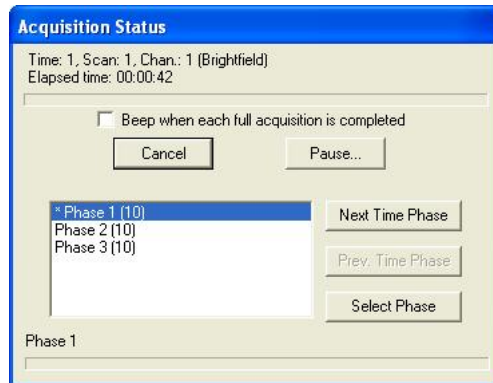
The **Pause...** button will pause the acquisition when the current “acquisition set” is completed – before the next **Time Point** (if **Multiple Time Points/Time Lapse** is selected), next **Site** (if not **Time Lapse** but **Multiple Sites** is selected, next **Scan (X/Y)** position (if not **Time Lapse** or **Multiple Sites**, but **XY Scan Areas** is selected), or next **Channel** or **Focus** (depending on the setting of **Channel** first, then **Focus**; or **Focus** first and then **Channel**). This is most important for time lapse acquisitions, where it assures that a complete “acquisition set” is acquired at each time point. The **Acquisition Status** dialog will be updated to indicate that the **Pause** request has been queued and the **Pause...** button is disabled:



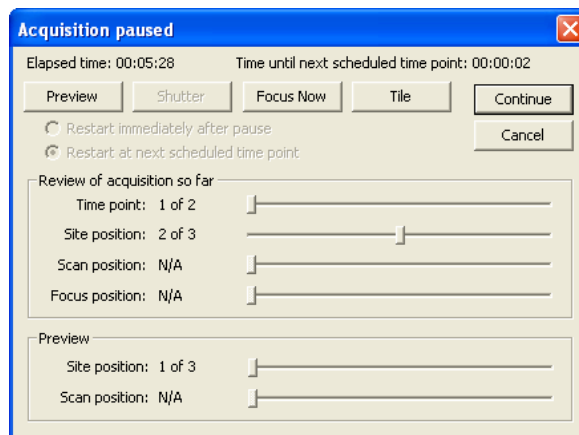
*Acquisition status while finishing acquisition set*

If you have entered a series of time phases, the Acquisition Status dialog displays each phase:

Advanced Menu: The AFA Tab Dialogs



When the current acquisition set is completed, the **Acquisition Paused** dialog is displayed:



Acquisition paused dialog

The **Elapsed time** field indicates the duration of the experiment so far. The **Time until next scheduled time point** field is a count-down timer to the next scheduled time point. As each scheduled time point passes, the count-down timer is reset to indicate the time to the next time point.

The **Preview** and **Shutter** buttons operate exactly as do their counterparts on the main *AFA* dialog. The **Focus Now** button operates exactly as does its counterpart on the *Focus* page of the *AFA* dialog, with the focus taken using the **Focus Channel** at the current preview position of all other dimensions. The **Z reference** location for that position will be updated for all future acquisition sets. The **Tile** button will use the *Image-Pro* function to tile the open workspaces so that all are visible.

The **Review of acquisition so far** controls are used to review the set of images acquired so far. (You will not see this group box if you are saving your images directly to disk.) Each of the sliders will navigate along the indicated dimension, in much the same way as the controls on the **Set Navigator** dialog. Dimensions may not yet be used, either because the experiment is not configured to acquire along that dimension, or because the acquisition was paused after acquiring only one time point or position. Unused dimensions will have the currently displayed position indicated as “N/A” (as with **Scan position** in the illustration), and the navigation slider will be disabled. Navigating along any dimension will automatically tile the workspaces in the same manner as the **Tile** button. This group of controls is *not* present if you have selected to **Save while Acquiring** on the *Experiment* page.

The **Preview** controls are used to select the location to view during the acquisition preview. The **Scan Position** and **Site Position** controls will operate exactly as do their counterparts on the *Preview* page of the *AFA* dialog. Note that there is no need for the **Channel** and **Focus** controls, as the current focus position of the focus channel will always be the location previewed during a pause.

The **Restart immediately after pause** and **Restart at the next schedule time point** controls determine how the acquisition is restarted. Since they are only significant when the acquisition includes multiple time points at scheduled times (e.g. **Use minimum possible time** is not selected), the **Restart immediately after pause** option will be selected and both controls disabled when this is not the case. For time lapse acquisitions, there are two choices as to when the acquisition restarts.

As an example, consider an acquisition with time points 30 minutes apart. The first time point is acquired at 0:00, the second at 0:30, and then you pause the acquisition. If the experiment is resumed prior to the next scheduled time point at 1:00, the experiment can continue as originally designed, so these options are disabled (with **Restart at the next schedule time point** selected). Once the next scheduled time point passes, the controls are enabled so that you can choose to resume immediately, or wait until the next scheduled time point. If the example acquisition is restarted at 1:10 with **Restart immediately** selected, the next acquisition will be immediately, at 1:10, and the time points after that rescheduled using the original time period, e.g. 1:40, 2:10, etc. If **Restart at the next scheduled time point** is selected, the resumed acquisition will wait until the next time point, or 1:30, and the future time points rescheduled to 2:00, 2:30, etc.

*Advanced Menu: The AFA Tab Dialogs*

**Continue** will continue the acquisition. Since the pause is deferred until completion of the “outermost” dimension position that is in progress, the next **Time point** (or **Site** position or **Scan** position) will be acquired in its entirety after the restart, using any focus positions that have been updated. **Cancel** will stop the acquisition permanently, in exactly the same manner as the **Cancel** button on the progress dialog.



## The Experiment Tab

In order to use *AFA* to its fullest potential, *Scope-Pro* and *Capture* settings should be established prior to using *AFA*. Otherwise, the current default settings in *Scope-Pro* and *Capture* will be used. (Refer to the *Scope-Pro User Guide* and your capture documentation for more details regarding settings and configuration information.)

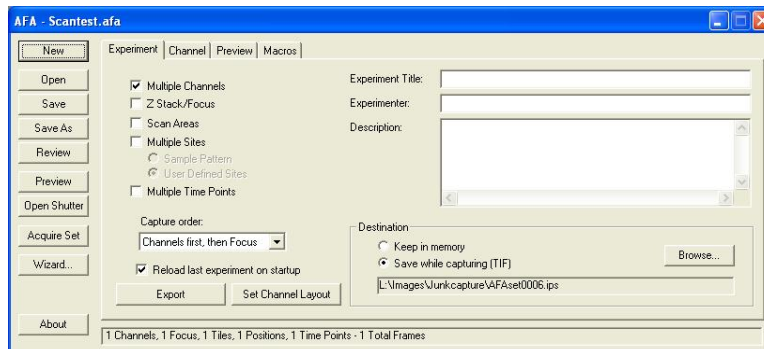
---

**Note:** When using *AFA* for the *first* time, a new settings file (\*.**AFA**) must be defined and optionally saved. Otherwise, you will not be able to access *AFA*'s tab dialogs. (Refer to *Chapter 2 - Using AFA* for information about creating a new settings file.)

---

## Experiment Tab Selections

The *Experiment tab* is the first dialog tab that you see the first time you select the *Advanced Acquisition* item from the *Advanced* drop-down menu on *Image-Pro Plus*'s menu bar. It replaces the *Acquire* tab in previous versions of *AFA*.



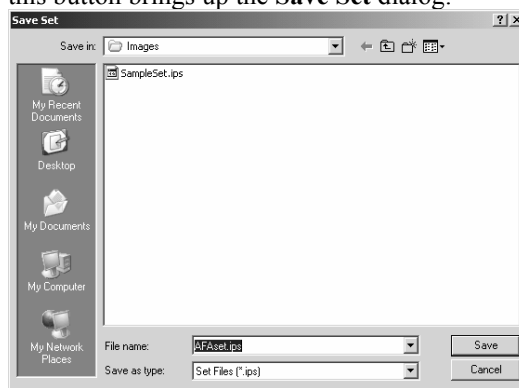
Only some of *AFA*'s tabs are visible the first time you select the *Advanced Acquisition* menu item. The others become visible as you select your *AFA* settings.

### Advanced Menu: The Experiment Tab

- **Multiple Channels.** Check this box to enable multiple channel acquisition. The **Channel** tab is always enabled, but AFA will only support defining a single channel if this is not selected.
- **Camera beamsplitter.** Check this box if you are using the *Optical Insights* beamsplitter. Checking this box activates the **Options** button.
  - **Options.** Clicking this button allows you to specify if the beamsplitter is a vertical two, a horizontal two, or a four-image splitting configuration. Your beamsplitter selections will be saved from AFA session to session.
- **Z Stack/Focus:** Check this box to enable multiple focus acquisition. You will see the **Focus** tab appear.
- **Scan Area.** Check this box to enable the **Stage** tab and define a scan pattern at selected wells or user-defined positions.
- **Multiple Samples:** Check this box to enable multi-well or multi-point scanning on the **Stage** tab. This moves the stage in X and Y positions.
  - **Sample Pattern:** Click this button if you want to scan multiple positions in a regular array, such as the wells of the multi-well plate, or multiple slides in a multi-slide stage table.
  - **User-Defined Positions:** Click this button if you are going to define the scan points individually on the **Stage** tab dialog.
- **Time Lapse:** Check this box to enable the **Time** tab. You will be able to repeat your acquisition selections at multiple time points.
- **Capture Order:** Use this list to select the capture order, which can be channels first and then focus, or focus first and then channels. Some systems (particularly those with manual filter sliders) will produce better results if an entire Z stack is captured for each channel before moving to the next channel. Others with good automated filter changers will produce better results if the channels are captured at each Z position before moving to the next, to keep them registered.
- **Reload last experiment on startup:** Check this box to reload the last-used experiment. The “last-used experiment” is only updated when you shut down *Image-Pro Plus*, and only when the AFA experiment has been loaded from a file or saved to a file. This ensures that experimental AFA settings that are not saved do not corrupt the last legitimate experiment.

*Advanced Menu: The Experiment Tab*

- **Destination:** Acquire the images, and keep them in memory, or write each image out to disk as it is acquired. If you have chosen to save while capturing, the text will display the set name used for image output, and the set will be re-saved after each image is added to it.
- The **Browse** button is used to indicate where your image sets will be saved and the name of the image set. The names of the individual images will be derived from the set name and all the set images will be saved in the same folder as the image set. Clicking this button brings up the **Save Set** dialog:



Please refer to your *Image-Pro Plus Reference Guide* for information about saving image sets.

- **Export:** The **Export** feature supports archiving and saving your AFA settings and acquired sets. Click this button to open the **Export** settings dialog.



- **Archive All...:** Clicking this button will archive the set and experiment information to the current folder of the current database. This button will be disabled if the experiment has

### Advanced Menu: The Experiment Tab

not been acquired and there is therefore no image set associated with the experiment..

- **Save as Text...:** Clicking this button will invoke a standard **Save As** dialog that allows you to save the experiment information to a text file. Currently, experiment information is limited to the *Experiment Title*, *Experimenter*, and the *Description*.
- **Copy to Clipboard:** Clicking this button will put the experiment information on the clipboard, where it will be available to be pasted into any text or document editor.
- **Send to Output...:** Clicking this button will send the experiment information to the **Output Window**, and show the window if it has not already been shown.
- **Send to Excel:** Clicking this button will send the experiment information to an Excel spreadsheet.
- **Done:** This dialog has a **Done** button instead of OK and Cancel. Click the **Done** button when you are finished exporting.
- **Set Channel Layout:** Clicking this button uses the current set from an AFA capture to record window positions, size, zoom, and scroll. When a new acquisition is taken after **Set Channel Layout**, the images will be arranged in that fashion. You should assign a useful name to each channel, but the channel names are not saved in the AFA settings file.

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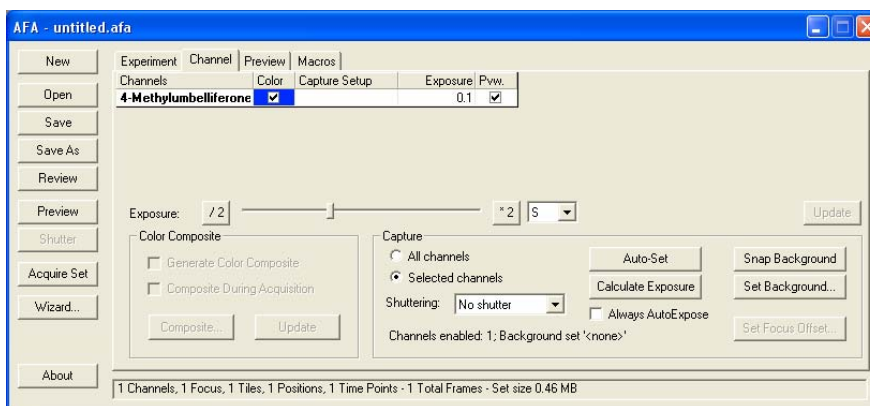
**Note:** You may find it easier to manage your *AFA* files if you store each set in its own subdirectory of your data directory.

---

Once the *Experiment tab* has been filled out, the other tabs become available. The next tab in the sequence of events is the *Channel* tab.

## The Channel Tab

**Channel tab** allows you to configure individual channels in the capture set by adjusting microscope, camera, background correction, and display settings for each channel. Clicking the **Channel** tab displays the following dialog:



The AFA **Channel** page has been revised in version 7.0 to enable you to create composite images during the acquisition process.

The main access to the channel information is from the channel grid. The width of the columns of the channel grid can be adjusted. A new channel is added when you click anywhere in the channel name column. An existing channel can be deleted by pressing the **Delete** key while the channel is selected. Right-clicking on the grid invokes a context menu that allows you to add a new channel or to delete the selected channel. If you add 6 or more channels, the grid will automatically add a vertical scroll bar. The controls are described below:

**Shutter Interval:** You can control a shutter through AFA using the options from this drop-down list:

- **Ignore:** Don't control shutter at all
- **All Moves:** Current behavior, close shutter at all transitions
- **Channels:** Leave open while changing channels

*Advanced Menu: The Channel Tab*

- **Z-Stack:** Leave open for an entire Z-stack
  - **Stage:** Open for entire time at a particular stage location
  - **Time Point:** Open for the time point, close while waiting for the next one
  - **Experiment:** Open when starting, close when ending
- **Channel name:** The channel name is established when you add a new channel by clicking anywhere in the channel name column. Initially the new channel will have an “untitled” dye and will have the current capture settings file assigned. A tool tip appears when you move your cursor over the channel name, displaying the characteristics of the selected dye. You should assign a useful name to each channel, but the channel names are not saved in the AFA settings file. Click on the **Channel** column to display a drop-down list of the currently defined dyes, so that you may select one. The **Browse** item in the list will display a **File:Open** dialog, so that you may select a dye for the current channel. The **Delete channel** item in the drop-down list allows you to remove this dye/channel from the list.

**Add/Edit dye,** will invoke the **Edit Dye List** dialog to select an existing dye or add a new one. A tool tip over the selection will display the characteristics of the selected dye, e.g.

FITC Em: 518 Ex: 496 Color: 0, 255, 0.

To confirm the selection of a dye, press <Enter> or click elsewhere on the grid.

---

**Note:** If a microscope setup with the identical name as the selected dye is found, that setup will also be selected.

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**TIP:** If you use related names for the dye file, *Scope-Pro* settings file, and *Capture* settings file, *AFA* will automatically select the *Scope-Pro* and *Capture* settings files that match the dyes that you select.

For example, if you regularly use FITC, there is an FITC.ipd dye file. By naming the *Scope-Pro* settings FITC.scp and the *Capture* settings FITC.vpf, you permit *AFA* to select the matching files automatically when you choose FITC as one of your channels.

- **Color:** The color patch displays the color of the selected dye, and also controls whether the channel is enabled for capture. The checkbox can be cleared to disable a channel for capture. Double-clicking the color patch (outside the checkbox) will invoke the **Edit Dye** dialog. Note that the dye cannot be changed from this dialog, as it can from the **Edit Dye List** dialog. You will only be able to fine-tune the displayed color of the currently selected dye.
- **Microscope Setup:** The microscope setup file (*Scope-Pro* settings file) can be selected from a drop-down list. The **Browse** item in the list will display a **File:Open** dialog, so that you may select a microscope settings file. The **Add/Edit microscope setup** item will invoke the *Scope-Pro Scope* page. When AFA is activated upon completion of setting up the microscope settings, the *Scope-Pro* dialog will be closed automatically.
- **Capture Setup:** A capture setup file (capture VPF file) can be selected from a drop-down list. The **Browse** item in the list will display a **File:Open** dialog, so that you may select a capture settings file. The **Add/Edit capture setup** item will invoke the currently selected **Capture** dialog. When AFA is activated upon completion of setting up the capture settings, the **Capture** dialog will be closed automatically. A warning will be displayed if the exposure time is changed, since exposure times in **Capture** are ignored by AFA.

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**Tip:** AFA configures *Scope-Pro* and *Capture* for each channel by reloading the specified settings file from disk each time that channel is visited. Any changes that you have made in either of these dialogs after saving the specified settings file will be permanently lost the next time AFA visits a channel. To avoid losing important changes to *Scope-Pro* or *Capture* settings, please keep this behavior in mind and remember to save your changes before returning to the AFA dialog.

**Note:** You will NOT be warned that changes will be lost when AFA loads a new settings file for *Scope-Pro* or *Capture*. Nor will you be prompted to save your changes

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- **Exposure:** The exposure time for the channel can be set in this column. You can double-click and edit the value directly (in seconds, e.g. 0.100 is 100 milliseconds, and 0.0001 is 100 microseconds). This column and the next 2 are moved from the *Exposure* page to this *Channel* page. The **Exposure** column will be hidden if the current capture device does not support exposure times. Note that microsecond exposure times are

### Advanced Menu: The Channel Tab

supported by this column and the other exposure controls if the current capture device supports it.

The / 2 and \* 2 buttons and exposure slider are added to provide a way to adjust the exposure time in addition to directly editing the value in the **Exposure** column. The controls will be disabled if the current capture device does not support exposure times. The slider adjusts the exposure time logarithmically, so that halving or doubling the exposure time moves the slider the same distance regardless of the current exposure time. The / 2 button will be disabled if ½ the current exposure time is less than the current capture device's minimum exposure time. The \* 2 button will be disabled if the twice the current exposure time is more than the current capture device's maximum exposure time.

- **Accumulate:** The number of frames to accumulate. The **Accumulate** column will be hidden if software integration is not selected in the current capture settings.
- **Divide:** The divisor once the specified number of frames have been accumulated. The **Divide** column will be hidden if software integration is not selected in the current capture settings.
- **Preview Channel:** This column indicates the channel currently being previewed. Only one channel can be selected.

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**Note:** Clicking anywhere on a channel row selects that channel as the preview channel to facilitate making adjustments to the channel. The current preview channel is indicated both by the check mark, and by the channel/dye name being displayed in **bold**.

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- **Focus Channel:** This column determines the channel used to focus. Only one channel can be selected.
- **Focus Offset:** This column determines each channel's offset from the focus position determined for/by the **Focus Channel**. The default value is 0.0, which indicates that there is no fixed offset in focus positions from one channel to another. This value can be double-clicked for editing on any channel other than the currently selected **Focus Channel**. See also the **Focus Offset** button (see below).
- **Apply objective information to all channels :** The **Apply objective information to all channels** control is a convenience to set all existing channels, and any channels added while the control is selected, to use the objective specified for the first channel. This option is selected by default.

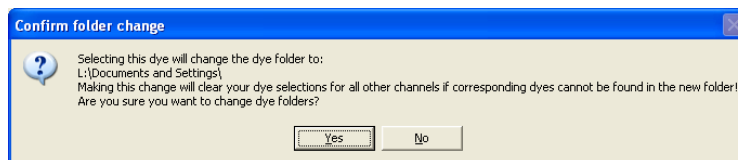


### Advanced Menu: The Channel Tab

- **Update:** Click the update button to confirm a selection and execute the change. This button is only enabled when you have selected an item in the **Channels**, **Microscope Setup**, **Objective**, or **Capture Setup** columns, but have not confirmed your choices by clicking **<Enter>**. Clicking **Update** has the same effect as clicking **<Enter>**.
- **Generate Color Composite :** The **Generate Color Composite** checkbox is used to determine whether a color composite should be made of the channels during acquisition.
- **Composite While Acquiring:** Check this box to generate the composite image after each set of channels is acquired, rather than after the acquisition process is complete.
- **Auto-Set :** The **Auto-Set** button invokes the same functionality as the **Auto Set** button on digital basic capture. It will be disabled if the current capture device is not a digital camera.
- **Set Focus Offset :** The **Focus Offset** button is added to invoke the **Focus Offset** wizard. This button is disabled if the current (preview) channel is also the *Focus Channel*. The *Focus Offset Wizard* is described in Chapter 1 of this manual.

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**Note:** Three of the columns work with external files: **Channels**, **Microscope Setup**, and **Camera Setup**. All of these columns include items to **Browse** to a new location for the type of file associated with the column (dye files, Scope-Pro settings files, lens information files, and Capture VPF files, respectively) and to **Add/Edit** a new file. Any of these selections can end up changing the active folder for that type of file, which may invalidate other selections on the grid. For instance, if you have selected the first channel's dye file from a given folder but select the second channel's dye file from another folder, the corresponding dye file for the first channel may not be available in the new folder. AFA will warn you before the new selection is accepted (after confirming the selection):



If you confirm the folder change by clicking **Yes**, the new selection will be accepted, and any invalid selections (of files that are not available in the new location) will be cleared. If you reject the folder change by clicking **No**, the selection will be canceled and none of the file selections will be modified

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**Note:** You can change the capture settings using the *Capture* dialog box:



The tabs and controls available in this dialog box will vary depending on the camera(s) and framegrabber(s) you are using.

Save the *Capture* (.VPF) file to retain the new configuration settings. Refer to the *Capture* section in the *Image-Pro Plus User Guide* for further detail on modifying the camera configuration.

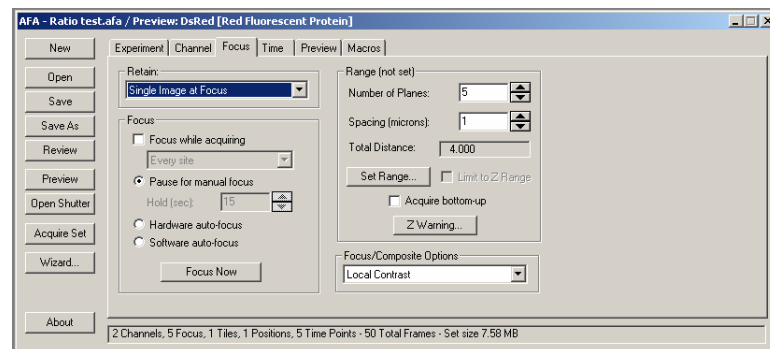
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## The Focus Tab

The *Focus* tab allows you to set the focus parameters for acquiring images. You can define how many planes to acquire, where and when autofocus occurs, and whether the selected positions are at the top, middle, or bottom of the stack.

### Focus Tab Selections

Clicking on the *Focus tab* displays the following dialog:



- **Retain:** This group box sets the output of the acquisition. Select the type of output required from the combo box:
  - **Single Image at Focus:** Choosing this option retains only one image at the point of focus.
  - **Best Image from Stack:** Choosing this selection will keep the best focused image from the stack.
  - **Composite Image from Stack:** Choosing this selection will enable you build a composite image from the best focus portions of the images in the stack.
  - **Full Stack:** Choosing this selection will keep the entire stack for each position. Note that this will be the best way to collect data for later use in with either *SharpStack/SharpStack Plus 7.0* or *3D-Constructor 7.0*.

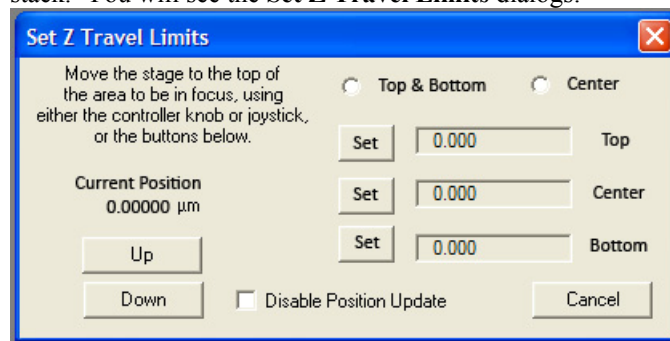
### Advanced Menu: The Focus Tab

- **Focus:** The options in this group box set up either auto or manual focusing during the acquisition. Note that if the focus is changed by the focus method, the next acquisition at that point will use the current position as a starting point, so that drifting objects can be tracked. The focus will use the **Preview** settings for which channel to view unless otherwise specified. The starting point for both manual and hardware focus will be the center of the Z range as specified in the **Range** group box.
- **Focus while acquiring:** Check or uncheck this box to enable or disable focusing during acquisition. The combo box sets the frequency of focus. This combo box contains the following settings:
  - *Every Frame:* Focus at every X/Y position in a scan area, sample pattern, or user-defined list of positions.
  - *Every Channel:* Focus every time the channel changes.
  - *Every Sample:* Focus at every X/Y position in a sample pattern or user-defined list of points. The microscope will be moved to the center of the scan area (if enabled) and focus there. Once focused, the microscope will move the beginning of the scan area and begin acquisition.
  - *First Sample:* Focus at the first X/Y position in a sample pattern or user-defined list of points. The microscope will be moved to the center of the scan area (if enabled) and focus there. Once focused, the microscope will move the beginning of the scan area and begin acquisition.
- **Pause for manual focus:** Pause during the acquisition for manual focusing. The preview window will appear when this is done. Note that this will add to the total acquisition time.
  - *Hold (sec):* Indicates how long the **Pause for manual focus** dialog will be shown.
- **Hardware auto-focus:** Click this button to focus using the hardware auto-focus capability of your stage, if available.
- **Software auto-focus:** Check this box to perform a software auto-focus at the specified location. This will acquire a stack, determine what is the best focal plane, based on the criteria specified in the *Focus/Composite Options* group box, and set the focus to that point.
- **Focus Now.** Click this button to update the focus position at the current preview position. The current channel will

*Advanced Menu: The Focus Tab*

automatically become the focus channel. For *manual focus*, a **Continue** button will appear in place of the *Hold (sec)*. After completion of the focus process, the Z position of the current preview position will be updated

- **Range:** The selections in this group box set the range of focus. This is used for stack acquisition, and to set the position and number of planes captured for the software auto-focus method.
- **Number of planes:** Use the spin buttons to set the number of focal planes in the stack. This must be a positive integer greater than zero.
- **Spacing (microns):** Type in or use the spin buttons to set the distance between each plane in microns. This will be in increments of calibrated Z distance, as supported by the resolution of the Z motor for this system. This must be a positive floating-point value greater than zero.
- **Total Distance:** This text reports the size of the stack (top to bottom) in microns.
- **Acquire bottom-up:** Check this box to set the Z stack acquisition from the bottom up instead of from the top down.
- **Z Warning:** Click this button to set a warning if the Z focus moves too far. The default is 1mm.
- **Set Range...:** Click this button to set the top and bottom of the stack. You will see the **Set Z Travel Limits** dialogs:



To determine the correct position, select either the **Top and Bottom** or **Center** radio buttons. Setting the top and bottom positions will set the center; setting the center will set the top and bottom based on the currently chosen AFA spacing and slice count. Note that the **Top and Bottom** can be set in either order,

### Advanced Menu: The Focus Tab

and that once both are chosen the Top/Bottom will be recalculated based on the best fit with the current slice spacing.

The current position can also be set by using the Top, Center, and Bottom fields, while using the Up and Down buttons to position the stage.

After setting the starting position, you will be asked to move the end of focus on the stack, and again choose **Continue** or **Cancel**. The positions that were marked will be used as the starting and ending points of the focus. Note that if these are not within the area specified by **Spacing**, the range will be increased so that the starting and ending points are included in the range.

Also note that the range and the number of planes may vary in a related fashion; if the number of planes is changed, the total distance (range) will change, and vice versa. The **Spacing** will be held if the Z range is not locked.

---

**Tip:** You can also set the center of a Z-stack by clicking **Set Range**, focusing on the center point, clicking **Set to Center**, and clicking **Continue** without moving the focus. This will allow you to specify the number of planes and plane searching independently.

You should be aware that the center point that you select in this process will not be imaged if you are using an odd number of planes. Also, the **Limit to Z Range** option (below) will not be available if you choose this method.

---

- **Limit to Z Range:** Check this box to lock the top and bottom of the Z range so that changing the number of slices or spacing adjusts the other to keep the stack within the set Z range. Unchecking this option allows the stack to grow larger than the set Z range if the number of slices or spacing is increased.
- **Focus/Composite Options:** These are the **Extended Depth of Field** options used to generate either the composite, or to evaluate the software focus. For more information about **Extended Depth of Field**, please refer to your *Image-Pro Plus Reference Guide*.
- **Z Warning:** This selection sets a safety limit for the Z motion. If a focus change exceeds this limit, *AFA* will issue a warning and ask for confirmation.

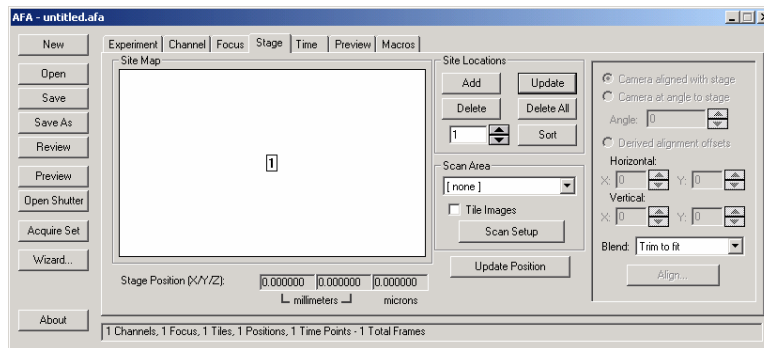
## The Stage Tab

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The **Stage** tab provides an interface to *Stage-Pro/Scope-Pro* and sets up the X and Y stage positions. The **Stage** tab is only visible when you have configured a stage in *Scope-Pro*, and selected multiple sites on the *AFA Experiment* tab.

### Stage Tab Selections

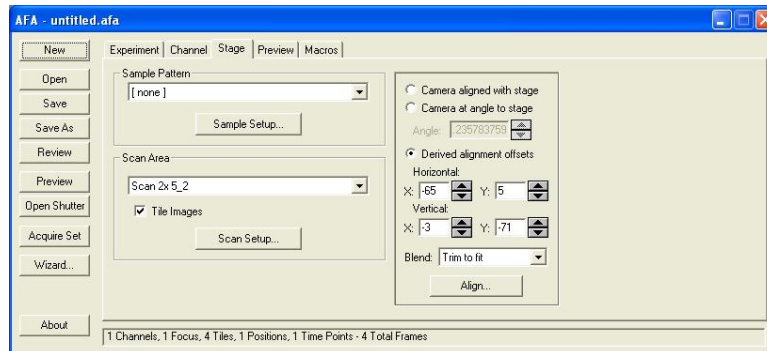
There are two possible forms for the **Stage** tab, depending on whether you have selected **Sample Pattern** or **User-Defined Positions** on the *Experiment* tab.



### Sample Pattern

If you have chosen the **Sample Pattern** on the *Experiment* tab, the **Stage** tab will look like this:

## Advanced Menu: The Stage Tab



In **Sample Pattern**, you need to choose which sample pattern to use. If scanning within a scan area is selected, you need to indicate which scan pattern to use.

---

**Note:** Any time AFA is prompted to change positions using a **Sample Pattern** or **Scan Area**, the selected settings file is reloaded from disk. If you make any changes to either of these functions in the *Stage-Pro* dialog, please remember to save your changes before returning to AFA.

---

- **Sample Pattern:** The **Sample Pattern** combo box allows you to select a pattern from the existing sample (well or slide) patterns. Note that this group box will not be visible if **Multiple Positions** is not selected on the *Experiment tab*.
- **Sample Setup:** Click this button to launch *Stage-Pro* and configure the sample patterns. For more information, please refer to your *Stage-Pro/Scope Pro Users Guide*.
- **Scan Area:** The **Scan Area** combo box allows you to select from existing scan patterns. Note that this group box will be not shown if the **Scan Areas** checkbox on the *Experiment tab* is not selected.

---

**Note:** *Stage-Pro* includes the current objective definition when saving scan area settings files. If you have a motorized nose piece, we recommend that you make note of the objective for which a particular settings file was saved. You can do this by simply including the objective name in the settings file name.

---

- **Tile Images:** Check this box to enable the system to capture data as large tiles. This will create a tiled image at each acquisition point using the “Scan Area” configuration.

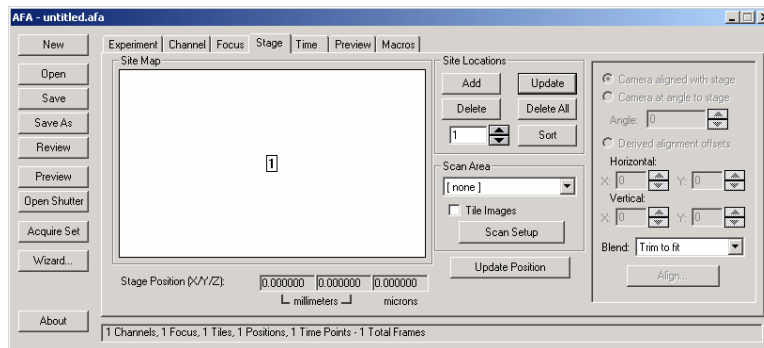


**Note:** If **Tile Images** is checked, the capture order must be **Channels first, then Focus** (see the *Experiment tab*). You will be asked to confirm this selection if **Focus First** has been previously selected on the *Experiment tab*. The *Image Tiling* selections are discussed in the following section.

- **Scan Setup:** Clicking this button launches *Stage-Pro* to configure the scan patterns. For more information, please refer to your *Stage-Pro/Scope Pro Users Guide*

### User-Defined Positions

This version of the *Stage* tab appears if **User-Defined Position** was chosen in the *Experiment tab*. Using this method, you will select a set of stage positions where the system will stop and acquire a sample image or traverse a scan area. You will see the following dialog:



- **Site Map:** The **Site map** shows a scaled (X and Y) map of the selected stage positions. These are created by moving the stage to a desired position and clicking the **Add** button. The first point will appear in the center of the Site map. When additional points are added to the map the map will be scaled down to fit all of the sampling positions in the **Site map** area. Clicking on a numeric position in the map will move the stage to that point. The selected point will be highlighted with a box around the number.

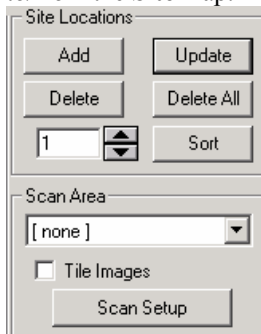
The **X**, **Y**, and **Z** boxes below the Site map show the selected point's XYZ position coordinates in microns.

- **Update Position:** Click this button to update the position information to show the current location of the stage. If the current

### Advanced Menu: The Stage Tab

location of the stage coincides with a sampling position, the corresponding position on the **Site** map will be boxed and the corresponding point selected in the **site location** group.

- **Site Locations:** This group box enables you to add or delete points to/from the **Site** map.



- **Add:** Clicking this button chooses the current stage location as a new position, and adds it to the list.
- **Update:** Clicking this button updates the stored XYZ values for the current position with the Stage's actual position.
- **Delete:** Clicking this button will remove the selected point from the list, renumber the positions, and update the display.
- **Del. All:** Clicking this button remove the all points from the list, and update the display. You will be asked to confirm this choice.
- **Sort:** Clicking this button will attempt to reorder (and renumber) the positions to minimize stage movement between them when going in order between positions. Note that this is not guaranteed to be an absolute minimum path, but it will likely be closer than an arbitrary orderings of the points.
- **Scan Area:** This combo box allows you to select a scan area pattern (as defined in *Stage-Pro*). This group box will not appear unless **Scan Areas** is checked on the *Experiment tab*.

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**Note:** *Stage-Pro* includes the current objective definition when saving scan area settings files. If you have a motorized nose piece, we recommend that you make note of the objective for which a particular settings file was saved. You can do this by simply including the objective name in the settings file name.

---

- **Tile Images:** Check this box to enable the system to capture data as large tiles. This will create a tiled image at each acquisition point.

---

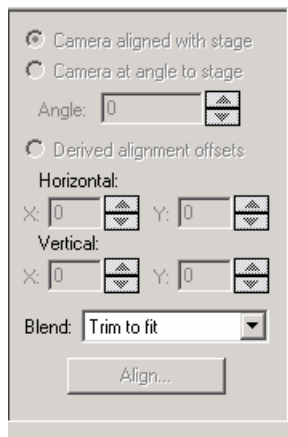
**Note:** If **Tile Images** is checked, the capture order must be **Channels first, then Focus** (see the *Experiment tab*). You will be asked to confirm this selection if **Focus First** has been previously selected on the *Experiment tab*.

---

- **Scan Setup:** Clicking this button launches *Stage-Pro* to configure the scan patterns. For more information, please refer to your *Stage-Pro/Scope Pro Users Guide*.

## Offset Tiling

**Offset Tiling** works with both the sample pattern and user-defined positions on the *Stage* tab. The offset tiling selections allow you to create tiled images when the stage is not perfectly aligned with the camera. To see these selections, you must choose **multiple sites** on the *Experiment* tab, and check **Tile Images** on the *Stage* tab.



- **Camera aligned with stage:** Click this button if your camera is squared with the motorized stage, using the **Stage Square** procedure in *Stage-Pro*. Images will be tiled from edge to edge with no offsets.
- **Camera at angle to stage:** Click this button if your camera is at an angle to the motorized stage. AFA calculates an angle between the camera and the stage, and the offsets for tiles are calculated from that. A 5% overlap

### *Advanced Menu: The Stage Tab*

is enforced between tiles. *Stage-Pro* is used to acquire the tiles, and the **Tiling** module is used to create the mosaic. The **Blend** combo box determines how tile overlaps are handled.

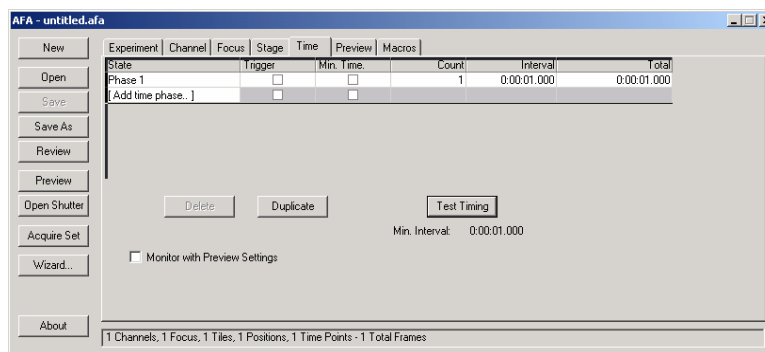
- **Angle:** Use the spin buttons to indicate the degree of the angle between the camera and the stage.
- **Derived alignment offsets:** These measurements indicate the offsets which are calculated for vertical and horizontal tiles. As in the **Angle to stage** section, the tiles are sent through the **Tiling** module with these offsets and the specified blending.
  - **Horizontal** indicates the horizontal X and Y offsets.
  - **Vertical** indicates the vertical X and Y offsets.
- **Blend:** Chose the correct blending technique for the offsets from the drop-down list.
- **Align:** Click this button to select an object on the slide for a test. AFA moves this object to the edge of the field of view and calculates the offset.

## The Time Tab

The **Time** tab sets the interval and number of exposures for time-lapse acquisition. The selections on the **Time** tab override any timing set on the **Image** page of the **Capture** dialog in *Image-Pro Plus*.

### Time Tab Selections

When you select the **Time** tab, you will see the following dialog:



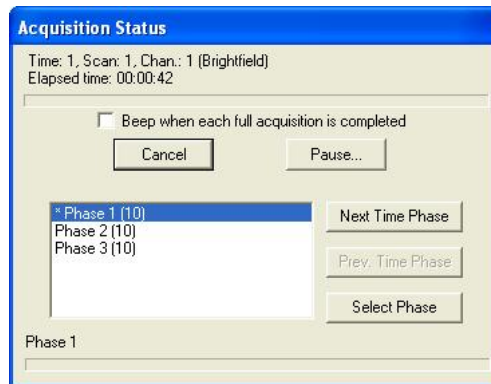
The new **Time** tab in *AFA 7.0* displays grid control, listing time lapse phases with different experiment speeds. The columns of this grid include **Phase**, **Interval**, check boxes for **Trigger**, and **minimum time**, **Count**, and **Total** settings. The 'phase' is a description of that portion of the experiment defined by you. As additional phases are added they will be given the default names 'Phase 1', 'Phase 2', etc.

- **State:** This column indicates the name of each phase. The **State** name is editable. If multiple time phases are present, the last line of the grid will show the total count and total time for the acquisition. These cannot be edited.
- **Trigger:** When **Trigger** is checked for a phase, a user event or macro call can be made to end the previous phase and start the desired one at any time.

### *Advanced Menu: The Time Tab*

- **Min Time** indicates the minimum time interval between acquisitions.
- **Count** indicates the maximum number of time points that will be acquired in that phase.
- **Interval** indicates the actual time between acquisitions. Clicking on the **Interval** column will bring up an Hours/Minutes/Seconds/Ms time setting dialog.
- **Total** indicates the total number of acquisitions ( $Count \times Interval$ )
- **Add:** Click this button to add a time lapse phase
- **Delete:** Click this button to remove a time lapse phase.
- **Duplicate:** Click this button to copy the current selection line or the last item.
- **Test Timing:** Click this button to test the time interval for acquisitions. It runs through the set of positions for the AFA capture (one loop each of each dimension, as in one run through the channels, one run through the Z, one run through the stage positions, etc.), and uses the time required for these to calculate how long a single pass through all items will take. This is used to provide you with an idea of the minimum time between sets of images for time lapse sequences. If the minimum time is 5 seconds, you can't actually capture any faster than one group of images every 5 seconds.
- **Monitor with Preview Settings:** Checking this box will bring up the **Capture Preview** window, using the currently selected preview channel and focus during any time delay before the beginning of each time point. It keeps the live preview running at the center focus and selected preview channel between time points.
- **Monitor with Preview Settings:** Checking this box will bring up the **Capture Preview** window, using the currently selected preview channel and focus during any time delay before the beginning of each time point.

The **Acquire** status dialog will be expanded to include a list of available phases, and controls for selecting the next, previous, and selected phase.



Macro calls are available to increment phases as well.

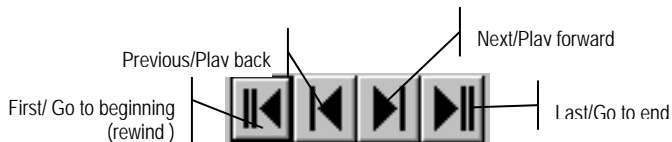
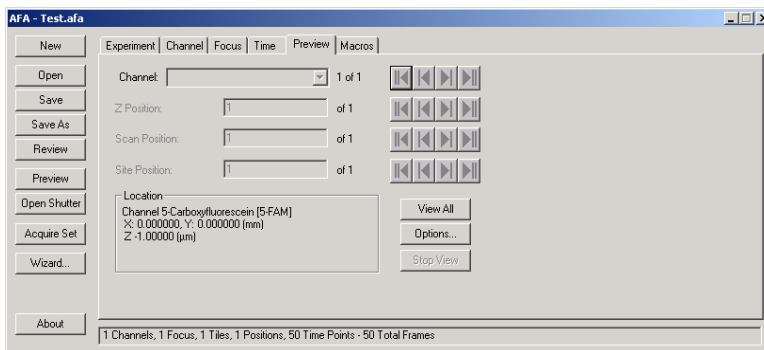
## The Preview Tab

Use the **Preview** tab with your current acquisition settings to preview the images as viewed with a particular microscope configuration. In this way, you can set up and test your capture configuration or monitor a live sample prior to recording the images.

**Note:** The microscope hardware must be turned **on** and connected for the **Preview** functions to work.

## Preview Tab Selections

Starting the preview will set the microscope to the configuration specified for the channels, and X, Y, and Z positions. When you select the **Preview** tab, you will see the following dialog:



The VCR buttons next to each channel allow you to step through the available positions for each channel. Entering a valid position number will move there directly. The position numbers will not warp at the ends of the dimensions.



### Advanced Menu: The Preview Tab

- **Channel:** Select the channel that you want to preview. The number of channels available depends on the number of channels selected on the *Channel* tab.
- **Z Position :** Select the Z position that you want to preview. ‘|<’ and ‘>|’ buttons allow you to step through the available positions..
- **Scan Position:** Select the X/Y position that you want to use for the preview.
- **Site Position:** Indicates the X, Y, and Z positions plus the name of the channel currently selected. This group box will display **Unknown** if the stage is not located at a preview position.
- **Sample Position:** Select the well plate or user-defined position where you want to start the preview.
- **View All:** Clicking this button will cycle through all existing variables except the channel selections. This will run in the same order as a timed acquisition, using the preview channel selected. A small status dialog will appear, which allows you to cancel the preview at any point.
- **Stop View:** Pressing the **Stop View** button halts the **View All** action. When **View All** is selected, the **View All** button is disabled and the **Stop View** button enabled.
- **Options:** Clicking this button will display the Preview Options dialog box:

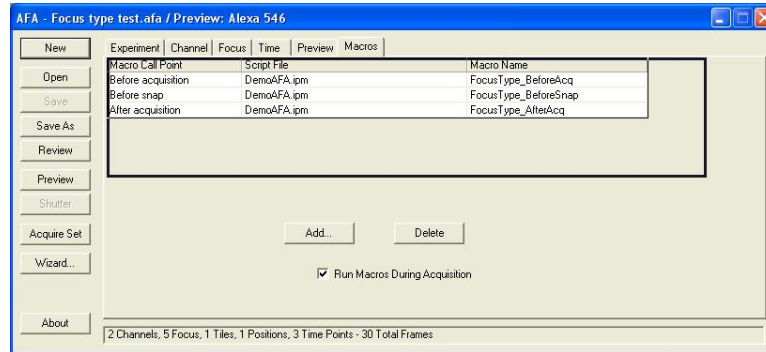


- **Seconds Time Delay:** Indicate how long to delay at each point when **View All** is selected.
- **Keep Current Channel:** Check this box to keep using the channel currently selected for the preview.
- **Keep Current Sample:** Check this box to keep using the sample location currently selected for the preview.

*Advanced Menu: The Preview Tab*

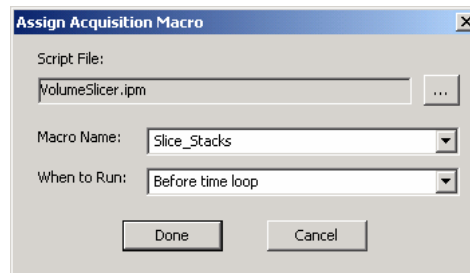
- **Keep Current Scan Position:** Check this box to keep using the **scan area position** currently selected for the preview.
- **Keep Current Z Position:** Check this box to keep using the Z position settings currently selected for the preview.
- **OK:** Click **OK** to set the **Preview Options**.
- **Cancel:** Click **Cancel** to erase your **Preview Options** changes.
- The new **Macros** tab aims to simplify the process of customizing AFA by giving you, the user, an entry point for adding a simple (often recordable) macro to the AFA loop at a defined point. This will eliminate the need for you to work through the multiple loops in Macro\_AFA, and figure out how to add your special piece of code.

## Macros Tab Selections



This tab presents a grid control with three columns, listing the **Macro Call Point**, or when the macro will be invoked, the script file, and the macro name. Buttons under this grid will allow the user to **Add** macros, and **Delete** currently selected macros.

The **Macro Call Points** are listed with names such as *Before acquisition*, *Before channel loop*, and *After snap*. Checking **Run Macros During Acquisition** will do just that. There are approximately 14 places where a macro may be called. Only those call points enabled by the current acquisition settings will be available in the **Add** dialog, although if a dimension is disabled any existing macros for that dimension will be listed in gray, as they will not be performed. The **Add** dialog is shown here:



- **Script File:** The name of the script file containing the requested macro is shown here. Select additional macros by clicking the ... button.
- **Macro Name:** The name of your requested macro is shown here. Select additional macros from the pull-down menu.

### *Advanced Menu: The Ratio Macro*

Note that macros will only be called in a full acquisition, not a background snap or autoexposure test.

## **The Ratio Macro**

---

The ratio macro provided with this release of AFA 7.0 provides a technique for using the relative intensities in two images to measure some environmental variable in the sample. Generally speaking, two images are taken of the sample, using fluorescent dyes that have either excitation or emission spectra that vary along with the environmental variable. For example, Ca<sup>++</sup> ratioing is used to measure calcium ions, via fluorescent dyes that change excitation spectra based on the local amount of free calcium ions. Dividing one dye image by the other cancels out the relative dye concentration, path thickness, and other variables, leaving just the relative response of the dyes to the calcium.

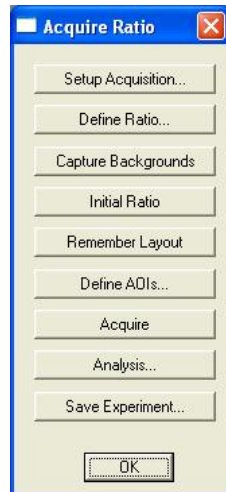
Other possible targets for ratio analysis include pH, magnesium concentration, and some less used targets such as cellular barriers to diffusion. The major requirement is that the amount of dye in the two inputs can be cancelled out by dividing the two images.

Ratio imaging provides one of the only known methods for truly quantitative measurements using fluorescent dyes, which otherwise suffer from photobleaching, oxidation, concentration, and other issues.

This feature comprises two macros: *Acquire Ratio* and *Analyze Ratios*.

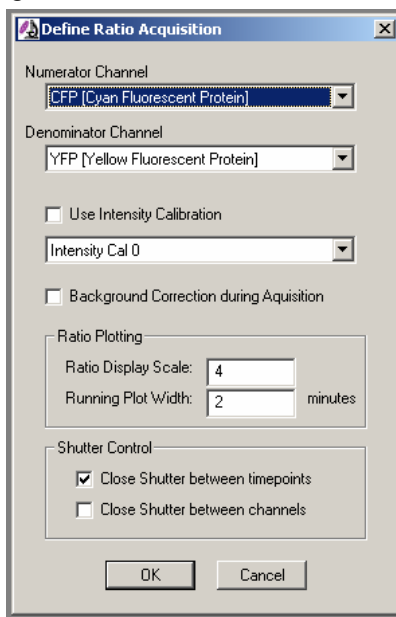
## The Acquire Ratio Macro

The *Acquire\_Ratio* toolbar dialog is shown here.



- **Setup Acquisition:** Clicking this button brings up the AFA dialog, along with a message asking you to configure at least two channels and a time lapse configuration. When you click **Continue** on the dialog the AFA window is closed.

- **Define Ratio...** Clicking this button brings up the ratio definition dialog, shown below:



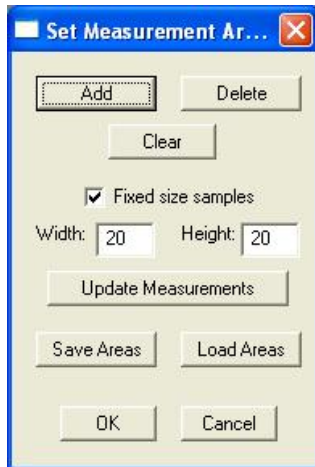
- The *Numerator* and *Denominator* channels are selected from the defined channels in the AFA settings file, defaulting to the first and second channels, respectively.
- *Intensity Calibration* is an optional checkbox. If you check this box, you must select an existing calibration (from the drop-down list of calibrations inside *Image-Pro*) that will be applied to the ratio image. This is useful if there is a calibration relating the ratio value to the environmental variable, such as calcium concentration or pH.
- The *Background Correction* checkbox allows you to use another set of images for background correction.
- The *Ratio Display Scale* sets the dynamic range of the floating point ratio channel from  $1/N$  to  $N$ . A factor of 4.0, as shown, will scale the image from  $1/4$  to 4. The floating point image will also have a log pseudocolor scale applied, which portrays values of 1.0 as green, values at the  $1/N$  as blue, and  $N$  as red.

### *Advanced Menu: The Ratio Macro*

This range of blue for low to red for high follows a fairly standard practice in ratio imaging.

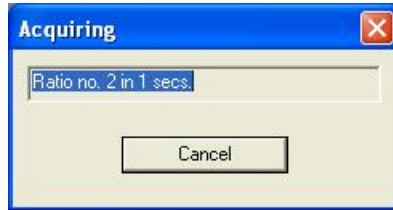
- The *Running Plot Width* simply indicates how many minutes to show on the rolling chart during acquisition.
  - *Close Shutter between timepoints* will **not** close the shutter while capturing multiple channels at one time point; it only closes between separate time points.
  - *Close Shutter between channels* will close the shutter after each snap, at one time point or several.
- **Capture Background:** Clicking this button prompts you to capture a background set, which is used if *Background Correction* in the ratio definition is selected.
  - **Initial Ratio:** Click this button to capture and calculate an initial ratio set. This captures one set of channels, calculates the ratio, and adds that as an additional channel to the set. The purpose of this is to provide an image on which to define the areas of interest for plotting and measurement.
  - **Remember Layout:** Clicking this button is equivalent to the **Set Channel Layout** function in AFA, but supports the additional ratio channel as well. The current layout of the channels in the active set (zoom and position) are recorded. When a new set is captured the images are laid out in the same fashion, providing the user with an arrangement that supports watching the data during capture. The layout positions are cascaded down the screen so that subsequent ratios will not be directly laid over the previous ones.
  - **Define AOIs:** Clicking this button provides you with a way to define the areas of interest on the initial or current ratio. These AOI's are used to measure average ratio values. The AOI definition dialog is shown below:

Advanced Menu: The Ratio Macro



- *Add* prompts you to define the AOIs.
- *Delete* lets you click on and delete an AOI.
- *Clear* deletes all defined AOIs. If no AOI is defined on the image, the default is for the ratio acquisition to measure and report the mean ratio value for the entire image.
- *Fixed Size Samples* provides for a single click interface that places elliptical areas of interest of the specified size on the image.
- *Update Measurements* will calculate the measurements on the current image with the present set of AOIs.
- *Save Areas* and *Load Areas* allow you to save or load a mask of the AOIs in a different file, so that a set of AOIs can be preserved from run to run without being overwritten.
- **Acquire** runs the AFA macro loop that captures channels and computes the ratio, creating an output image set. A progress dialog is shown here.



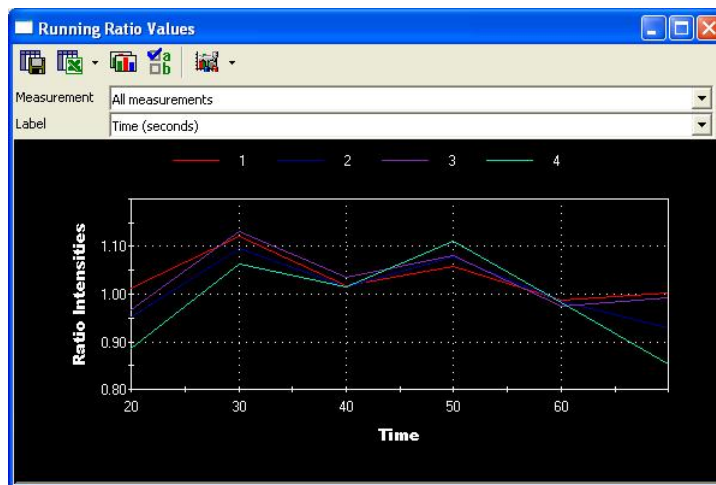


The next dialog is shown if the AFA acquisition defines multiple time phases, such as fast and slow acquisition.



If any of the buttons in the progress dialog are clicked, the macro will respond accordingly as soon as the current set of channels is completed. During acquisition a running graph is shown.

The background of the chart is set to black, with white foreground, so as to minimize screen glare in the laboratory. The width of the running chart is as set in the **Ratio Definition** dialog above.



- **Analyze...** brings up the **Analysis** dialog, described in the next section.

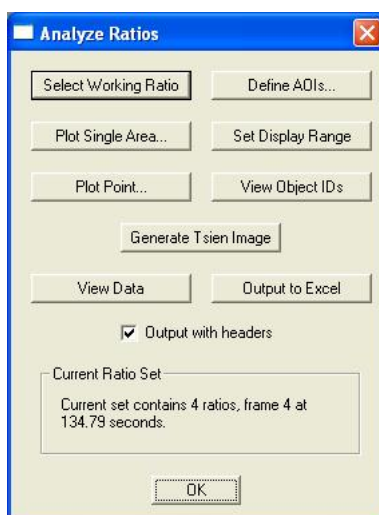
*Advanced Menu: The Ratio Macro*

- **Save Experiment...** simply runs *Save Set* in template mode, allowing the user to write the image set to disk.

## Analyze Ratios

The *Analyze\_Ratios* macro allows you to measure ratio images over time, examine multiple areas of interest, and send the output to a file or to an Excel spreadsheet.

The *Analyze\_Ratios* dialog can be reached either from the Acquire Ratio toolbar described in the previous section, by calling the macro by name: **RatioApp\_51.ipm**. The main window is shown below.



- Clicking **Select Working Ratio** prompts you to select an image from an existing set. If that set contains a floating point channel, that channel is identified as the ratio channel. If not, you will be prompted to define a ratio using channel names, using a dialog similar to the **Define Ratio** dialog. If background correction is selected, you will also be prompted for the selection of a background set.

If a set without a floating point channel is selected, a floating point ratio channel is calculated, added to the set, and displayed. The currently defined AOIs are loaded and plotted on the image, and a chart of all AOIs over time is generated. If no areas of interest are defined, the mean ratio value of the entire image is calculated and plotted.

- **Define AOIs**: Clicking **Define AOIs** brings up the area of interest dialog

### Advanced Menu: The Ratio Macro

- **Plot Single Area:** Clicking **Plot Single Area** prompts you to select one of the areas of interest on the ratio image. That area is charted on a separate chart, with the original trace in the full chart highlighted.
- **Plot Point...** Click this button to select one or more points in the image, and display a plot of the ratio at that point over the set. This is a useful way to explore the data set. This continues until you click on the prompt dialog to cancel this mode.
- **Set Display Range:** Clicking **Set Display Range** changes the image display range from the entered value  $N$  to  $1/N$ , and recalculates the log pseudocolor display. This is useful if the current display range is saturated or doesn't display small changes.
- **View Object IDs :** Clicking **View Object IDs** toggles the object IDs between the area number and the ratio value in those areas. This is useful for identifying the current area plotted or in relating the data to Excel or file output.
- **Generate Tsien Image** creates a *Tsien* style display image. This view, first created by Roger Tsien, uses the mean of the numerator and denominator images for intensity and the pseudocolor of the ratio for hue. The output image shows the ratio as color and the underlying dye intensity as brightness. Note that this is a qualitative image, and that the true ratio value is no longer directly available after creating this RGB image.
- **View Data and Output to Excel** write out the data for the currently defined areas to the Output Window and to Excel respectively. Output with headers affects how this data is written; if checked, headers for the object area and the seconds for each image are added to the output.

Note that the bottom of the **Analyze** dialog contains a live display. If the active image is the ratio channel of the active set, information about the image will be displayed, including the elapsed time for the image from the start of acquisition. When the active frame of the ratio changes the measurements will be redone, and the AOI labels will reflect their values.

If the *Numerator* or *Denominator* image is active, **Numerator** or **Denominator** will be displayed. If the active image is not part of the currently selected set, that will be displayed as well.

## **Label with Frame Times**

*Label\_With\_FrameTimes* macro is a simple macro that burns in annotation containing the frame time into the image.

This macro is contained macro file *RatioApp\_51.ipm*. There is no interface other than that. It will run through the frames of a sequence and label the images with the frame time, using the annotation tool. It is supplied simply as a utility function.

## **Set Normalization Macro**

The Set Normalization macro takes an input set, and converts it (if needed) into a standard 'normalized layout'. A 'normalized layout' consists of one image workspace per channel - if the input set consists of many channels stuffed into a single workspace (the default load structure for a number of multidimensional file formats) or more workspaces than channels (as with loading many individual frames from an AFA acquisition), the macro will convert the set. If the set has the right number of workspaces the macro will exit immediately.

Normalized layout sets are required for the Ratio Analysis macro, and make channel/channel operations much more convenient; it makes it possible to divide one channel by another for all time points, for example.

Note that the set must be 'complete' - i.e., each dimension must be fully filled, one frame per coordinate for the size of the set. You can't have a Z stack at only one time point, for example; the set navigator can't handle it, and the macro will complain (and exit) if the input set is incomplete for some reason.

The new set will have one channel per workspace (as many workspaces as channels, in other words), with individual frames iterating by Z, then scan position (mosaics), then stage position, and then time, in that order. For example, if the set has Z stacks over time, the frames in each workspace will iterate through Z at the first time point, then the second time point, and so on.

The result is a new set, with the various set information (channel names, comments about the set, etc.) copied over.

## **The FRET Macro**

Fluorescence Resonance Energy Transfer (FRET): FRET, also known as Förster Energy Transfer (FET), is a technique for measuring the proximity of tagged molecules in a microscope sample. In this technique, items of interest are tagged with fluorescent dyes. These dyes, the 'donor' and the 'acceptor', are chosen so that the emission spectra of the donor overlaps the excitation band of the acceptor. If these dyes are in close proximity, the energy of an excited donor molecule can directly transfer to an acceptor molecule, resulting in acceptor emission under donor excitation. This provides a signal indicating distance on a scale of 1-10 nanometers, and can provide information on molecular interactions and conformations on a scale far below the diffraction limit of the microscope optics.

Images are taken using the donor excitation and emission, the acceptor excitation and emission, and finally the donor excitation and the acceptor emission. In a perfect scenario, the only signal in the FRET image would be that of the resonance energy transfer. In reality, the signal is contaminated by spectral cross-talk from the donor and acceptor spectra, where the acceptor is excited directly by the donor excitation, or where the donor emits in the acceptor emission spectra. All methods of FRET analysis include correction of this cross-talk factor in one way or another.

This signal strength is affected by a large number of factors, including the transfer quantum efficiency of the dyes, the rotational alignment of the molecules, solvent effects, and so on. The FRET macros as written provide a raw FRET value, with the interpretation of the results to a molecular distance left to the end user as a calibration on that raw FRET value.

The FRET macro provided with AFA 7.0 requires both *AFA 7.0* and *Image-Pro Plus 7.0*. These macros support FRET post-processing on captured images, sequences, and sets.

When you select the FRET calculation macro, you will see the following dialog:

**FRET Calculations**

Calculation Method: Youvan Fc  $F_c = (\text{fret} - \text{bk}[\text{fret}] - \text{cf}[\text{don}] * (\text{don} - \text{bk}[\text{don}]) - \text{cf}[\text{acc}] * (\text{acc} - \text{bk}[\text{acc}]))$

Set inputs

Inputs

Chose Set Update Image Lists

Donor: Ratio save 5 C0 4T.seq

Acceptor: Ratio save 5 C1 4T.seq

FRET: Ratio save 5 C2 4T.seq

Backgrounds

Correct for backgrounds  Image Backgrounds

Measure from images

Donor: 3488.417

Acceptor: 3354.87

FRET: 2.300493

Spectral Bleedthrough

cf[ donor ]: 0 Measure Donor CF

cf[ acceptor ]: 0 Measure Acceptor CF

Calculate Close

- **Calculation Method:** This selects from one of four emission FRET equations for calculating the raw FRET values; the Youvan, Gordon, Xia, and the Acceptor only methods. These are described as follows:

## Advanced Menu: The Ratio Macro

### *Youvan*

Requires three images, three backgrounds, and correction factors derived from four calibration images

$$F_c = (fret - bg[fret]) - cf[donor] * (don - bg[don]) - cf[acc] * (acc - bg[acc])$$

$F_c$  = FRET calculation according to Youvan

fret = FRET image

don = Donor image

acc = Acceptor image

bg = background intensity

cf = correction factor

### *Gordon*

$$F_n = F_c / ((don - bg[don]) * (acc - bk[acc]))$$

### *Xia*

$$F_n = F_c / \sqrt{((don - bg[don]) * (acc - bk[acc]))}$$

### *Acceptor alone*

$$A_c = F_c / (acc - bk[acc])$$

The area to the right of the combo box for the **Calculation Method** displays the equation for the selected method.

- **Set Inputs:** This check box selects the behavior of the **Inputs** section of the dialog. If checked, the **Choose Set** button is enabled, and the **Donor**, **Acceptor**, and **FRET** dropdown lists select channels in the chosen set. If unchecked, the dropdown lists select from the currently open images or sequences.
- **Update Image Lists:** Update the dropdown selections for all images, in order to handle newly opened or captured images that are not in the current selection lists.
- **Correct for Backgrounds:** Enables background correction, as in the method equations. If unchecked, no background correction will be done, and the method equation display will change to indicate that.
- **Image Backgrounds:** This controls the behavior of the **Donor**, **Acceptor**, and **FRET** selections for backgrounds. If checked, these are dropdowns that select background images, and the



**Measure Background** button is disabled. If unchecked, the **Measure Background** button is enabled, and the selections are numeric values for backgrounds.

- **Measure Background:** Prompts you to select an AOI in one of the images that is used to select a background region. The average intensity for that region in each channel is used as the background value for FRET calculation.
- **Spectral Bleedthrough:** This option allows you to select one of the cross-talk values for the FRET equations, as the ratio of the cross-talk to the Donor and Acceptor signals, respectively. These are floating point values between 0.0 and 1.0, generally fairly small.
- **Measure Donor CF/Measure Acceptor CF:** Prompts you to select reference images taken with the three filter sets, labeled with Donor or Acceptor dyes only, respectively. These are used to calculate the cross-talk between the two dyes and the FRET filter set.
- **Calculate:** Perform the FRET calculation as specified by the dialog, creating a floating point image or sequence. If **Set Inputs** is checked, this is added to the set as a new channel. A user-specified intensity calibration can be then applied to the FRET channel to convert this raw value to a calibrated measure of efficiency or molecular distance.

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**Note:** The standard FRET capture recommendation is for equal exposure time for all three images (or sequences); this allows the cross-talk calculation to proceed unscaled. The FRET macro as currently implemented scales backgrounds and cross-talk factors so that the three images can have different exposure times, if the camera properly attaches the exposure time to the image.

---

## Photobleaching FRET Calculation (pbFRET)

This macro is used to process previously acquired images using the Photobleaching FRET technique. In this technique the input images are taken in only the donor channel. First a starting *donor* image is acquired. Next, the *acceptor* channel is photobleached to completion, which is usually defined as a greater than 80-90% decrease in fluorescence. Finally, a second donor channel is acquired. The increase in donor fluorescence due to the removal of the energy absorbing Acceptor is used to calculate the FRET efficiency at every pixel. This gets around cross-talk and registration errors, at the expense of having to photobleach the acceptor. This method is generally used only with single images, as it is not possible to perform time lapse experiments in this fashion.

When you select *pbFRET\_Calculation*, you will see the following dialog:

**pbFRET Calculation**

Method  
$$BL = (1 - (\text{don}[\text{bef}] - \text{bkg}[\text{bef}]) / (\text{don}[\text{aft}] - \text{bkg}[\text{aft}])) * 100$$

Inputs  
Donor Before: Ratio save 5 C0 4T.seq  
Donor After: Ratio save 5 C2 4T.seq  
 Subtract Backgrounds

Backgrounds  
 Image Backgrounds  
Bkgr. Before: 0  
Bkgr. After: 0  
Measure Backgrounds

Update Image Lists  
Calculate Close

- **Method:** This displays the equation for the pbFRET calculation, as determined by the rest of the dialog.
- **Donor Before:** The image or sequence of the initial donor fluorescence.
- **Donor After:** The image or sequence of the final donor fluorescence.
- **Subtract Backgrounds:** Checking this box enables background correction, as in the method equations. If unchecked, no background correction will be done, and the method equation display will disable to indicate that.
- **Image Backgrounds:** This controls the behavior of **Background Before** and **Background After** selections. If checked, these are dropdowns that select background images, and the **Measure Background** button is disabled. If unchecked, the **Measure Background** button is enabled, and the selections are numeric values for backgrounds.
- **Measure Background:** Prompts the user for an AOI in one of the images that is used to select a background region. The average intensity for that region in each channel is used as the background value for pbFRET calculation.
- **Update Image Lists:** Update the dropdown selections for all images, in order to handle newly opened or captured images that are not in the current selection lists.
- **Calculate:** Perform the pbFRET calculation as specified by the dialog, creating a floating point image or sequence.

## AFA Demonstration Macros

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You can also run the *AFA* demonstration macros to view highlighted features of *AFA*.

The macro file **AFA Demo .scr** contains several macros (see below). Select the macro you want to run from the **Macro** command on the *Image-Pro Plus* menu bar.

**Macro\_AFA:** This macro uses IpBasic commands to step through the various loops of a complex acquisition. This allows the user to run an acquisition based on previously set parameters, with the additional option of modifying it to add processing of their own.

**Demo\_AFA:** Walks the user through the various tabs of the *AFA* program, showing setting various parameters and talking about (text dialogs popping up describing various items) what the settings mean.

**Setup\_Focus\_Interval :** : Allows you to insert timed intervals in the *AFA* acquisition macro. To use, the focus should be on, the desired autofocus settings are enabled, and the time lapse should be on.

**Insert\_Pause :** : This macro inserts the **Pause after Focus** macro, which inserts a 1/10 second pause after focus .

**AFA\_Ratio:** Demonstrates adding additional parameters to an *AFA* acquisition. This macro runs through a two-channel *AFA* acquisition, and adds a floating-point ratio on a third channel.

## SharpStack

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*SharpStack* is available in four configurations. The base *SharpStack* product included with *Image-Pro Plus* and *Analyzer* provides **No Neighbor**, **Nearest Neighbor**, and **Inverse Filter** methods. *SharpStack Plus*, *Confocal* and *Total* all add two additional deconvolution methods, **2D-** and **3D-blind deconvolution**. *SharpStack Confocal* and *Total* also add **Super Resolution** and **Custom Noise** options. In *SharpStack Plus*, the **Widefield Fluorescence** and **Transmitted-light Brightfield** modalities are available, while in *SharpStack Confocal* the **Laser Scanning Confocal**, **Spinning-disk Confocal**, and **Two-Photon Fluorescence** modalities are available. *SharpStack Total* includes all modalities.

### What is Deconvolution?

The word *deconvolve* means “to untangle or unwind”. In most microscopes, the lens aperture causes light to spread out from a point source, or diffract. The light coming from out-of-focus planes causes image contamination. So, in the context of image processing and analysis, deconvolution is the process of systemically removing image noise or haze from an image.

Classic deconvolution in microscopy attempts to determine this point spread function and remove the contamination through mathematical methodology. Deconvolution involves the reassignment of photons to their points of origin within the image volume while deblurring actually removes the out of focus photons completely from the image stack. *No Neighbor* and *Nearest Neighbor* filters are examples of deblurring algorithms, while the *Inverse Filter* is an example of a deconvolution algorithm.

There may be other sources of noise and sharpening or deblurring techniques may be employed. Both techniques have come to be called “deconvolution.” Deconvolution can often reveal fine details of an image that are not directly observable.

*SharpStack* offers three different methods to perform deconvolution:

- No Neighbor

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- Nearest Neighbor
- Inverse filter

The **No Neighbor** method does not require data from other planes in the image stack. This is not as accurate as the **Nearest Neighbor** algorithm, as it uses a smaller amount of data, but is faster. It is also the only option for single images, and is the default selection if a single image is deconvolved.

The **No Neighbor** algorithm reverses the blur (known as the “Airy disk”) that the microscope imposes on objects. This has the effect of improving the resolution and to some degree removing the out-of-focus information.

The **Nearest Neighbor** method analyzes a minimum of three neighboring slices. There is no upper limit to the number of slices that you are able to use. The **Nearest Neighbor** algorithm uses the images from planes in the stack above and below the image plane of interest to estimate the amount of blurring in the central image. When this data is subtracted, the image of interest is sharpened, as in **No Neighbor**.

Note that the **Nearest Neighbor** algorithm, while fast, is rather sensitive to illumination variations. Flat field correction, intensity normalization, and care during acquisition will help produce the best images.

The **Inverse Filter** method uses a point spread function (PSF) to determine the amount of blur imposed by the microscope in all three dimensions. The **Inverse Filter** algorithm deconvolves the entire PSF to deblur the center object. This is more accurate than the other two methods, but slower and more memory intensive, as it performs Fourier Transforms in three dimensions.

*SharpStack Plus* , *Confocal* and *Total* provide two additional deconvolution methods:

- 2D Blind
- 3D Blind

Deconvolution is essentially a very specific high pass filtering of the data in three dimensions, matched to the acquisition parameters so as to remove out of focus blur. In order to get good results from deconvolution it is important to have consistent illumination and low noise conditions.

Otherwise, these external variations will dominate the results and hide or distort the reconstruction of the samples; the errors in the data get amplified too.

### *Advanced Menu:SharpStack*

Flat field correction of lamp shading, intensity normalization for fading dyes or lamp variations, and background subtraction can all help improve the final results, regardless of the algorithm used.

If you have purchased *SharpStack Plus*, *Confocal*, or *Total*, you will have two additional deconvolution methods.

**Blind Deconvolution** is considered to be the most accurate algorithm available. Blind deconvolution does not require the calibration and measurement of the Point Spread Function (PSF). Blind deconvolution can be used with widefield or brightfield fluorescence.

**2D Blind deconvolution** allows you to apply the Blind (Adaptive) deconvolution to a single two-dimensional widefield or brightfield image without requiring previous knowledge of the microscope parameters or the image parameters. The 2D deconvolution algorithm is also capable of improving the resolution of an image for the restoration of features at a sub-pixel resolution level. You may process multi-frame (time series) image sets, individual color channels or intensity images. The 2D algorithm is able to suppress noise while retaining quantitative accuracy (total number of photons) in the image, thus allowing you the ability to make valid quantitative measurements.

**3D Blind deconvolution** is an iterative and constrained algorithm. It is iterative in the sense that it repeats the same computational operations many times while converging to the enhanced image solution. It is constrained in the sense that it only accepts deconvolved images that have the correct mathematical properties of non-negativities, (that is, it does not allow the tracer concentration to have negative values) and smoothness (suppresses snowy-like noise due to low-light levels).

**3D Blind deconvolution** is a method of deconvolution that adapts itself to the real PSF of the microscope system (which can be significantly different from the theoretical PSF and from the previously measured PSFs) due to specimen and instrument variations. The *SharpStack* Blind Deconvolution system is able to adapt to PSF changes within a specimen itself. Thus, the deconvolved results are superior to those methods which utilize theoretical or previously measured PSFs.

*SharpStack Confocal* and *Total* add two new options:

- Super Resolution
- Custom Noise

*SharpStack Confocal* and *Total* also support:

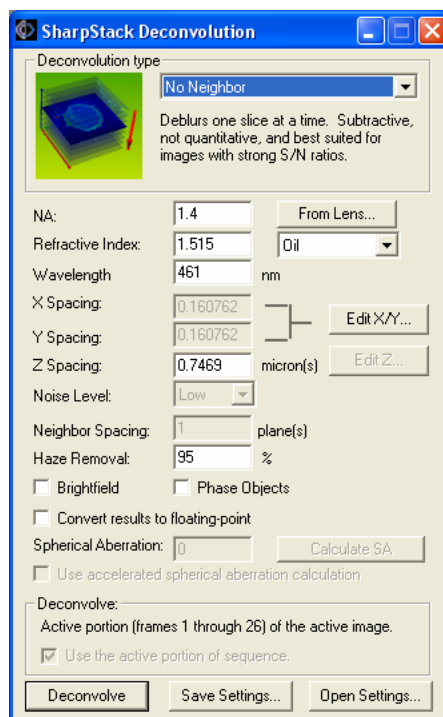
- Laser Scanning Confocal

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- Spinning-disk Confocal
- Two-photon Fluorescence..

## The SharpStack Deconvolution Dialog

When you select *SharpStack* from the *Advanced* menu, you will see the *Deconvolution* dialog. The contents of this dialog will change according to the type of deconvolution that you select:



**Type:** This combo box allows you to select the type of deconvolution process. Your choices are:

- **No Neighbor:** When selected, the stack or image will be deblurred using the **No Neighbors** algorithm. This is the only option for single images, and is the default selection if a single image is deconvolved.

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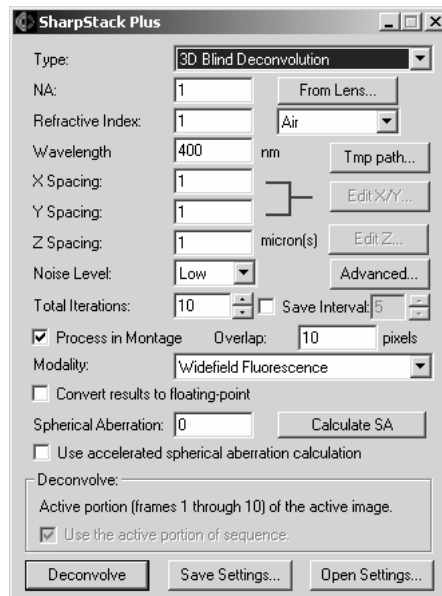
**Note:** **No Neighbor** is the only valid option when the active image is not part of a Z stack.

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- **Nearest Neighbor:** When selected, the stack or image will be deblurred using the **Nearest Neighbor** algorithm.
- **Inverse Filter:** When selected, the stack or image will be deconvolved using the *Inverse Filter* algorithm.

If you have purchased *SharpStack Plus*, *Confocal*, or *Total* you will have these additional deconvolution methods available:



- **2D Blind:** *2D Blind deconvolution* allows you to apply the Blind (Adaptive) deconvolution to a single two-dimensional widefield or brightfield image without requiring previous knowledge of the microscope parameters or the image parameters.
- **3D Blind:** *3D Blind deconvolution* is a method of deconvolution that adapts itself to the real PSF of the microscope system (which can be significantly different from the theoretical PSF and from the previously measured PSFs) due to specimen and instrument variations.

**NA:** This number indicates the numerical aperture of the lens used in capturing the images. This affects the effective resolution and the point spread function used in the deconvolution. This information is generated from the AFA or other acquisition program.

**Refractive Index:** This number indicates the refractive index of the lens immersion medium. The representative values for air, oil, water, or

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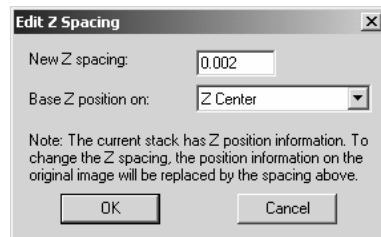
glycerin can be entered automatically, by selecting that medium from the list. This information is generated from *AFA* or another acquisition program.

**Emission Wavelength:** This number indicates the emission wavelength used in imaging the sample, expressed in nanometers. For brightfield imaging, a value in the middle of the range is preferred, for example 550 nm as a center wavelength for visible light.

**X Spacing:** This is the calibrated **X** distance per pixel in microns. This will be editable only if the active image is not calibrated, in which case the **Edit** button will be disabled. When the image is calibrated, this will be a read-only display of the calibration, and the **Edit** button will be enabled. Clicking the **Edit** button will display the **Spatial Calibration** dialog from *Image-Pro Plus*, which allows you to edit the image's spatial calibration.

**Y Spacing:** This is the calibrated **Y** distance per pixel in microns. This will be editable only if the active image is not calibrated, in which case the **Edit** button will be disabled. When the image is calibrated, this will be a read-only display of the calibration, and the **Edit** button will be enabled. Clicking the **Edit** button will display the **Spatial Calibration** dialog from *Image-Pro Plus*, which allows you to edit the image's spatial calibration .

**Z Spacing:** This is the calibrated **Z** distance between images, measured in microns. Clicking the **Edit** button displays the following dialog:



Enter your new **Z** spacing and position values and click **OK**.

**Haze Removal:** This indicates the amount of haze removed from the sample during background correction. This value can range from 1% (almost no correction) to 100% (full correction). This control will be hidden if the **Inverse Filter** type is selected. In most cases, a value of 90% – 99% is used.

**Neighbor Spacing:** This number determines which neighbor plane will be used by the **Nearest Neighbor** deblurring. Under normal conditions, this should be set to 1, where the adjacent planes (the plane just above

and just below the processed plane) will be used. However, if the stack is acquired with a very tight Z spacing (so there is very little difference between adjacent planes, and very little point spread), a larger value (2 to 5) may produce better results. This value is disabled for **No Neighbor** and **Inverse Filter** deconvolution types.

**Process in Montage:** Checking this box determines whether the inverse filter will be applied to sub-regions or to the entire image stack. Processing on sub-regions speeds up the algorithm. It also may speed up the processing time when computer memory is limited. This control will replace **Haze Removal** when the **Inverse Filter** type is selected, and will be hidden otherwise. Some images may produce discernable discrepancies between the subregions.

**Overlap:** This control allows you to edit the montage overlap, from 1 to 100 pixels (a value larger than the width of the image is not valid). Note that the overlap may be ignored even when **Process in Montage** is specified, if the image is small enough that montage processing is not necessary.

**Brightfield :** Checking this box inverts the brightfield image, creating a bright object on a dark background so that it will emulate fluorescence.

**Phase Objects:** Checking this box indicates that a phase object is being processed. This is the case if no stain is used with a brightfield microscope and the condenser is stopped down to cause a contrast due to phase character.

Using **phase objects** allows the deconvolved pixels of a brightfield specimen to take on values that are brighter than the background, which can happen with phase objects due to a lens effect in the sample. Although this feature is designed primarily for brightfield phase-object samples, it is found to be helpful in rare occasions with fluorescent samples, for preserving very fine low intensity speckle that surrounds bright objects. Hence, this flag can be set for fluorescent samples as well although the term “phase object” does not accurately apply. If it is set for fluorescent samples (i.e., when the phase-object flag is set and the brightfield flag is not set), then the deconvolved data values will be allowed to take on values that have less intensity than the background. In this case, in effect, the minimum value is allowed to be negative.

These settings will be initialized to the appropriate values for the stack if the values are available as properties of the stack or in the *Set Manager*, otherwise they will default to the last settings used.

**Convert results to floating-point:** Checking this box will convert the results of the processing to a floating-point image.

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**Spherical Aberration (SA):** This control specifies the spherical aberration correction, from -15 to +15 (no correction is zero). The values used for SA calculations are “waves.” SA is used only with **Inverse Filter**, and **2D-** and **3D-** blind methods that will be available if you have *SharpStack Plus*, *Confocal* or *Total*. You may obtain an estimated value using the **Calculate SA** button. This will fill in the SA value with the estimate, which then can be used in conjunction with the inverse filter. An estimated value between -3 and 3 indicates that there is probably no significant spherical aberration and should not be used (enter 0 for no correction). You may be able to see some improvement in the correction when the values are from -3 to -1 and from 1 to 3, but would generally be imperceptible. If you have already performed a SA estimate calculation for the conditions under which the image was collected, you may enter that previously calculated SA value directly.

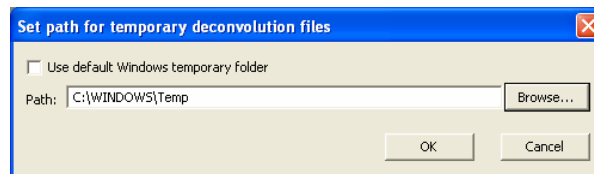
**Calculate SA:** This button calculates the spherical aberration and displays the results in the **Spherical Aberration** control so the user can see whether the wave values are reasonable (please see the discussion of **Spherical Aberration** at the end of this section).

**Use accelerated spherical aberration calculation :** Checking this box increases the speed at which these calculations are performed. However, there might be some sacrifice of accuracy.

**Noise level:** The noise level can be set to **Auto**, **Low**, **Medium**, or **High**. The appropriate setting depends on the noise level in your image set. Best results must be determined empirically.

If you are using *SharpStack Plus*, *Confocal*, or *Total*, you will have the following options for **2D-** and **3D-Blind** deconvolution:

**Tmp. Path..** The **Tmp Path...** button sets the folder for temporary files generated during deconvolution.



If the **Use default Windows temporary folder** box is checked, the temporary files are saved in the *Windows* default directory. You may also select a different folder using the **Browse...** button.

**Total Iterations:** The **Total Iterations** field allows you to choose the total number of iterations performed during a deconvolution. The higher this number the more times the data will be processed, the more time it will take, and generally will provide improved

### *Advanced Menu:SharpStack*

resolution. Beyond a certain number of iterations, depending upon the imaging modality (widefield or confocal), the deconvolution may provide unstable results. 60 is the recommended number of iterations.

**Save Interval:** The **Save Interval** control allows you to select the number of iterations that will occur between storage of deconvolution results on the disk. The possible values are integer from 1 to the number of **Total Iterations**. For example, if the number of **Total Iterations** is 20 and the **Save Interval** is 5, the deconvolution will be saved at 5, 10, 15 and 20 iterations. The deconvolution application automatically checks for available disk space before beginning deconvolution. If the **Save Interval** box is not checked, no intermediate results will be saved.

**Modality:** The microscope **Modality** can be one of the following:

- Widefield Fluorescence
- Transmitted-light Brightfield

**Advanced:** The **Advanced...** button opens an advanced/expert options dialog, which include specific 2D and 3D deconvolution parameters. The advanced options are described in the next section.

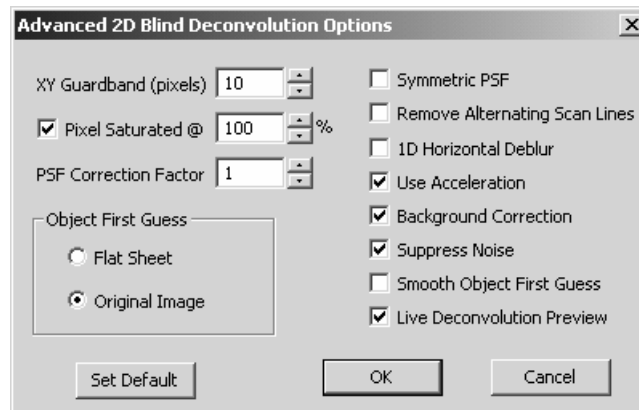
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**Note:** As a general rule, **DO NOT MODIFY THE ADVANCED SETTINGS** until you have attained expertise in using *SharpStack*. Advanced settings should not be adjusted from their original default settings except in rare cases. Do not change them casually.

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## Advanced 2D Blind Deconvolution Options

When you select **2D Blind** and click the **Advanced** button, you will see the following selections:



**XY Guardband (pixels)** : **XY Guardband (pixels)** size defines the width in pixels of the border surrounding each subvolume. This border is the region where subvolumes are processed to seam them together. This guardband prevents artifacts at the seams.

**Pixel Saturated:** The **Pixel Saturated** is a flag that indicates if the image contains saturated pixels. The value defines the percentage of maximum intensity necessary for the pixels to be considered saturated.

**PSF Correction Factor:** The **PSF Correction Factor** defines the number of PSF iterations per cycle. The default setting is 1.

**Object First Guess:** The **Object First Guess** is the initial estimate for the blind deconvolution. There are two options: **Flat Sheet**, **Smoothed Raw Image**, and **Original Data**. The default is **Original Data**.

- **Flat Sheet** uses a volume filled with a constant value equal to the average of the entire image stack as the first guess. For very noisy data, this is an appropriate starting point for the deconvolution.
- **Smoothed Raw Image** uses a smoothed version of the original image as the object for the first guess.

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- **Original Data** uses the original image data as an initial estimate. This uses the input image as the first guess at the restored image. This is the default selection.

**Symmetric PSF:** Checking this box forces the PSF to be symmetric when rotated by 90 degrees.

**Remove Alternating Scan Lines:** Checking this box toggles the routine for removing scan line artifacts on or off.

**1D Horizontal Deblur:** Checking this box will deblur the image only in the horizontal direction. Use this selection when **Remove Alternating Scan Lines** isn't sufficient.

**Use Acceleration:** Checking this box accelerates the process of detecting the spherical aberration of the data. Accuracy is slightly better with acceleration NOT selected, but the tradeoff of speed with the acceleration on is greater than the accuracy compromise.

**Background Correction:** Checking this box corrects background subtraction using the minimum intensity value.

**Suppress Noise:** Checking this box toggles noise compensation on or off.

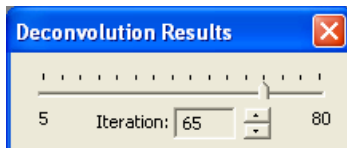
**Smooth Object First Guess:** Checking this box toggles smoothing of the initial image estimate on or off.

**Live Deconvolution Preview:** When the **Live Deconvolution Preview** box is checked, the deconvolution process is shown live on the preview image.

**Set default:** Clicking the **Set default** button sets the default parameters for the active modality.

## 2D Blind Deconvolution Results

When a deconvolved image is created, the **Deconvolution Results** dialog is shown:



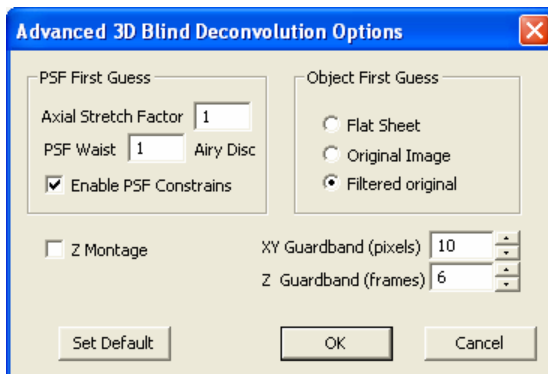
This dialog allows you to select the image for viewing and processing after a number of iterations.

The **iteration** can be changed starting from the number defined in the **Save Interval** to the number of **Total Iterations**. The value can be changed with **Save Interval** steps.

This dialog is not shown if the active image is not a result of blind deconvolution.

## Advanced 3D Blind Deconvolution Options

When you select **3D Blind** and click the **Advanced** button, you will see the following selections:



**Axial Stretch Factor:** The **Axial Stretch Factor** is used to calculate the theoretical PSF (Point Spread Function) that is lengthened along the Z axis to more accurately initiate the first guess for a confocal data set. The default **Axial Stretch Factor** is 1 for widefield data and 3 for confocal data.



**Enable PSF Constraints:** Checking this box removes the limitations placed on the Point Spread Function (PSF). It is recommended that the **Enable PSF Constraints** box be unchecked (disabled).

**PSF Waist :** This value indicates the size of the narrowest part of the PSF, usually measured in *Airy Disc* diameters. The **PSF Waist** default setting is 1 for both widefield data and confocal data.

**Z Montage: Checking the Z Montage** box breaks the data set into sections along the optical axis and deconvolves these subsections separately. Its default setting is disabled (unchecked). This option has a similar effect as the **Process in Montage** option in the main Deconvolution dialog, which breaks the data into subvolumes along the XY dimensions.

**XY Guardband (pixels):** This value indicates the size in pixels of the width of a border surrounding each subvolume. This border is the region where subvolumes are processed to seam them together. This guardband prevents artifacts at the seams.

**Z Guardband (frames):** This value specifies the number of slices that will be added at the top and bottom of the subvolume. This guardband prevents artifacts at the seams.

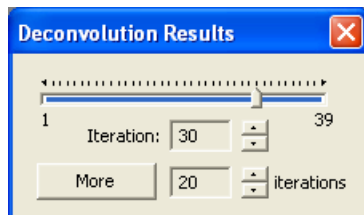
**Object First Guess :** The **Object First Guess** is the initial estimate for the blind deconvolution. There are two options: **Flat Sheet, Smoothed Raw Image**, and **Original Data**. The default is **Original Data**.

- **Flat Sheet:** The **Flat Sheet** option uses a volume filled with a constant value equal to the average of the entire image stack as the first guess. For very noisy data, this is an appropriate starting point for the deconvolution.
- **Filtered original** uses a filtered version of the original image as the basis for the first estimate. This is the default selection.
- The **Original Image** uses the original image data as initial estimate. This uses the input image as the first guess at the restored image.

**Set Default:** Clicking the **Set default** button sets the default parameters for the active modality.

### 3D Blind Deconvolution Results

When a deconvolved image is created, the **Deconvolution Results** dialog is shown:



This dialog allows you to select the image for viewing and processing after a number of iterations.

The **iteration** can be changed starting from the number defined in the **Save Interval** to the number of **Total Iterations**. The value can be changed with **Save Interval** steps.

Clicking the **More** button will apply additional iterations to the image.

This dialog is not shown if the active image is not a result of blind deconvolution.

**Deconvolve:** This group box indicates the type of **Z** stack that the deconvolution will be applied to when the **Deconvolve** button is clicked.

**If the image is part of a set:** The *Set Manager* will be asked to provide the set ID for the image. The position of the active image's current frame will be inquired, and also the length of the **Z** dimension at that position in the set. The **Z** stack this represents will be the stack to be processed. The **Deconvolve** group will report the deconvolution as applying to the **current Z stack of the set <set name>**.

**If the image is a sequence and has Z position information:** The **Z** positions of the frames of the sequence will be checked to see if the positions repeat, indicating more than one time point, channel or X/Y position is contained in the active sequence. The set of unique **Z** positions that contains the active frame will be processed.

The **Deconvolve:** group will report the deconvolution as applying to frames **<start> to <end> of the image**.

**If the image is a sequence and Z position is not known:** The active portion of the sequence will be processed. (Note that the active portion and Apply to Sequence is ignored for the first two cases.) The

**Deconvolve** group will report the deconvolution as applying to the **active portion of the image**, and the **active portion** checkbox will be checked and disabled.

**If the image is not a sequence:** The image will be treated as a single frame, which means that only the **No Neighbors** algorithm can be applied. The **Deconvolve** group will report the deconvolution as applying to the **image frame**, and the **active portion** checkbox will be hidden.

**Use the active portion of sequence:** Checking this box overrides the default behavior based on the set membership and/or property information of the image. It can also be used to select a small set of frames to test the deconvolution parameters and preview the resulting deconvolution.

**Deconvolve:** When you click this button, the currently selected deconvolution methodology with the active associated parameters will be applied to the image stack. The results will appear in a new workspace.

**Save Setting...:** Click this button to save your deconvolution parameter settings in a \*.DCV file.

**Open Settings...:** Click this button to open a deconvolution parameter settings \*.DCV file.

**From Lens...:** This button (not shown) will only appear when *SharpStack* is run from within *Image-Pro Plus 5.0*. It will appear next to the numeric aperture (NA) entry box, and will allow the user to select an existing lens information file or define a new one. The NA and Refractive Index (RI) will be taken from the selected lens information file and will replace the current NA and RI settings.

## 3D Constructor

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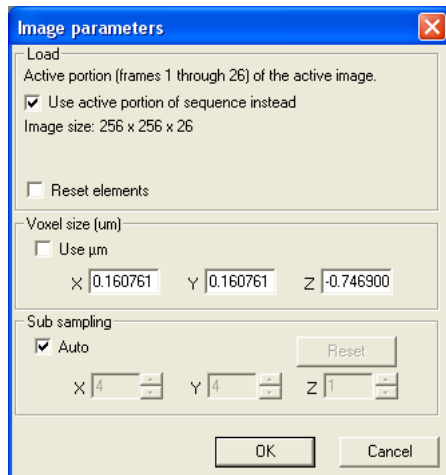
*3D Constructor v. 7.0* includes a volume renderer, animation tools, and measurement tools. Volume rendering enables you to view an image stack, or a portion of an image stack, from any angle. You can also adjust the transparency and shading of the image. Animation allows you to create a moving image sequence. The measurement tools allow you to make manual and automatic measurements of object dimensions and volumes.

In volume rendering, light emission and light absorption parameters are assigned to each point of the volume. Each of these points is called a *voxel*; that is, a 3-dimensional version of a pixel. Voxels have height, width, and depth, as well as intensity characteristics. Simulating the transmission of light through the volume makes it possible to display your data from any view in any direction.

The *3D Viewer* window allows you to examine an image stack in a variety of different ways: by volume, in thick or thin slices, and as an animated display. In the *3D Viewer*, all geometric objects are shown in 3D space. It can be regarded as a virtual camera which can be moved to an arbitrary position within or around the 3D scene.

The first time that you select *3D Constructor* from the *Advanced* menu, you will see the **Image parameters** dialog, which loads the active image into the *Viewer*:

The **Image parameters** dialog determines the rendering of the active stack.



- The **Load** group box determines which images to load:
  - **Use active portion of sequence instead:** The default behavior loads the current Z stack of the current set. This checkbox allows you to use only a portion of the active sequence, instead of the current stack. The **image size** is given as width x height (in pixels) x depth (in number of frames). (Please see the note under **Time** if you want to view a single time point.)
  - **Load As:** If you are loading a color image, a drop-down list box appears, giving you a choice about how to display your color image: Color images can be displayed as:
    - **Color Image:** All channels are loaded into an indexed color volume (The sequence is converted to a palette class using a palette containing the most popular colors in the sequence).
    - **Red Channel:** The red channel of the image is displayed and a red palette is created.
    - **Green Channel:** The green channel of the image is displayed and a green palette is created.
    - **Blue Channel:** The blue channel of the image is displayed and a blue palette is created.

If you are loading a black and white sequence, it is displayed unmodified and a gray palette is created.

- **Reset Elements:** When the **Reset Elements** checkbox is selected, all added elements, such as the manual measurements, volume measurements, external objects, slices, etc. are removed and the measurements options are reset before loading the volume.
- **Hi-color:** If the **Hi-color** control is active a color image will be loaded in **Hi-color** mode. Color presentation of images in this case may be better, but this mode uses four times more video memory for rendering compared to palette mode, and palette adjustments (opaque limits, color channels) will involve automatic reloading of the volume. When this option is *off*, the volume is loaded in 256-color palette mode.

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*Note:* 256-color palette mode supports interactive brightness/contrast/gamma adjustments throughout the palette settings.

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- **Voxel size:** This group box defines the dimensions of one voxel (a pixel in 3 dimensions). The values displayed are the last ones

### Advanced Menu:3D Constructor

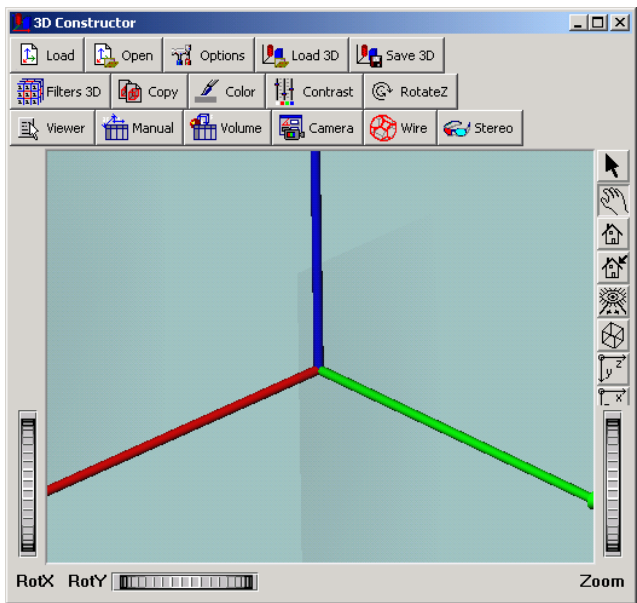
used. If the image does not have a spatial calibration, and no system calibration has been set, the values are 1, 1, 1. If the image is spatially calibrated, the X and Y values and the units are taken from the calibration. If the active sequence or stack includes information about the Z frame position, the Z size is set from the distance between first and second frame in the sequence. If Z information is absent, Z size is set to the average value of X and Y sizes. These values can be modified to achieve the desired volume ratio.

- **Use  $\mu\text{m}$  (microns):** If this box is checked, the values are presented in microns. If the box is not checked, the units of the image's spatial calibration are used. (For more information about spatial calibration, please refer to your *Image-Pro Plus Reference Guide*.)
- **Sub sampling:** This group box defines the sub-sampling values for loading images. Default values are 1,1,1. The values can be set to higher numbers for larger images, to decrease loading time and increase performance. However, the image quality in this case will be lower.
- **Auto:** Selecting the **Auto** checkbox activates automatic sub-sampling mode. In this mode, the sub-sampling is calculated based on the size of the image. This provides faster loading and rendering of Volume images, but the maximum size of a loaded volume in any direction is limited to 64 voxels. By default this option is *on*. Advanced users can switch this option Off and set sub-sampling manually.
- **Reset:** Clicking the **Reset** button clears all your volume settings and sets 1:1:1 sub-sampling. The **Reset** button is disabled when **Auto** is on.

### 3D Viewer Tools and Buttons

There are a number of tools, buttons, and icons to help you examine the data in the **3DViewer**.

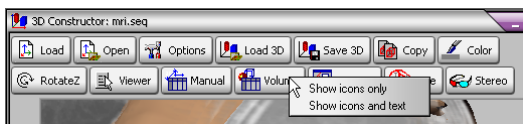
Once you have entered the required information in the **Image Parameters** dialog, and clicked **OK**, you will see the following dialog:



The **3D Viewer** dialog contains controls to zoom, rotate, and modify the image. The following controls appear at the top of the window:



In version 7.0 of **3D Constructor**, you have the option of viewing both icons and text on the toolbar buttons, or just the icons. To turn the button text on or off, place your cursor over one of the buttons and click the right mouse button. Then you can choose **Show icons only** (above) or **Show icons and text**, as in the picture below:



**Show icons and text** is the default for the main *3D Viewer* toolbar. The default for other *3D Constructor* toolbar is **Show icons only**.

---

**Note:** The *3D Viewer* documentation in this section sometimes refers to a “camera”. This does not mean a physical camera, but a virtual camera, as if the *Viewer* were actually the view-finder in a typical camera. Changes in the *Viewer’s* perspective or point of view can be considered as changes in the position of the camera.

---



**Load new volume:** Clicking this button loads the active sequence from *Image-Pro Plus* into the *3D Viewer*. You can also use this button to change the image stack under analysis.



**Open volume from disk:** Clicking this button opens the image file from disk, creates a color composite sequence (if possible and the “*Generate color composite automatically on image open*” option of the **Preferences** dialog of *Image-Pro Plus* is active) and loads the image to into *3D Constructor*.



**3D Constructor Options:** Clicking this button opens a dialog with active 3D elements and controls to adjust these elements.



**Load 3D Constructor file:** Clicking this button loads rendering settings (\*.ren) and 3D elements from a settings file. It also allows you to load *3D Constructor* experiment files (\*.S3D). The settings files contain all of the *Viewer* settings that you can adjust from the **Options** dialog (which are discussed later in this chapter).



**Save 3D Constructor file:** Clicking this button saves rendering settings (\*.ren) and 3D elements to a settings file and 3D experiment information to an experiment file (\*.S3D).



**Filters:** Clicking this button opens the **3D Filters** dialog.

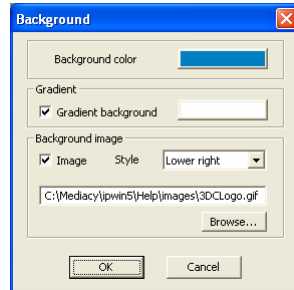


**Copy to Clipboard:** Clicking this button copies the active view to the clipboard as a picture.





**Background options:** Background options allow you to select the background color of the 3D viewer. Clicking this button displays the following dialog:



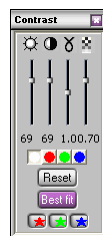
**Background color** defines the background color when the **Gradient background** is off. (default)


When the **Gradient background** control is *on*, (box is checked) the color gradient is drawn as a background for a scene. The **Background color** appears at the top of the window and the second color appears at the bottom of the window.


When **Image** options is *on* (box is checked), an image will be displayed as the background. The following image formats are supported: BMP (Windows only), GIF, JPEG 2000, JPEG, PGX, PNG, PNM, SGI RGB and TIFF.





**Brightness, Contrast, Gamma:** Click this button to see the **BCG** dialog.



 slider adjusts volume brightness.

 slider adjusts volume contrast.

 slider adjusts volume gamma.

 control adjusts volume transparency.

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The color buttons  define the channel to adjust: All, Red, Green and Blue.

The **Reset** button resets the controls to their default settings.

The **Best fit** button sets the contrast and brightness controls according to the optimized display range of the image.

The color checkboxes  allow you to switch the color channels on or off.



**Rotation Z:** Click this button to rotate the image around the **Z** axis of the viewer (which is perpendicular to the screen). This button rotates the view clockwise by 15 degrees. If you hold down the <Ctrl > or <Shift>keys when you click the button, the view is rotated counterclockwise.



**Show manual measurements data table:** Click this button to show the manual measurements data table.

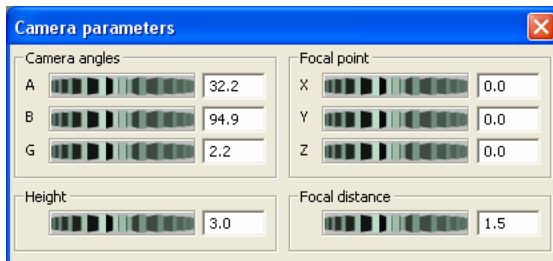


**Show volume measurements data table:** Click this button to show the volume measurements data table.



**Show camera parameters dialog:** Click this button to display the **Camera Parameters Dialog**, which allows you to adjust the settings for the camera viewer.

You will see the **Camera Parameters** dialog:



This dialog displays the major parameters used to set the camera viewer:

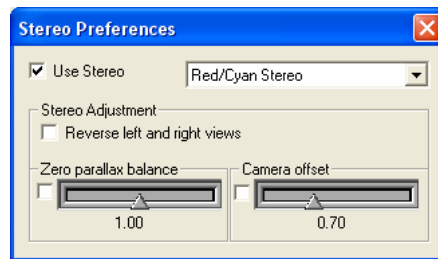
- **Camera angles:**
  - A** – alpha angle, the angle between the camera orientation vector and the Z axis.
  - B** – beta angle, the angle between the projection of the camera orientation vector on the XY plane and the Y axis.
  - G** – gamma angle, the angle of camera rotation around the orientation vector. This angle also controls the Z-rotation (the same as the **Rotation Z** button in the toolbar).
- **Height angle/ Height:** The height angle for perspective cameras defines the height of the viewing angle for the camera. For orthographic cameras, this control defines the height of the viewing area in world coordinate units.
- **Focal Point:** Focal point defines the point at which the camera is pointing
- **Focal distance:** This indicates the distance between camera and focal point. The distance and focal point coordinates are in world coordinates units.



**Toggle wire frame mode:** Clicking this button turns the wire frame display mode on or off.



**Toggle stereo mode:** Clicking this button turns the stereo display mode on or off. If this mode is on, you will see the **Stereo Preference** dialog:

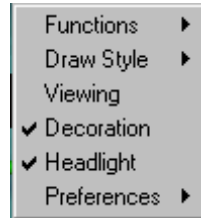


Use the controls on this dialog to adjust your stereo preferences.



**Viewer options:** Clicking this button invokes the viewer popup menu. You can also see these options by right-clicking on the image in the *3D Viewer* (these options are discussed in the next section).

### Advanced Menu:3D Constructor



The popup menu items allow you to change most of the viewer properties, such as *drawing mode* (**as is**, **wire-frame**, etc.). There are also popup menu items corresponding to most of the viewer buttons on the right toolbar (**Home**, **Set Home**, **View All**, etc.).

The *Preferences* submenu contains additional viewing options, including stereo support.



Each of these viewing options is discussed later in this chapter.

The following set of buttons appears on the right side of the *Viewer* window. If the window is too small, not all of these buttons may be visible.



**Select/Pick Button:** Use this button to select object manipulation or *Pick* mode (and deselect camera or *View* mode). The cursor shape will change to an arrow. In this mode, the user is manipulating and holding objects in the *Viewer* window. If you are in *Pick* mode, clicking the **<Alt>** key will change to *View* mode. All of the functions associated with the *View* mode can then be accessed using **<Ctl>** and **<Shift>** as well.



**View Button:** Use this button to select camera or viewer mode (and deselect object manipulation or pick mode). The cursor shape will change to a hand icon. In this mode, the user is moving the camera in 3D space, which changes the perspective of the rendering in the *Viewer* window.



**Home Button:** This button returns the camera to its home position.



**Set Home Button:** This button resets the home position to the current camera position.



**View All Button:** Use this button to reduce or enlarge the scene graph, so that it fills the *Viewer* window (but leaves sufficient room for the image to rotate).



**Camera Alignment Buttons:** Use these buttons to move the camera to the X, Y, or Z position. (Also see the section on *Keyboard Shortcuts* at the end of this chapter.)



**Projection Buttons:** Use one of these buttons to select the type of camera used by the *Viewer*. These buttons will toggle between the two available camera types: perspective or orthographic.

The *3D Viewer* also uses the wheels (virtual trackball) at the right, left, and bottom to move the image around within the viewer window. These controls are only available with the perspective camera. They are labeled **Rot-X** to move in the X direction, **Rot-Y** to move in the Y direction, and **Zoom** to move in or out of the 3D view. **Dolly** moves your viewpoint back and forth toward or away from the 3D image. **Pan** moves your viewpoint right or left.

The *Viewer* also uses some special mouse controls:

- **<Left>** Click the left mouse button to rotate the virtual trackball.
- **<Ctrl>+<Left>** or **<Shift>+<Left>**. Use one of these combinations to pan across the image in the viewer.
- **<Ctrl>+<Shift>+<Left>** Use this combination to move back and forth (dolly) toward or away from the image.

### 3D Viewer Options

The *Viewer Options* popout menu contains the following items:

- **Functions:** This selection displays the following choices (equivalent to the buttons on the right side toolbar):
  - **Home** - returns the camera to the home position.
  - **Set Home** – resets the home position to the current camera position.
  - **View All** – reduces or enlarges the screen graph so that it fills the viewer window, but leaves sufficient room for the image to rotate.
  - **Seek** – sets the viewer in Seek mode. After selecting this item, the cursor changes to a “target” shape. Click on the desired point with the left mouse button. After clicking on the point, the camera will be automatically moved to view this location, i.e. the “seek point”. This function works only on orthogonal and oblique slices in viewing mode. (See the *Seek to Point* option, below).
- **Draw Style:** This selection gives you a long list of choices for displaying the volume in the *3D Viewer*.
- **Viewing:** Toggles the viewer between *Viewing* mode (“hand” cursor) and *Selection* mode (“arrow” cursor). Viewing mode allows you to use the mouse for rotating and moving the object. When this mode is switched off, the *Viewer* is in Selection mode, where you can manipulate the image with the handles on the VOI or slices.
- **Decoration:** This selection turns the display of the wheel controls and the right side toolbar buttons on or off.
- **Headlight:** This selection switches the headlight *on* or *off*. The headlight is the directional light that follows the path of the camera.
- **Preferences:** Clicking on the *Preferences* menu item displays the following choices:
  - **Seek to Point** - Related to the **Seek** option in the *Functions* sub-menu. When the **Viewer** is in **seek** mode, clicking the left mouse button picks a point, and the viewer changes its

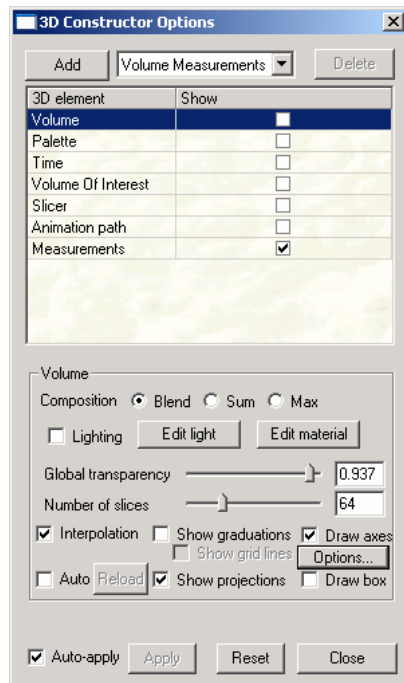
*Advanced Menu:3D Constructor*

orientation and position to look at that point. This routine determines whether to orient the camera towards the selected point (**Seek to Point on**), or towards the center of the object's bounding box (**Seek to Point off**) of the object or slice.

- **Auto-Clip Planes** - Sets the auto clipping plane. When auto clipping is *on*, the near and far planes closest/furthest from the camera are dynamically adjusted to be as tight as possible around the objects being viewed.
- **Spin Animation** - Enable/disable the spinning animation feature of the **Viewer**.
- **Rotation Axes** – Shows or hides the X, Y, and Z axes in the **Viewer**.
- **Stereo Support** invokes stereo support dialog. When **Stereo** mode is on the viewer generates images for left and right eyes, presenting stereo view of the scene. Raw stereo requires *OpenGL* hardware stereo support.

## 3D Constructor Options

The *3D Constructor Options* dialog allows you to control which aspects or dimensions of the image stack are displayed in the **Viewer**.



This dialog includes a list of the 3D elements that are available in the current session.

- **Add:** Click this button to add more elements to your list, in addition to those that are automatically created when you start the **Viewer**. The drop-down list box contains names of the various elements that are available to be added. Multiple instances of these elements may be added to your list.
- **Delete:** Click this button to remove the selected element. The button is disabled if the element can not be deleted.
- **Show:** Select this check box to show or hide any of the elements in the viewing window. A set of controls corresponding to the selected element is shown below the list.



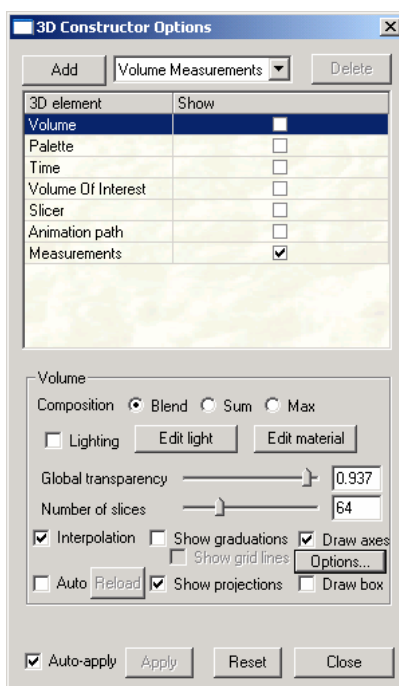
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- **Apply:** Click this button to apply your most recent changes to the volume in the viewing window.
- **Auto-Apply:** Select this check box to enable or disable image updates when the parameters are changing. If the updates are slow, you should uncheck **Auto-apply** and apply changes using the **Apply** button.
- **Reset:** Click the **Reset** button to return all parameters to their default settings. All the added elements, such as iso-surface, ortho-slices, or oblique slices will be deleted.
- **Close:** Click this button to close the *3D-Constructor* dialog box.
- **3D element:** This group box lists the names of the currently available 3D elements. Highlight the selected element with your cursor to see the options available with it. An element can be edited by double clicking on the name and modifying it. **Volume, Palette, VOI, Slicer,** and **Animation** are all created by default and can only exist as single entities. You cannot add features to these elements, or delete the ones created by default. Each of the available elements is described in the following sections.

## Volume

You can display the controls for any one of these elements, without activating that element, by clicking on that element's name outside of the checkbox.

The **Volume** controls allow you to display of the volume in the *3D Viewer* window. Highlighting the **Volume** element displays the following controls:

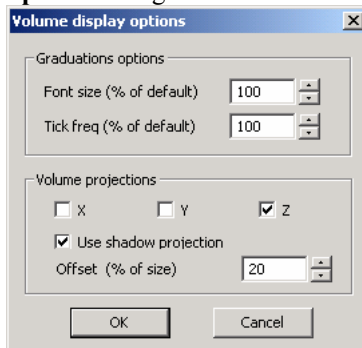


- **Composition:** These radio buttons select the image composition method.
  - **Blend:** Blending averages the values of the voxels in a straight line through the volume of the object.
  - **Sum:** This method displays the sum of the value of the voxels in a straight line. It is most useful for dark images.
  - **Max:** *Maximum Intensity Projection (MIP)* takes the value of the brightest voxel in the same straight line as used by the **Blend** option.

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- **Global transparency:** This slider controls the degree of transparency of the volume. Moving the slider to the left makes the entire volume progressively more transparent. The global transparency value is updated as the slider moves, if you have selected **Auto-Apply**. You can also type a value in the edit box.
- **Number of slices :** This slider controls the number of slices drawn for 3D and 2D multi-texture map rendering. Zero means to automatically select the number of slices. Rendering fewer slices will generally improve performance (with reduced image quality).
- **Interpolation:** Select this check box to switch on linear interpolation on texture maps, which increases the visible resolution.
- **Draw box:** Select this check box to display a bounding box that encloses the entire volume.
- **Draw axes :** Select this check box to display axes that show the orientation of the volume (Red for the X axis, Green for the Y axis, Blue for the Z axis). The origin of the axes is at the 0,0,0 location.
- **Show graduations:** Selecting this check box displays any graduation marks on the rendered cube.
- **Show grid lines:** Selecting this check box displays grid lines inside a graduated cube.
- **Show projections:** When illumination is shown, checking this box projects a virtual shadow on the volume in the viewer. The virtual shadow follows the apparent origin of the light source and displays the volume projections along the x-, y-, and z-axes.

- **Options:** Click this button to see the **Volume Display Options** dialog. You will see the following dialog:



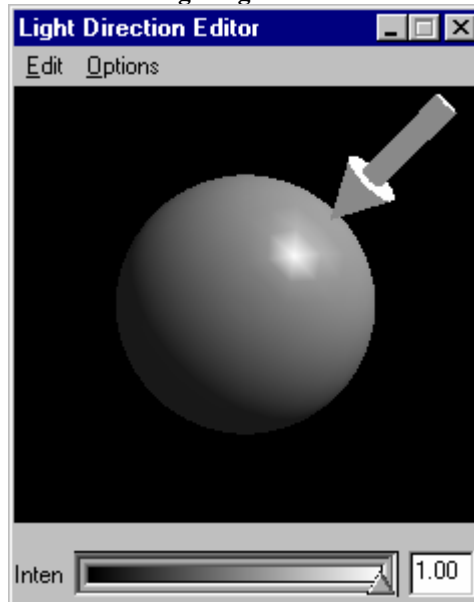
- The **Graduations options** are calculated based on the volume size. The default values for both **Font size** and **Tick frequency** are set to 100%. You can adjust them using the spin buttons. Tick frequency can be adjusted only by factors of 100.
- The **Volume projections** group box controls the display of the projections on the volume in the viewer. The **X**, **Y**, and **Z** check boxes indicate which projections (along which axes) are shown. When **Use shadow projections** is checked, the projections are shown as volume shadows. The **Offset** defines the distance of the projections from the volume boundaries in percent of size along each dimension. **Auto:** Checking this box turns the **Auto-reload** mode on or off. When the control is on, the volume is reloaded automatically when it after it was modified in *Image-Pro*. This option can be used to test various 3D filters. In this case the volume will be reloaded after applying a filter or after an **Undo** operation
- **Reload:** Click this button to reload the image with the possibility of changing color channel, voxel size and sub-sampling. The **Reload** dialog is the same as **Load New Volume**. The difference is that **Load New Volume** loads the active workspace from *Image-Pro* and the **Reload** button reloads the image already opened in the *3D Viewer*.
- **Lighting:** The check box controls a directional light, initially aimed at the volume from the +Z axis. This light rotates with the volume. Using texture map rendering, lighting is approximated.

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*Note:* Turning the light on and off or changing the light direction may require you to reload the textures, which may change the performance of your rendering.

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- **Edit light:** Clicking this button activates the directional light editor. **Edit light** only affects the image displayed in the *3D Viewer* if the **Lighting** check box is selected.



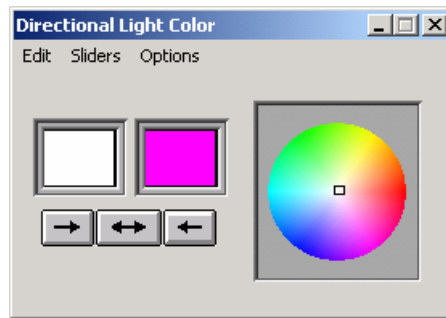
The gray sphere shows how the light will shine on the volume. The yellow arrow shows the current direction of the light. You can use your cursor to grab this arrow with the mouse and drag it to change the light direction. When you are finished with the editor, click the **X** button in the window banner to close the editor and register your changes.

The *Edit* menu allows you to bring up a color editor to change the headlight color. It also allows you to copy the current headlight properties to the *Windows* clipboard, and apply them to the scene later.

The *Options* menu contains only one item: *Always on Top* allows you to keep the **Light Direction Editor** dialog on top of the other windows in the *Image-Pro* workspace while you are editing.

### Directional Light Color:Edit

The *Edit* menu on this dialog brings up the **Directional Light Color** dialog. This dialog allows you to change the intensity and direction of the light on the volume in the *Viewer* window. Use the arrows under the color windows to select the area and direction for your color changes.



*Edit: Continuous.* Selecting this option indicates that the color changes will be automatically reflected in the **Light Direction Editor** dialog and on the volume in the *Viewer*.

*Edit: Manual:* Selecting this option indicates that you will have to manually update the color changes in the **Light Direction Editor** and on the volume in the *Viewer* by clicking and dragging the yellow arrow.

*Edit:WYSIWYG:* This common acronym for “what you see is what you get” indicates that the changes are dynamically reflected in the volume in the workspace.

*Edit:Copy:* Click this option to copy the color values to the *Windows* Clipboard.

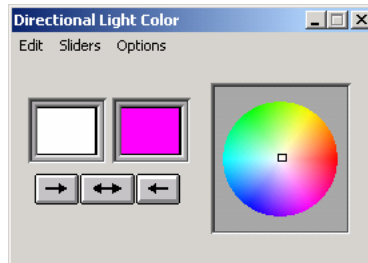
*Edit:Paste:* Click this option to paste the color values from the *Windows* Clipboard.

*Edit:Help:* Click this option to bring up the context-sensitive help.

### Directional Light Color:Sliders

This menu controls how many sliders are available to help you control the color and light of the volume editor.

*Sliders: None.* No sliders are available if you select this option.



*Sliders: Value.* One intensity value slider is available:



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*Sliders:RGB:* Three sliders, one for each RGB channel, are available:



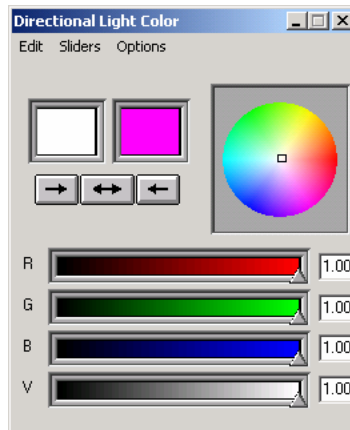
*Sliders:HSV:* Three sliders, one for each HSV channel, are available:



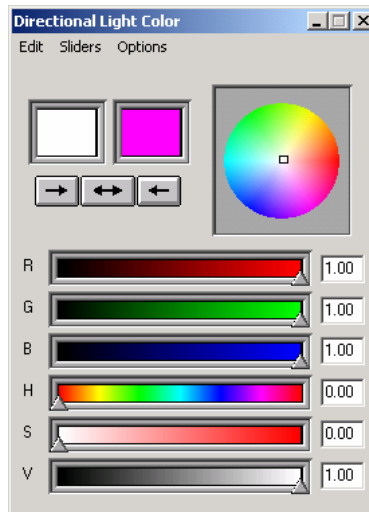


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*Sliders: RGB V:* Four sliders, three for RGB and a fourth for intensity value, are available:



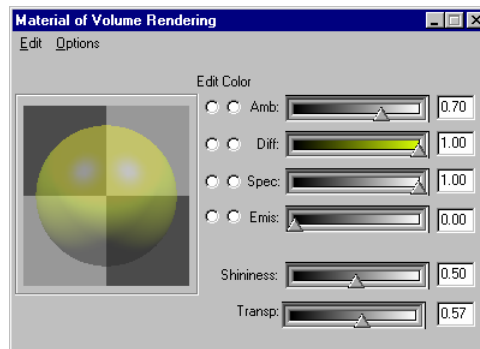
*Sliders:RGB HSV:* Six sliders, three for RGB and three for HSV channels, are available.



## Advanced Menu:3D Constructor

*Options:* The *Options* menu contains only one item: *Always on Top* allows you to keep the **Directional Light Color** dialog on top of the other windows in your workspace.

- **Edit Material:** Clicking this button on the **Volume** element in the *3D Constructor Options* brings up the material editor, which allows you to modify the display of material properties of the volume.



Your choices on this dialog include:

- **Amb: Ambient Reflection** uses the available light normally present to light the entire surface plot image. Ambient light is of uniform brightness caused by multiple reflections of light from the many surfaces present in most environments. This component presents the surface color, which is set on the **Surface** tab. When **Ambient Reflection** is set to 100, Diffuse to 0 and Specular to 0, the image appears as it does in the *unshaded* mode.
- **Diff: Diffuse reflection** uses diffused light to soften and smooth the surface of the surface plot image. Dull matte surfaces exhibit diffuse reflection, scattering light equally in all directions so that all the surfaces appear to have the same brightness from all viewing angles. When **Diffuse Reflection** is set to 100, Ambient to 0 and Specular to 0, the image appears as it would in moonlight. Objects do not show their own surface color in this case.
- **Spec: Specular reflection** uses a “spotlight” effect to focus bright light on the surface plot image. Specular reflection is best observed on a shiny or glossy surface; the highlighted effect is caused by specular reflection. When Ambient Reflection is set to 100, Diffuse to 0 and

**Specular** to 100, the image appears in a glass or candy-like material, because glass has very low diffuse reflection.

- **Emit: Emitted Reflection** is used when the illumination or intensity of emitted light (glow) will change in the time that it takes to acquire all of the planes.
- **Shininess**: Use the slider to adjust the “reflectivity” of the surface plot image. Matte surfaces have lower reflectivity, shiny or glossy surfaces have higher reflectivity. The range of possible values is 0 -100.
- **Transparency**: Transparency defines how transparent the object or volume is. Transparency range is from 0 to 1, where 0 is opaque and 1 - completely transparent (invisible)

The **Materials Editor** dialog contains a different series of sliders, which allows you to select the type of material and colors for viewing the iso-surface.

### Material Volume Rendering:Edit

*Edit: Color*: Selecting this option brings up the color editor that was described in the previous section.

*Edit:Material*: Selecting this option brings up the **Material Palette** dialog:



This feature allows you to choose the color for the volume displayed in the *3D Viewer*. (Be careful, as you can easily lose the colors by selecting incorrectly.)

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*Material Palette:Edit* This menu allows you to access the color and material editors described previously. You can also copy and/or paste information to and from the *Windows* Clipboard.

*Material Palette:Palette.* This menu allows you to select from the available color palettes.

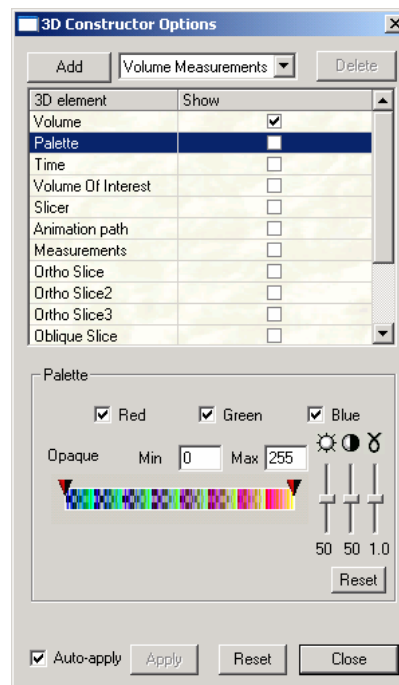
*Material Palette: Options:* The *Options* menu contains only one item at this time: *Always on Top* allows you to keep the *Material Palette* on top of the other windows in your workspace.

- **Accept:** Click this button on the **Directional Light Color** dialog to accept the color, lighting and materials changes that you've selected.

## Palette for Color Stacks

The **Palette** specifies the color map that is used in volume rendering. For color images the color map is loaded from the sequence itself, for gray images any palette can be selected.

When the **Show** item for **Palette** is selected in the 3D elements list, the palette appears on the left side of the image. The **Palette** displays these volume controls for a color image:

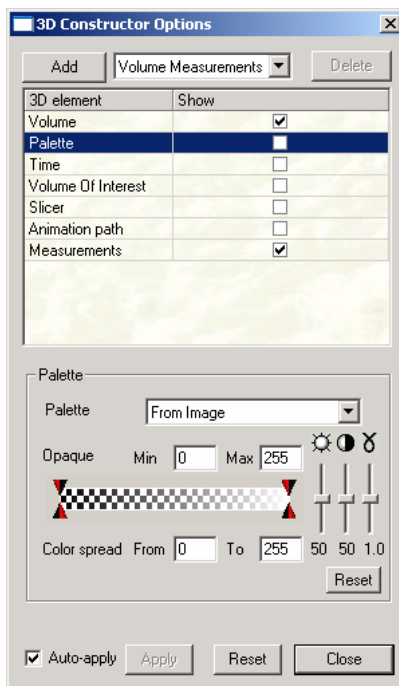


- **Palette:** The **Palette** combo-box allows you to select of one of the predefined color maps, a palette loaded from the image, or a user-defined palette.
- **Red, Green and Blue:** Check the appropriate box to show or hide the visible color channels in the volume displayed in the **3D Viewer**.
- **Opaque Min/Max:** These controls allow you to keep the color map range the same, but force all entries below **Min** and above **Max** to be completely transparent. This is a powerful tool for identifying and highlighting interesting structures in the volume.

The values can also be changed using the top cones on the color map as sliders.

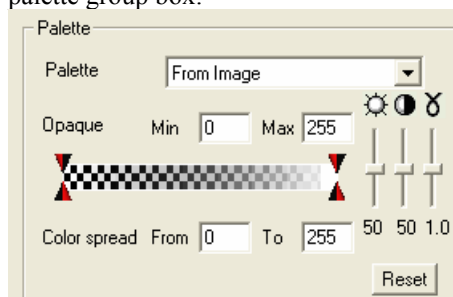
## Palette for Grayscale Stacks

For a grayscale image, the following **Palette** controls are available:



- **Palette:** The **Palette** combo-box allows you to select of one of the predefined color maps, or a palette loaded from the image, or a user-defined palette. There is a wide variety of palettes displayed in the drop-down list. These include:
  - **Gray** – This palette has constant transparency, for use with gray-scale images.
  - **Temperature**
  - **Physics**
  - **Standard**
  - **Glow**
  - **Blue\_Red**

- **Seismic**
- **From Image**
- **White** – This palette may also be used only with grayscale images. It is similar to the gray palette, except that it has a descending transparency, which provides better visualization on volumes with gradually increasing intensity levels.
- **Negative** – The negative palette should be used with brightfield images having bright backgrounds and dark objects. Applying this palette hides the background and shows the objects more clearly.
- **Image Tint** – Image Tint applies a palette according to the emission wavelength property associated with the image. The wavelength can be set either in the **Set/Property Info** page or by applying a dye tint to the image. If the image does not have an Emission wavelength property, the white palette is used.
- **Rainbow** – The Rainbow palette represents a color spread from 380 to 645 nm. This palette can help by visually separating objects that have slight variations in intensity from each other.
- **Rainbow Transparent** – This palette is based on the rainbow palette but has a descending transparency.
- **Rainbow Inverted** – This palette is based on the rainbow palette but has an ascending transparency, and can be used with brightfield images.
- **User-defined** – The user-defined color palettes may be loaded and saved using the **Load** and **Save** buttons in the palette group box:



- **Brightness, Contrast, Gamma** – These controls allow you to adjust the brightness, contrast, and gamma of the selected volume.




**Brightness:** Move this slider up to increase, or down to decrease, the image brightness. A value of 50 represents no change to the image values.



**Contrast:** Move this slider up to increase, or down to decrease, the image contrast. A value of 50 represents no change to the image values.



**Gamma:** Move this slider up to increase the Gamma value (increase dark area contrast) or down to decrease the Gamma value (increase bright area contrast). A value of 1 represents no change to the image values.

The color buttons  define the channel to adjust: All, Red, Green and Blue.

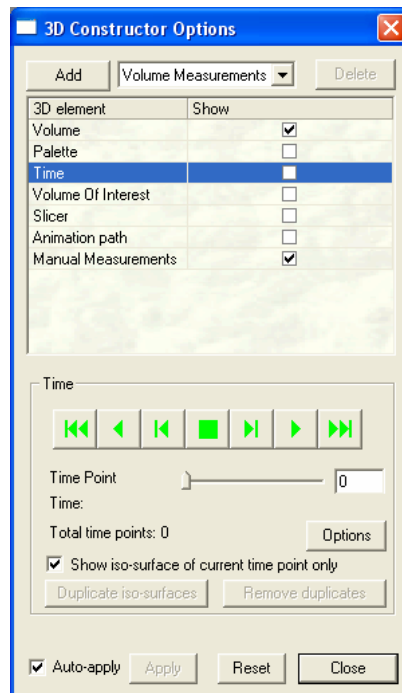
The *Reset* button resets the controls to their default settings.

- **Opaque Min/Max:** These controls allow you to keep the color map range the same, but force all entries below **Min** and above **Max** to be completely transparent. This is a powerful tool for identifying and highlighting interesting structures in the volume. The values can also be changed using the top cones on the color map as sliders.
- **Color Spread:** These controls allow you to compress the application of the palette to a smaller range of the color map.



## Time

3D Constructor allows you to include **Time** as a factor in your analysis. For the **Time** function to display and play back properly, the 4D (includes Time) stack must be opened in the *Set Manager*. When loading a 4D stack, the **Use Active Portion of Sequence** option should be turned *off* (unchecked). If this option is *on* (box is checked), you will see each individual z-stack for each time point in the viewer. From the **3d Constructor Options** dialog, select the **Time** check box.



### Advanced Menu:3D Constructor

The **Time** toolbar contains the following controls;



Go to the first time point



Play sequence in reverse



Go to the previous time point



Stop



Go to next time point



Play time sequence forward



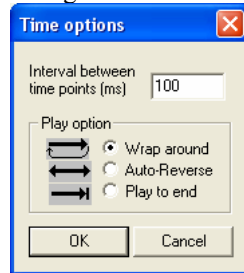
Go to the last time point

- **Time:** The **Time** group box provides additional information about the time sequence.
  - **Time point:** The slider bar indicates the number of the current time frame.
  - **Total Time Points:** This number indicates the total number of time points used.

The group box also indicates the acquisition time of the current frame in the sequence.

- **Show iso-surface of current time point only:** When the **Show iso-surface of current time point only** option is *on* (default), all frozen iso-surfaces of any other time points are hidden. If the option is off, all time points are shown. This option does not have any affect if there are no frozen volume measurements.
- **Duplicate iso-surfaces:** Clicking this button replicates existing volume measurements for every time point.
- **Remove duplicates:** Clicking this button removes all Volume measurements elements with time points other than 0. This action reverses **Duplicate iso-surfaces**.

- **Options:** Clicking the options button displays the **Time Options** dialog:



- **Interval between time points:** This box displays the interval between time points (frames) in milliseconds.
- **Play Option:** These radio buttons indicate how 3D Constructor should play the time sequence:
  - **Wrap Around:** Start over from the beginning
  - **Auto-Reverse:** Automatically play the sequence in reverse.
  - **Play to end:** Plays the sequence to the last frame.
- **OK:** Applies your changes to the time sequence file.
- **Cancel:** returns you to the **3D Constructor Options** dialog without applying or saving any changes.

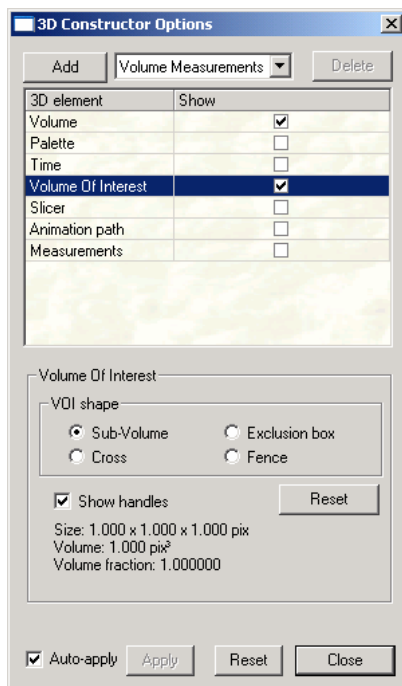
---

**Note:** To view a single time point in a 4-D data set, you must first load the data set using *Set Manager*, and turn *off* the **Use Active Portion of Sequence** check box.

---

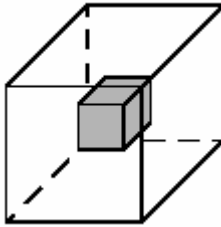
## Volume of Interest (VOI)

**Volume of Interest (VOI)** modifies the visible portion of the data volume in the Viewer window. Selecting the **Volume of Interest** check box displays the following set of controls:

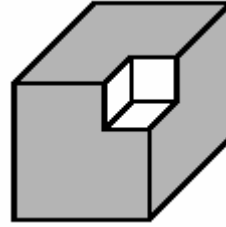


- **VOI shape:** This set of radio buttons controls the shape of the VOI (Volume of Interest).
  - **Sub-Volume:** This button indicates that the VOI is an inclusion box which specifies the part of the volume that should be displayed in the **Viewer**.
  - **Exclusion box:** The VOI can also be an exclusion box which specifies a region of the volume that will not be displayed. This is very useful for cutting away part of the volume.

Examples of the sub-volume and the exclusion box VOI shapes are shown here. The gray sections indicate which parts will be visible in the *Viewer*:

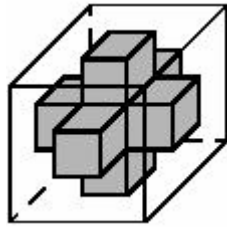


*Sub -Volume*

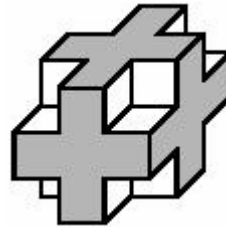


*Exclusion box*

The cross and fence shapes are shown here:



*Cross*



*Fence*

- **Show handles:** This check box controls visibility of the dragger tool that allows you to move and modify the VOI.

**To use the dragger tool:**

1. First make sure the *3D Viewer* window has focus (click in it if necessary).
2. Then make sure the *Viewer* is in selection mode (cursor is an arrow shape).
3. Click on the arrow.

**To Resize the VOI:**

1. Move the cursor onto one of the green handles
2. Press the left mouse button and drag. The VOI cannot be larger than the data volume.

**To Move the VOI:**

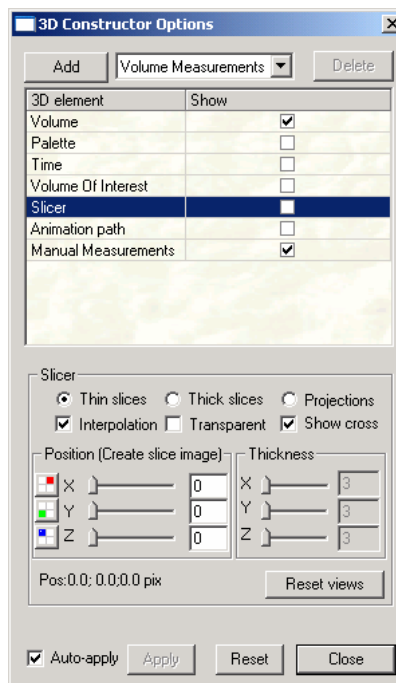
- 1.** Move the cursor onto one of the sides of the dragger box.
- 2.** Press the left mouse button and drag. The VOI cannot move outside the data volume.

The size of the VOI (XYZ) and the volume are given in spatially calibrated units. Volume fraction shows the ratio between total volume of the image and the VOI.

- **Reset:** Click this button to reset the VOI to the full size of the data volume.

## Slicer

The **Slicer** controls the display of image slices or planes in the *3D Viewer*. Checking the **Show** box for the **Slicer** changes the *Viewer* window to show the original volume in the lower right of the four panels. The other three panels show orthogonal slices through the volume. Selecting the **Slicer** checkbox displays the following controls:



- **Thin Slices:** If you select **Thin slices**, thin transparent slices of the corresponding planes are shown. The properties of the slices can be changed using the **Interpolation** and **Transparent** check boxes.
- **Thick Slices:** If you select **Thick Slices**, thick transparent sections of the volume are displayed. The thickness of the sections can be controlled by changing the X, Y and Z values in the **Thickness** group box. This thickness is indicated by Red, Green and Blue cylinders on the views.

Advanced Menu:3D Constructor

- **Projections:** If you select **Projections**, the X, Y, and Z projections of the volume are displayed. Transparency, composition, lighting and interpolation of the projections are controlled by the **Volume** element of the **Options** dialog.  
Transparency, composition, lighting and interpolation of the slices are controlled by the **Volume** element of the **Options** dialog
- **Interpolation:** Select this check box to turn on linear interpolation of the slices, which increases the visible resolution.
- **Transparent:** Select this check box to control the display of alpha values in the data volume. Transparent means voxels with non-zero alpha values are completely opaque and voxels with zero-alpha values are completely transparent.
- **Show Cross:** Checking this box switches on/off the crosshairs of the slice positions. (This option is disabled in the *Projections* mode).
- **Pos:** The **Pos** controls show the current position of the slicer in calibrated units.
- **Position/Thickness:** Clicking on one of the following buttons creates a slice image in the current slicer position:



- ZY slice



- XZ slice



- XY slice

The pixel size of the generated image in X and Y directions corresponds to the pixel size of the original volume. The image dimension in Z direction is scaled according to the voxel size ratio to X:

$$ScaleZ = \text{VoxelSizeZ} / \text{VoxelSizeX}$$

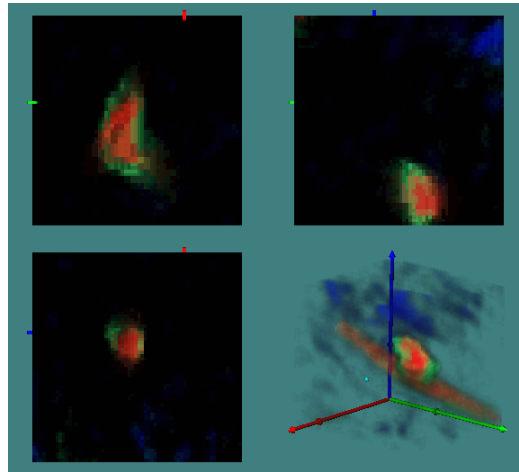
The output image is spatially calibrated as the original volume. When the **Interpolation** control is *on*, the output image is generated using bilinear interpolation between pixels. When the control is *off*, no interpolation is applied.



### Displaying a Volume

The position of the planes can be controlled by adjusting X, Y, and Z sliders or using the left mouse button of the images.

When the **Show** control of the **Slicer** is on, the *3D Viewer* shows the volume from 4 points of view:



The top left panel shows the XY plane of the volume. The top right panel show the ZY plane, and the bottom left panel displays the XZ plane of the volume. The bottom right panel shows the position of volume that you can control, using the commands below:

<ALT> + <Left> = Zoom in and out

<ALT>+ <CTRL> + Left or

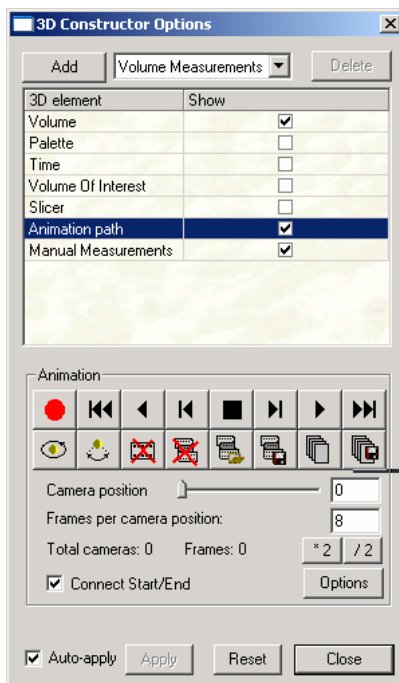
<ALT>+<SHIFT> + Left = Pan (translate up/down/left/right)

You can also use the **Pan** tool to manipulate the volume display. Views of the **XY** plane are zoomed and moved all together when any one is selected.

- **Reset Views:** Click the **Reset View** button to return to the original view; i.e. undoing any **Zoom** or **Pan**.

## Animation

The **Animation** feature allows you to create, record, and play animation files (**\*.anm**) in the *Image-Pro Plus* workspace. When you select the **Animation path** from the list of 3D elements, you will see the following screen:



The **Animation** dialog includes the **animation toolbar**, **camera position** controls, buttons used to control the positioning of frames in the animation file, the check box **Connect Start/End** to interpolate camera positions between the end and start of animation and the **Options** button. Text on the bottom shows total number of frames and camera positions in the current animation file.

The **Animation** toolbar contains the following controls:



Record the current camera position



Go to the first camera position



Play sequence in reverse



Go to the previous camera position



Stop



Go to next camera position



Play sequence forward



Go to the last camera position



Create circular animation. This is done by placing the cameras in an evenly spaced, circular path perpendicular to the *Viewer* window, and half-way between the top and bottom of the window.



Create “rocking” animation. This animation rocks the image around the vertical axis back and forth by approximately 30 degrees.



Delete the current camera position



Delete the entire animation



Load animation from file



Save this animation to a file.



Create an animation sequence in the *Image-Pro* workspace

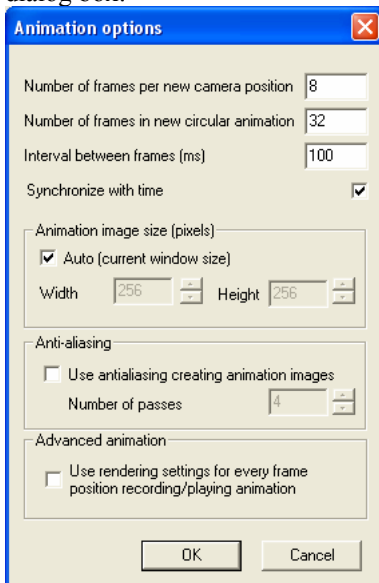


Save this animation to an avi file.

- **Camera position:** Use this slider to change the camera position. When the camera position changes, the

corresponding view is shown in the *3D Viewer* window. You can also enter a camera position in the edit box.

- **Frames per camera position:** Indicates the current number of frames for each camera position. The buttons are used to double (\*2) or half (/2) the number of intermediate frames.
- **Connect Start/End:** Select this check box to connect the beginning and end of the animation file to create a continuous loop.
- **Options:** Click this button to display the **Animation Options** dialog box:






- **Number of frames per new camera position** defines the number of frames, base and intermediate, which will be generated between the new and next camera positions. The number of intermediate frames is one fewer than this number. For example if this number is 8, the program will add one base camera position and generate 7 intermediate positions.
- **Number of frames in new circular animation** defines how many frames will be generated when you create a new circular animation file.
- **Interval between frames (ms)** defines the time delay between frames during animation file playback.

- **Synchronize with time:** When this option is *on* (box is checked) the time points are changed when the animation is played, so that the first time point is synchronized with the first animation frame. If the **Connect Start/End** option is *off*, the last frame of animation is synchronized with the last time point. When the **Connect Start/End** option is *on*, the time is played forward and then back in one animation cycle.
- **Animation Image Size** defines the size of created animation image. When the **Auto** (default) box is checked, (*on*) the size of the animation image is defined by the current 3DConstructor window size. When the option box is unchecked (*off*), the size of the animation image will have the dimensions defined by the **Width** and **Height** controls. The size is in pixels.
- **Anti-aliasing** increases quality of edges on created images. When the “**Use anti-aliasing...**” box is checked (option is *on*), the multi-pass anti-aliasing is activated during the creation of animation images. Every image is rendered according to the **Number of passes** from slightly different camera positions, averaging the results.
- **Use rendering settings ...** this control can be activated to create advanced animations. When the control is *on* (box is checked) clicking the **Record** button, in addition to setting the camera position, also records the current rendering settings for *3D Constructor*. During the playback the settings are restored for every frame, which allows you to create the animation by changing various parameters, e.g. slice position, transparency, color, surface level and so on. Note that the rendering settings are restored only for the base frames and not interpolated in between frames, as is done for the camera position. If the animation requires a more smooth transition from one state to another, more intermediate frames should be recorded. When the control is *off* (box is not checked ) only the camera positions are recorded and played.
- **OK:** Applies your changes to the animation file.
- **Cancel:** returns you to the **Rendering Options** dialog without applying or saving any changes.

### Creating a New Animation


To create a new animation sequence, please follow these steps:

1. Make certain that the current animation is empty (click the **Delete animation** button  if necessary).
2. Set the camera mode (  Perspective or Orthogonal) in the viewer. Note that if you want to create a *fly-through* animation you have to select the perspective camera.

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**Note:** A *fly-through* animation is one where the camera moves through the rendered object in much the same way that an airplane flies through a cloud.

---

3. Using the mouse and viewer window controls, set the desired view of the volume.
4. Click the **Record camera position** button .
5. Move the camera to the next view position and click **Record** again.
6. Record all the camera positions that you want to have in the animation file.
7. Save your animation file.

### Creating a Circular Animation

To create a circular animation file,

1. First, record at *least* two camera positions by clicking the **Record camera position** button. This is optional.
2. Click the **Create Circular Animation** button.   
The new animation will contain the number of frames defined in the **Number of Frames in Circular Animation** control of the **Animation** options. The animation will have one frame per camera position.

### *Advanced Menu:3D Constructor*

The circular animation file uses a set of camera positions that go around the object, from the viewpoint of the first camera position and in the plane defined by the first two camera positions and the center of view. The distance of the first camera to the focal point defines the radius of the circular animation file.

---

**Note:** If you do not create two camera positions, the animation will be created in the XZ plane, at the center of the volume, where the XY plane is in the *Viewer* window.

---




#### **Creating a Seesaw Animation**

To create a seesaw animation:

1. Record the position of the first viewpoint
2. Record the position of the last viewpoint.
3. Check the **Connect Start/End** check box.


#### **Playing an Animation**

To play your animation:

1. Click the **Play forward**  or **Play in reverse**  button.
2. You can stop animation by clicking on the image in the viewer window or clicking the **Stop**  button.

#### **Editing an Animation**

If you want to edit the camera position:


1. Select the camera position you want to edit,
2. Click the **Delete current camera position** button 
3. Adjust the camera to the desired view

4. Click the **Record camera position** button .


The new camera position will be inserted in place of the old one.

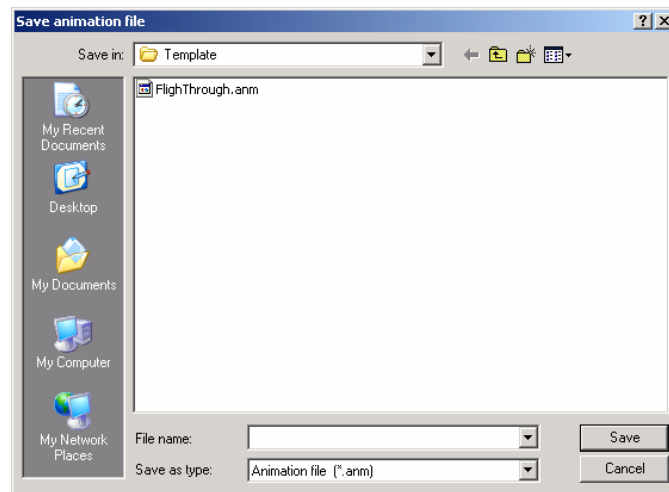
You can change the number of intermediate frames in any camera position by editing the value in the **Frames per camera position** window. If you want to increase the quality of the whole animation file, and make it smoother, double the number of frames in the whole animation file by clicking the **\*2** button.

If you want to decrease the number of total frames, for example before creating a sequence in the *Image-Pro* workspace, you can click the **/2** button. This will reduce the number of frames in each camera position by half.

To delete a camera position click the **Delete current camera position** button .


### Saving and Loading Animation files

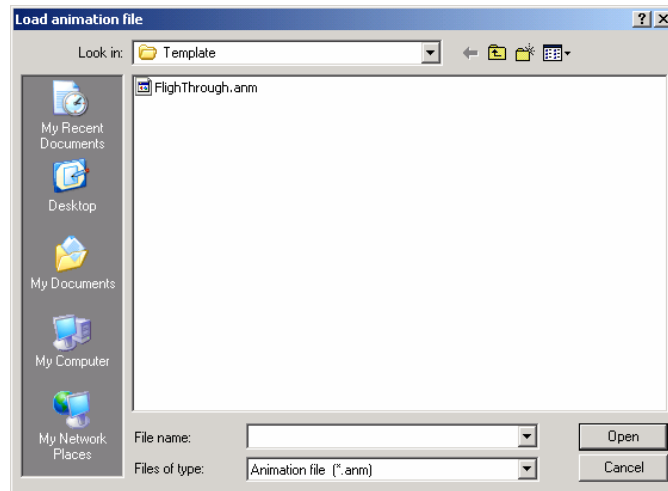
To *save* an animation sequence, click the **Save animation to file** button . You will see the **Save animation file** dialog:



Select the name and path for the animation file, and click **Save**.




To *load* an animation file, click the **Load animation from file** button . You will see the **Load animation file** dialog:



Select the name of the file you want to load, and click **Open**.

### Creating an animation sequence in an Image-Pro Workspace

To create an animation sequence in an *Image-Pro Plus* workspace, you will need to:

1. Create or load an animation using one of the techniques discussed previously.
2. Resize the *3D Viewer* window to the size you need
3. Click the **Create animation file** button .

---

**Note:** The resulting animation sequence can be saved as either a sequence file (**\*.seq**) or as an AVI movie (**\*.avi**) in *Image-Pro Plus*.

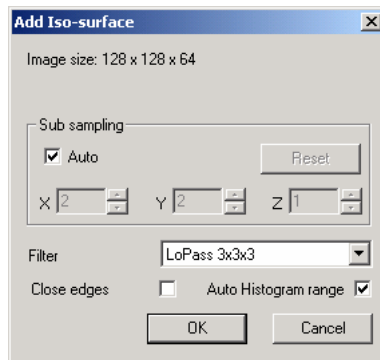
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## Volume Measurements/Iso-Surface

Iso-surfaces provide a very quick, yet often sufficient, method for reconstructing polygonal surface models. By correlating transparency with the local orientation of the surface relative to the viewing direction, complex spatial structures can be understood much more easily. If you are making volumetric measurements, you will need to define at least one iso-surface.

Iso-surfaces are user definable. This means, that, unlike other features of *3D Constructor* (volume, palette, time, animation, etc.) you can create more than one iso-surface per session.

When you select **Volume Measurements/Iso-Surfaces** from the drop-down list box and click the **Add** button, you will see the following dialog:



- **Add iso-surface for:** Here you can select which channel of a color image will be used to create the iso-surface. Your choices are:
  - Gray image
  - Red channel
  - Green channel
  - Blue channel
  - Red-Green co-localization
  - Red-Blue co-localization
  - Blue-Green co-localization
  - Red-Green-Blue co-localization

- Red – Green – Blue co-localization (3-channel co-localization)

If you select a co-localization channel, an iso-surface of co-localized objects will be built. The surface level defines minimum threshold levels for both (or all three) co-localization channels. For example, if the surface level equals 150 for Red-Green co-localization, the iso-surface will be created for objects with voxels where the red and green levels are greater than or equal to 150.

- **Sub sampling:** Use sub-sampling for large volumes to improve the speed of the rendering function.
- **Auto:** Selecting the **Auto** checkbox activates automatic sub-sampling mode. In this mode, the sub-sampling is calculated based on the size of the image. This provides faster loading and rendering of Volume images, but the maximum size of a loaded volume in any direction is limited to 64 subsampled levels. By default this option is *on*. Advanced users can switch this option *off* and set sub-sampling manually.
- **Reset:** Clicking the **Reset** button clears all your volume settings and sets 1:1 :1 sub-sampling. The **Reset** button is disabled when **Auto** is on.
- **Close edges:** Select this check box close the surfaces of the objects where they touch the bounding box of the volume. Note that when **Close edges** is turned *on*, additional walls of pixels of intensity 0 are added around the volume, forcing the surface to close when it has values of edge voxels above the threshold. When using *Sub-voxel precision*, the position of the surface faces are created in between voxels at the positions where bi-linear approximated intensity levels of neighboring voxels equal the threshold level.

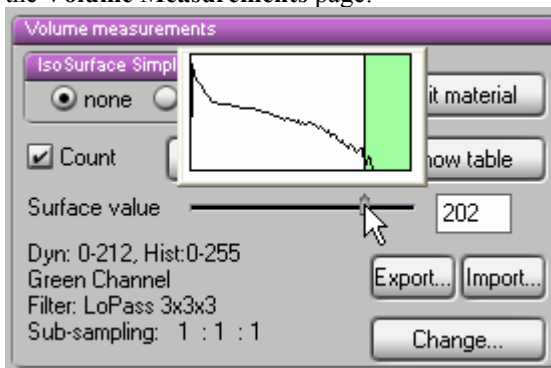
When **Close edges** is turned *off*, the surface is closed on the sides that touch edges regardless their intensity value. (The edges must be closed to measure volumes.)

This difference in the algorithm used to close edges for measurement may lead to the difference of 1-2% (depending on object size) between the results. (For scientifically valid measurements the objects that touch edges should be discarded from the analysis using **All borders** option in the **Volume measurements options** dialog).

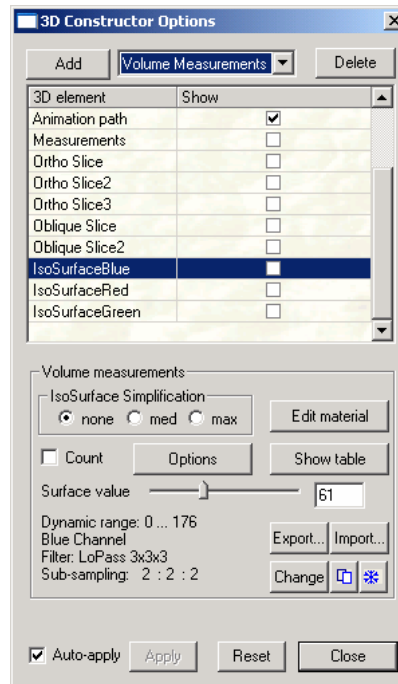
*Advanced Menu:3D Constructor*

With *Voxel precision* (the way 2D objects are measured in *Image-Pro Plus*), the results are identical to the results obtained here.

- **Filters:** Filters can be used to smooth the surface. A filter can be selected from the following list:
  - None
  - LoPass 3x3x3
  - LoPass 5x5x5
  - LoPass 7x7x7
  - LoPass 9x9x9
  - Gauss 5x5x5
  - Gauss 7x7x7
  - Gauss 9x9x9.
- **Auto Histogram Range:** When this box is checked, the histogram range is scaled according to the dynamic range of the volume values. When the box is not checked, the histogram range is scaled according to the display range of the active image. Information about the histogram is displayed on the **Volume Measurements** page:



Once you have added your iso-surface to the list of 3D elements, you will see the following dialog:



- **Simplification:** Using **Simplification** reduces the number of triangles in the iso-surface. It improves rendering performance while examining the volume. Simplification can be set to **none**, **med** and **max**. When **None** is selected no simplification is applied. **Med** corresponds to median level of surface simplification, and **max** indicates the maximum simplification level.

---

**Note:** While increasing simplification improves rendering speed, it also reduces the accuracy of the rendered volume. If you are incorporating a high degree of sub-sampling and simplification, you should be aware that erroneous results may be produced.

---

- **Edit Material:** Clicking this button displays the **Edit Material** dialog which allows you to change the color and other characteristics of the iso-surface. The **Edit Material** selections are described earlier in this chapter.

### Advanced Menu:3D Constructor

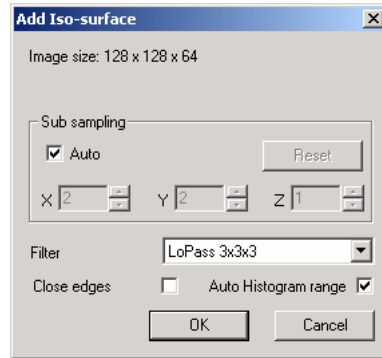
- **Surface Value:** This slider controls the dynamic range of the data volume displayed in the **Viewer**. The default value is the middle value in the dynamic range.
- **Export:** The **Export** button saves the iso-surface as a VMRL image to a file with extension **\*.IV**. The file can be loaded into *3D Constructor* using the **Import** button. This method allows you to compare the parameters of different volumes.
- **Import:** The **Import** button loads the saved iso-surface file as a VMRL image to a file with extension **\*.IV**.

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*Note:* *3D Constructor \*.iv* files are VMRL type 1, and can be used with other 3d programs.

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- **Count:** Clicking this button activates the volumetric measurements of the surface. If the **Count** control is active, the 3D objects in iso-surface are re-measured every time the threshold level or iso-surface parameters are changed .
- **Options:** Click this button to show the **Volume Measurement options**, discussed later in this chapter.
- **Show Table:** Click this button to show the **Volume Measurements Data Table**, discussed later in this chapter.
- **Freeze:** When this option is *on*, the iso-surfaces are “frozen” and cannot be changed when changing or adding time points.
- **Duplicate:** Click this button to create a new volume measurement using the same parameters as the current one. The new iso-surface will differ only by color. The **Freeze** option must be *off* to create a duplicate. Repeating the sequence of operations *Freeze, Duplicate, Next Time Point* will create new volume measurements for all time points and collect them in one data table.
- **Change:** Clicking this button brings up the **Add Iso-Surface** dialog with the current parameters displayed, as shown in the following dialog:

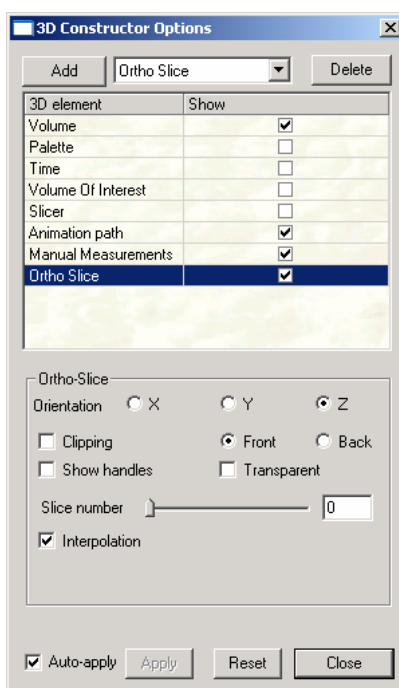


You can edit and change any of the values displayed here, using the spin buttons and drop-down list box.

## Ortho Slices

Ortho Slices are used to display a slice through the X, Y, or Z plane orientation in the *3D Viewer*. Voxels on the plane of the slice will appear as a different color.

When you select **Ortho Slices** from the drop-down list box and click the **Add** button, you will see the following area on the **3D Rendering** dialog:



- **Orientation:** Use these buttons to select the display of the orthogonal slice in the *3D Viewer*; it can be aligned with the **X**, **Y**, or **Z** axis.
- **Clipping:** Select this check box to enable the clipping plane associated with the slice. Clipping makes the voxels in front or behind the slice invisible. You can choose to clip data from either the **Front** or **Back**, using the appropriate radio button.
- **Show handles:** This check box controls visibility of the dragger tool that allows you to move and modify the location of the orthogonal slice.

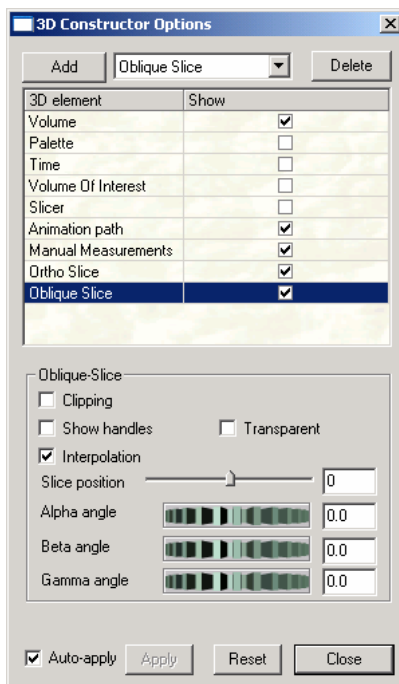


**To use the dragger tool:**

1. First make sure the *3D Viewer* window has focus (click in it if necessary).
  2. Then make sure the *Viewer* is in selection mode (cursor is an arrow shape).
- **Transparent:** Select this check box to display values in the data volume. Transparent indicates that the object data on either side of the slice is not hidden by the plane of the slice.
  - **Slice Number:** This dialog shows the slice number where the slice is currently positioned. It can also be used to drag the slice through the data volume. The slice is moved dynamically as the slider moves. You may also type a slice number in the edit box.
  - **Interpolation:** Select this check box to turn on linear interpolation of the slices, which increases the visible resolution.

## Oblique Slices

Oblique slices are used to display slices of the volume which are not precisely aligned with the X, Y, or Z plane. When you select **Oblique Slices** from the drop-down list box and click the **Add** button, you will see the following area on the **3D Rendering** dialog.



- **Clipping:** Select this check box to make the voxels on one side of the slice invisible
- **Show handles:** This check box controls the visibility of the dragger tool that allows you to move and modify an oblique slice. Clicking the <Ctrl> key and the left mouse button reorients the handle to the next plane. It is possible to reorient the manipulation center of the plane by moving the handle with this key-click combination.
- **Transparent:** Select this check box to control the display of values in the data volume. Transparent indicates that the object

data on either side of the slice is not hidden by the plane of the slice.

- **Interpolation:** Select this check box to turn on linear interpolation of the slices, which increases the visible resolution.
- **Slice position:** This slider moves the slice in any direction along the X, Y, or Z axis on the active image. Moving this slider allows you to control the **Slice position** numerically. The control defines the distance from the center of the volume in percent of maximum dimension. The value can be changed from -100 to 100. Three angles define orientation of the slice plane. The angles may range from -180 to 180 degrees.
  - **Alpha angle** is the angle between the plane's normal axis and the Z axis.
  - **Beta angle** is the angle between the object's normal projection on the XY plane and the Y axis.
  - **Gamma angle** is the rotation angle around the normal position of the object. Changing this angle does not affect the slice position, though it can be adjusted using the dragger.

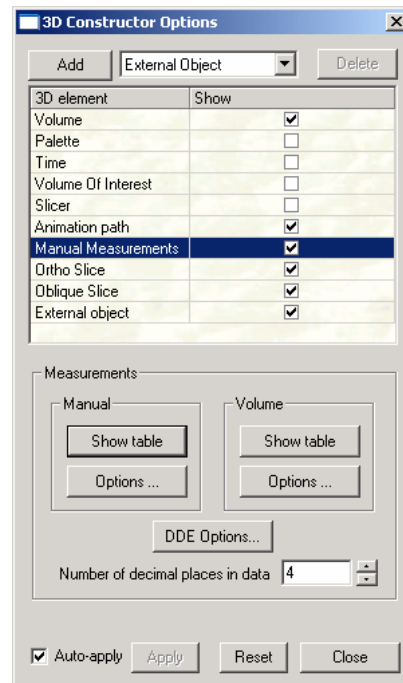
**To use the dragger tool:**

1. First make sure the *3D Viewer* window has focus (click in it if necessary).
2. Then make sure the *Viewer* is in selection mode (cursor is an arrow shape).
3. Click on the side handle to move the Slicer through the volume.
4. Click on the top handle to re-orient the slicer window within the volume.

## Manual Measurements

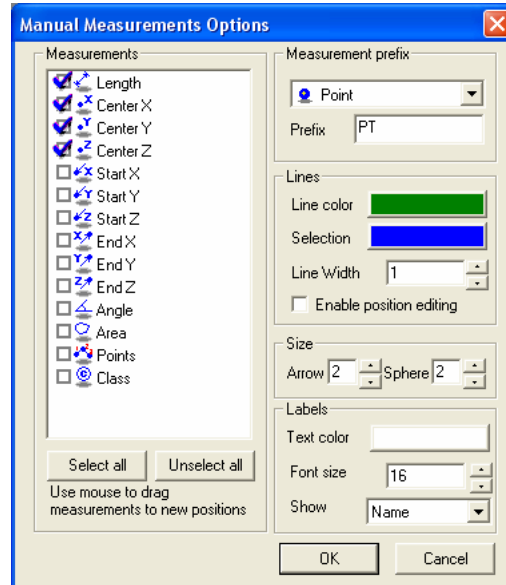
The **Manual Measurements** option creates and displays a table of all the available measurements. **Manual Measurements** is a standard feature of the element list in the **3D Constructor Options** dialog.

When you highlight this selection, you will see the following dialog:



You have a choice of **Manual Measurements** or **Volume Measurements** to be displayed in the appropriate **Data Table**. These tables are discussed in the following pages.

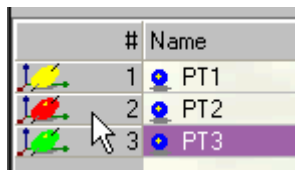
- **Number of decimal places in data:** The number in this box defines the number of digits after the decimal point which will be displayed in the **Data Table**.
- **Manual Measurement Options:** Clicking this button displays the **manual measurements options** window, as shown here:



- **Measurements:** The **Measurements** list contains all available measurements. Check the box next to each measurement to include that item into the **Measurements data table**. The order of measurements can be changed by dragging the measurements to new positions.
- **Measurement prefix:** **Measurement prefix** defines the default prefix for different types of measurements. In the example above, the default measurement is *point*.
- **Lines:** The **Lines** group box contains display options for different measurement elements.
  - **Line color** and **Selection** define colors for the normal (default) and selected (on the active measurement) state of the elements. New elements are created using the default **Line color**. The colors of manual measurement elements can be set individually by double clicking the corresponding color icon in the data table
  - **Line width** defines the size of the lines and poly-lines connecting the manual measurement points in pixels.
  - When the **Enable position editing** option is on the positions of manual measurement points can be edited by dragging the points on the image in the selection mode. If the editing is not necessary or the polylines have many points, switch this

### Advanced Menu:3D Constructor

option *off* to increase performance while selecting manual measurements. When the option is *off*, no spheres and draggers are drawn for every point.



#	Name
1	PT1
2	PT2
3	PT3

To change colors of a group of measurements select the measurement elements, click the right mouse button, and select the ***Set color to selected items*** from the context menu.

- **Size:** The **Size** group box contains controls that allow you to draw arrows to represent the end-points of lines and poly-lines, and to draw spheres to represent single points.
- **Labels:** The **Labels** group box contains options for measurement labels displayed on the image.
  - **Text color** and **Font size** set text color and size for the labels.
  - **Show:** This combo-box displays the type of labels. The labels can be:
    - **Name** – only the measurement name is displayed.
    - **First measurement** – the value of the first selected measurement will be displayed as a label (you can drag any measurement to the first position to be displayed as a label)
    - **None** – no labels are displayed.

## Manual Measurements Data Table

The table contains the list of manual measurements. The values are in calibrated units. Selecting this option is the same as clicking on the



button in the **3D Viewer** toolbar.

#	Name	Length	Center X	Center Y	Center Z
1	PT1		33.0000	26.8750	29.7500
2	LN1	25.0405	57.6077	39.1741	57.6350
3	LN2	42.0226	41.3234	27.5486	59.9269
4	PL1	63.6586	53.3162	23.9981	58.9928
5	AN1	48.1431	44.8407	7.6836	58.4280
6	PT2		36.7728	34.6834	59.0642
Mean value		44.7162	44.4768	26.6605	53.9666
Standard ...		15.9681	9.5047	10.8536	11.8877
Minimum		25.0405	33.0000	7.6836	29.7500
Maximum		63.6586	57.6077	39.1741	59.9269
Range		38.6180	24.6077	31.4906	30.1769
Sum		178.8647	266.8609	159.9629	323.7966
Number of...	6.0000	4.0000	6.0000	6.0000	6.0000

The **Manual Measurements Data Table** toolbar contains the following buttons:



**Load measurements:** Clicking this button loads the manual measurements parameters file from an \* .m3d file.



**Save measurements :** Clicking this button saves the manual measurements parameters file to an \* .m3d file (in binary format).



**Save table:** Clicking this button saves the data table in text, tab-delimited, comma-delimited, or HTML format.



**Send table to Excel:** Clicking this button sends the data to an Excel spreadsheet (via DDE).



**Measurement options:** Clicking this button opens the **Manual Measurement Options Dialog**, where you can select and rearrange the manual measurements, set the default prefix for measurements, set colors, and other options (see the **Options** dialog earlier in this section).



**Show statistics :** Clicking this button shows or hides the measurement statistics.

### New Measurement Group



**Select measurements:** Clicking this button allows you to select measurements on the image, and also permits you to edit the position of external objects. If you are in 'selection mode', the points you select will appear as colored spheres on the object in the viewer. These spheres serve as handles to allow you to drag the points to new positions.



**Add point measurement:** Clicking this button activates the point tool



**Add line measurement:** Clicking this button activates the line tool. A line is added after 2 clicks.



**Add polyline measurement:** Clicking this button activates the polyline tool. Click the right mouse button to see the line. This feature allows you to combine two polylines into one.



**Add angle measurements:** Clicking this button activates angle measurements. An angle is added after 3 clicks.



**Add an outline of object crossed by a plane:** Click this button to create a closed polyline feature that is an outline of a plane crossing an object.

To add an outline, you have to click twice on the imaginary line along which you want to create the outline of the object. The outline plane passes through the selected two points, and through



the position of the viewing camera, so that the outline is always created in the plane perpendicular to the screen plane.

The object is viewed correctly only in *Perspective* projection view. A crossing plane designated in *Orthographic* view may not look perpendicular.



**Add a polyline along object surface connecting two points:**

Clicking this button creates a polyline feature that connects two points along the surface of the plane that passes through the selected two points and the camera position. This feature also allows you to select two polylines, and combine them into one.



**Split polyline into lines:** Clicking this button creates a new line measurement from every segment of the selected poly-lines/tracks. Analyzing the length of every line provides information related to the velocity of the object moving along the track.



**Extract points from lines/polylines:** Clicking this button creates a new point measurement for every coordinate of the selected polylines/tracks. It can provide information about the object coordinates on different time points (the same information can be obtained in the **Volume measurements data table**).

### Derived Measurements Group



**Get center of the object:** Clicking this button creates a point measurement of the center of the selected objects.



**Measure distance between centers:** Clicking this button creates a line measurement between centers of selected features. Note that this function is active only when two features are selected.



**Measure perpendicular distance between point and line: :**

Clicking this button creates a line measurement between a point and a line as a perpendicular line. Note that this function is active only when one line and one or more points are selected.



**Measure angle between lines:** Click this button to create an angle measurement between two lines. Note that this function is active only when two lines are selected. The angle between 3D vectors is calculated directly from the result of vector multiplication:

1. The angle is created from two 3D vectors defined by two selected line segments.
2. The second line is translated so the position of the *first* point of the *second* line is moved to the position of the *second* point of the *first* line.
3. The new structure includes three points: the 1st point of the first line; the 2nd point of the first line; and the 2nd (translated) point of the second line.
4. These 3 points define the plane where the angle is measured between the vectors 2-1 and 2-3.



**Create a polyline including points of all selected measurements:** Clicking this button creates a polyline that includes all the points in the selected measurements. For example, if two lines are selected, the new polyline will include four points.



**Measure distance between point and surface of volume object:** Clicking this button creates a line feature between the selected point and selected 3D object. The 3D object can be selected either in the **Volume measurements data table**, or on the image itself in **Select measurements/select objects mode**



**Show all measurements:** Click this button to show all measurements (whether they are selected or not).



**Hide unselected measurements:** Click this button to show only the selected measurements and hide the rest.



**Hide selected measurements:** Click this button to hide the selected measurements



**Delete all measurements:** Click this button to delete all measurements.



**Delete selected measurements:** Clicking this button deletes the selected measurements.

The names of the measurements can be edited in the **Measurements data table**. The measurements can be sorted by clicking on the column header.

To add measurements, you should:

1. Activate **Selection mode** on the *3D Viewer* (Click the arrow button on the top of right-side toolbar). **Selection mode** on slicer views is active by default.
2. Check the **Show** check-box of the **Measurement** element of *3D Constructor* options table.
3. Activate one of the **Add** tools: **Add point measurement**, **Add line measurement**, **Add polyline measurement**, or **Add angle measurement**.
4. Click on the image (volume or slicer views) and add points to the element.

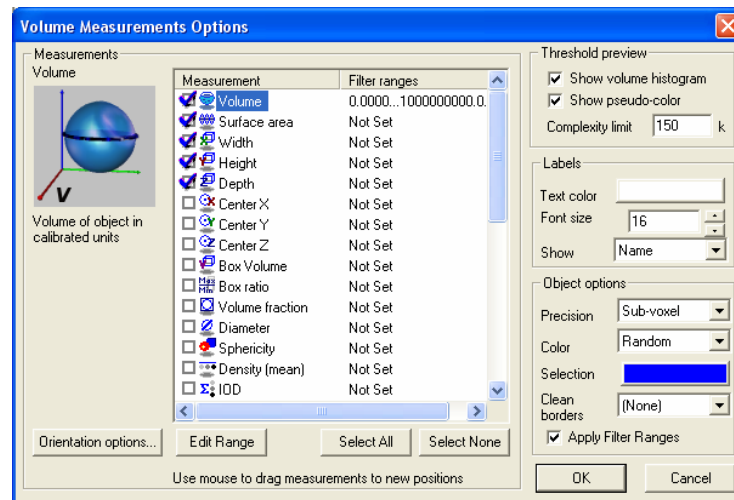
Clicking on **Ortho-slice**, **Oblique slice** or **Iso-surface** adds a point at the intersection of the mouse click ray and the element.

Clicking on **Volume** adds a point on the first non-transparent voxel of the volume. (To click on features inside the volume, you can increase the minimum opaque level to hide outer layers). If the volume is transparent, no point is added.

Note that on three **Slicer** views you have to use **Ctrl-click** to add a point. A **polyline** is closed by clicking the **right** mouse button.

## Volume Measurements

Volume measurements for 3D objects are activated by checking the **Volume Measurements** box on the **Measurements** page, or by selecting **Volume Measurements** from the drop-down list box, or by clicking on the **Volume Measurements** selections from the **Measurements** group box.

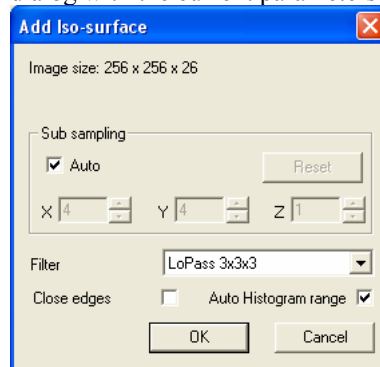


- **Iso-Surface Simplification:** Using **Simplification** reduces the number of triangles in the iso-surface. It improves rendering performance while examining the volume. Simplification can be set to **none**, **med** and **max**. When **None** is selected no simplification is applied. **Med** corresponds to median level of surface simplification, and **max** indicates the maximum simplification level.
- **Edit Material:** Clicking this button displays the **Edit Material** dialog which allows you to change the color and other characteristics of the iso-surface.
- **Reset Outline:** Click this button to reset the outline measurements and return to the normal Volume Measurements mode. When an **Outline** is loaded the **Level** control is hidden and **Simplification** controls are disabled.

The Outline IV file must contain one **Coordinate3** node contains 3D coordinates of surface points of all objects and one or more

**IndexedFaceSet of IndexedTriangleStripSet nodes** for every object, which describe a closed surface. Every object may include a **Material node**. The **Outline** in VRML format must contain **VRMLIndexedFaceSet** nodes for every object.

- **Options:** Click this button to show the **Volume Measurement options**, discussed below.
- **Show Table:** Click this button to show the **Volume Measurements Data Table**, discussed below.
- **Surface Value:** This slider controls the dynamic range of the data volume displayed in the **Viewer**. The default value is the middle value in the dynamic range.
- **Export:** The **Export** button saves the iso-surface to a file with extension **\*.IV**. The file can be loaded into 3D Constructor using the **Import** button.
- **Import:** Click this button to load 3D object outlines that you saved using the **Export** button. Importing saved outlines allows you to use the same outline settings on different objects and images for comparison.
- **Count:** Clicking this button activates the volumetric measurements of the surface. If the **Count** control is active, the 3D objects in iso-surface are re-measured every time the threshold level or iso-surface parameters are changed .
- **Change:** Clicking this button brings up the **Add Iso-Surface** dialog with the current parameters displayed.



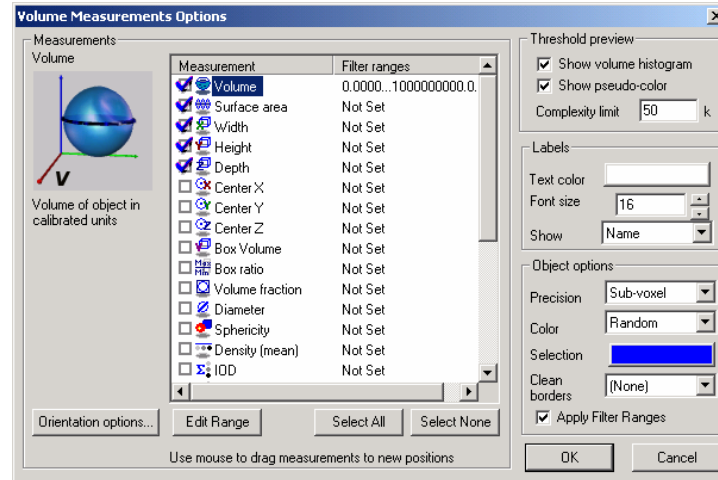
You can edit and change any of the values displayed here, using the spin buttons and drop-down list box.

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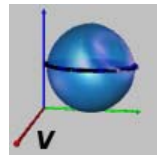
When **Count** is active, the current iso-surface is segmented and volume parameters of every isolated object are calculated. The list of volume measurements and other parameters are defined in the **Volume measurements options** window, which can be opened either from the measurements page (shown above) by clicking the **Options** button, or from the **Volume measurements data table** by clicking the **Show Table** button.

## Volume Measurement Options

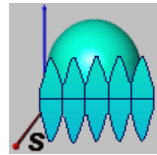
Clicking the **Volume Measurement options** button displays the dialog shown here:



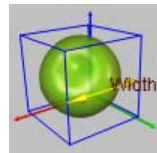
The **Measurements** list includes the following measurements:



**Volume:** Volume of object in calibrated units

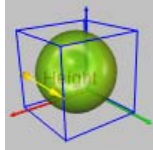


**Surface area:** Surface area of object in calibrated units

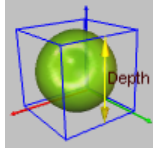


**Width:** Size of bounding box in X direction

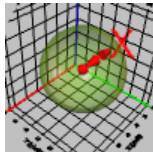
Advanced Menu:3D Constructor



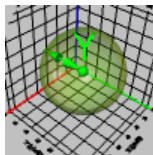
**Height:** Size of bounding box in Y direction



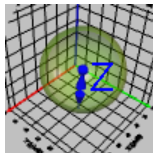
**Depth:** Size of bounding box in Z direction



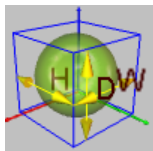
**Center X:** X coordinate of the center of object



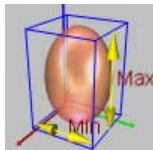
**Center Y:** Y coordinate of the center of object



**Center Z:** Z coordinate of the center of object

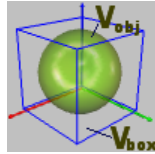


**Box Volume:** Volume of object's bounding box  
(  $V = W * H * D$  )

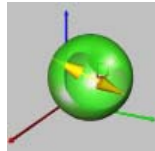


**Box ratio:** Ratio between maximum and minimum size of the bounding box. (  $R = Max / Min$  )

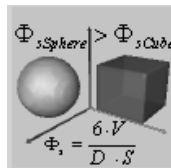




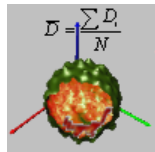
**Volume fraction:** Ratio of object's volume to the box volume ( $R = V_{obj} / V_{box}$ )



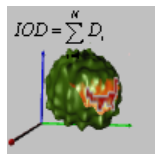
**Diameter:** Equivalent diameter of object.



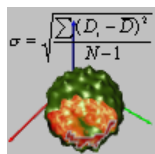
**Sphericity:** Sphericity of object, calculated as 6 volumes of object divided by equivalent diameter and surface area of object. For a spherical object this parameter equals 1, for all other shapes it is less than 1.



**Density (mean)** Mean density of object

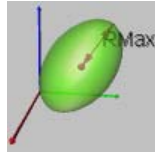


**IOD** Integrated Optical Density of object. Indicates the average intensity/density of each object.

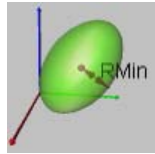


**Density (st.dev.)** Standard deviation of optical density of an object

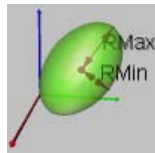
Advanced Menu:3D Constructor



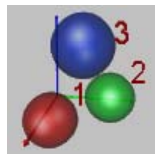
**Radius (max):** Maximum distance between an object's centroid and surface



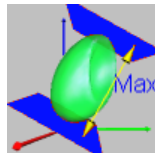
**Radius (min)** Minimum distance between an object's centroid and surface



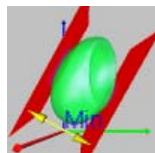
**Radius Ratio** : Ratio between Radius (max) and Radius (min)



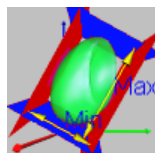
**Class:** Class to which object belongs. The classification can be established using the **Histogram** window.



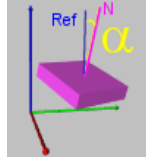
**Feret (max)** Maximum distance between two parallel planes enclosing an object.



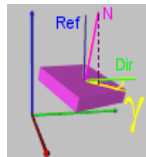
**Feret (min):** Minimum distance between two parallel planes enclosing an object.



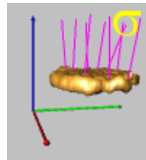
**Feret Ratio:** Ratio between Feret (max) and Feret (min).



**Alpha angle** is the angle between the Reference vector and the surface normal (in degrees).



**Gamma angle** is the angle between the *Direction vector* (Dir) and the projection of the normal (Proj) to the plane created by the direction vector (Dir) and the vector (Perp) perpendicular to Ref-Dir plane. The angle can be in range from -180 to 180 degrees.

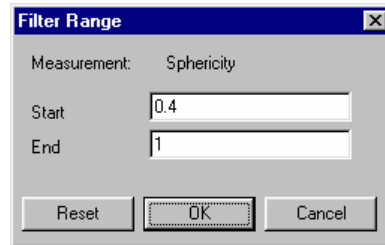


**Surface deviation** is calculated as the deviation of end-points of triangle normals. The calculations are done as following: all triangle normal vectors of surface are normalized (length set to 1) and the average distance from the mean position of the end-points to all other vectors is calculated. Uniform surface will have deviation of 0. The maximum deviation of 1.336 will have a sphere

Checking the box in front of each measurement activates the measurement. The order of the active measurements can be changed by dragging the measurement name to a new position.

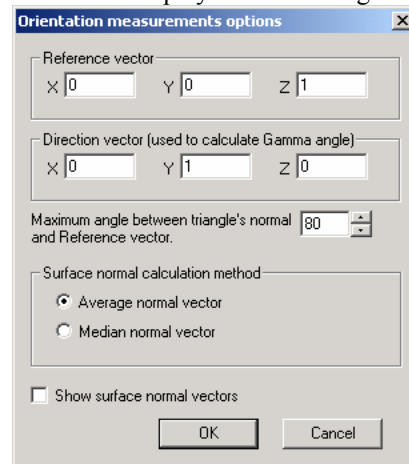
- **Select All:** Clicking the **Select All** button selects all the measurements.
- **Select None:** Clicking the **Select None** deselect all the measurements
- **Edit Range:** Every measurement may have a **Filter range**, which can be set by clicking the **Edit range** button or double-clicking on the measurement's name.

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If a **Filter Range** is set, objects with parameters below **Start** or above **End** values are excluded from the view. The **Filter Range** can be switched off by clicking the **Reset** button.

- **Orientation Options:** Orientation of an object in 3D space can be defined using at least 2 angles from a perpendicular vector. In *3D Constructor 7.0*, these angles are called **Alpha** and **Gamma**. Clicking this button displays the following dialog:

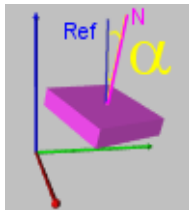


**Surface normal vector:** Every surface in *3D Constructor* consists of triangles; every triangle has its own normal vector. If the angle between triangle's normal vector and the reference vector exceeds the *maximum angle range*, the triangle is excluded from the surface normal vector calculation. The default value is 80 degrees, which excludes triangles on the sides of the object (angles around 90 degrees) and triangles on the opposite side of the object (angles greater than 90 degrees). The maximum value for this parameter is 180.

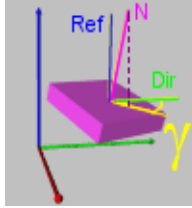
- **Reference vector:** The reference vector is defined by the X,Y and Z components. The default vector of 0,0,1 is parallel with the Z axis. The alpha angle is calculated between this vector and the surface normal vector.

### Advanced Menu:3D Constructor

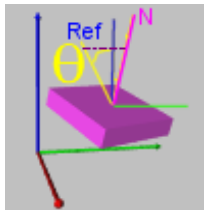
- **Direction vector:** The direction vector is defined by the X,Y and Z components. The default vector of 0,1,0 is parallel with the Y axis. The gamma angle is calculated between this vector and projection of the normal vector to the plane of the direction vector and the vector perpendicular to the plane defined by the reference and direction vectors.
- **Maximum angle** between triangle normal and reference vector: This number defines which areas (triangles) of the object surface will be taken into account when calculating the surface normal.
- **Calculation method:** There are 2 methods provided to calculate the normal vector of the surface:
  - **Average vector**, which is calculated as area weighted average normal vector of all surface triangles (within *angle range*). This method is relatively fast in calculation, but sensitive to deviations on the surface orientation. Small bumps and pits on the surface can slightly affect the vector.
  - **Median vector**, where median vector from all triangles is selected as surface normal. Median vector is the vector, which has minimum sum of distances to all other vectors. This methods provides very stable results, small bumps and pits of the surface do not affect the result. The vector represent the normal direction of the surface which covers most of the object. This method is very computation expensive and can be much slower that average method for big objects (if each object contains more than 10,000 triangles).
- **Show Surface Normal vectors:** The **Show Surface Normal vectors** option toggles showing surface normals on the image. Check this box to show the surface normal vectors, or uncheck the box to hide them.
- **Orientation measurements:** The following new measurements have been added to the list of available volume measurements of *3D Constructor*:



- *Alpha angle* – angle between the Reference vector and surface normal (in degrees).

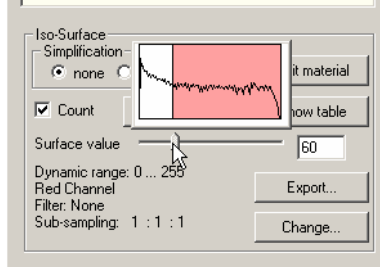


- **Gamma angle** - angle between the *Direction vector* (Dir) and the projection of the normal (Proj) to the plane created by the direction vector (Dir) and the vector (Perp) perpendicular to Ref-Dir plane. The angle can be in the range of -180 to 180 degrees.



- **Theta angle** - angle between the *Reference vector* (Ref) and the projection of the normal (Proj2) to the plane perpendicular to the *Direction vector* (Dir). The angle can be in the range of -180 to 180 degrees.

- **Threshold preview** group box includes controls that define behavior of dynamic threshold preview.
- **Show Volume Histogram:** When the **Show volume histogram** is checked, a popup histogram window is displayed above the threshold slider.



The color on the histogram corresponds to the channel color. The vertical scale is logarithmic.

- **Show pseudo-color:** When the **Show pseudo-color** box is checked, the volume shows the outlines of the object above the threshold value in pseudo-color. The pseudo-color threshold preview is available for either color or single channel images. The preview quality depends

on the parameters of the currently-loaded volume. Using Hi-color mode for color images increases preview quality, but decreases performance ( because images are loaded in non-paletted mode and changing threshold requires 3d Constructor to rebuild the whole volume in its entirety).

In two cases pseudo-color preview is not available for color images: when an iso-surface is created for a grayscale image, and when the volume and iso-surface are based on different channels of a color image. For example, when the Volume contains Red channel data and the iso-surface is based on the Green channel.

- **Complexity Limit:** The value in this box limits the number of triangles in a created surface. *3D Constructor* estimates complexity of the created surface, and if the complexity is greater than the value defined in the **Complexity limit** , a warning message is displayed. The warning message recommends that you use some tools, such as using sub-sampling and filters, to reduce the complexity, but it will allow you to proceed to create the surface anyway.
- **Labels:** The **Labels** group box contains options for measurement labels displayed on the image.
  - **Text color** and **Font size** set text color and size for the labels.
  - **Show:** This combo-box displays the type of labels. The labels can be:
    - **Name** – only the measurement name is displayed.
    - **First measurement** – the value of the first selected measurement will be displayed as a label (you can drag any measurement to the first position to be displayed as a label).
    - **None** – no labels are displayed.
- **Object Options:** The **Object options** group box contains options for 3D objects.
  - **Precision:** The **Precision** control defines whether the surface outlines of 3D object are created with **Sub-voxel** or **Voxel** granularity. The default option is **Sub-voxel**. This option allows creating smooth surface outlines that can be created in sub-voxel space and the position of this outline depends how close the threshold value is to the intensity value of the voxel.

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When the **Voxel** option is selected, the surface outlines and all the volumetric measurements are built from voxel cubes. Voxels with intensity greater than or equal to the threshold level are completely included in the object.

- **Color:** The **Color** control defines what color the objects will have. Two choices are available: **Iso-Surface** and **Random**. If **Iso-Surface** is selected the objects inherit color from the iso-surface. If **Random** is selected, the colors for objects are generated randomly, so every object has a different color.
- **Selection:** The **Selection** control defines the color for selection box, which is shown when object is selected.
- **Clean Borders:** **Clean borders** includes two options: **(None)** and **All borders**. If **All borders** is selected, the objects that touch the bounding box of the volume are excluded from the analysis.
- **Apply Filter Ranges:** When the **Apply filter ranges** box is checked, objects with parameters that are outside the filter ranges of one of the measurements or do not satisfy the **clean borders** option are filtered out from the view and data table.

## **Volume Measurements Data Table**

Clicking the **Show Table** button under **volume measurements** displays the parameters of 3D objects that are collected in the **Volume measurements data table**. Clicking on the column headers (measurement labels) will sort the objects according to that measurement. Double-clicking on a column header will reverse the sort order.



#	Name	Volume	Surface area	Width	Height
7	1.7	5515.8315	1521.8634	22.6244	22.6244
15	2.7	5431.8665	1507.5072	22.6244	22.6244
23	3.7	4020.8060	1233.1849	20.6258	20.6258
30	4.6	4160.5465	1263.5687	20.6258	20.6258
37	5.6	3999.9537	1228.8280	20.6258	20.6258
43	6.5	4164.3123	1264.7056	20.6258	20.6258
47	7.2	4022.6964	1233.7743	20.6258	20.6258
54	8.3	4021.3888	1233.1752	20.6258	20.6258
61	9.2	5558.7303	1529.7639	22.6244	22.6244
69	10.2	3999.2154	1228.8322	20.6258	20.6258
Mean value		4489.5347	1324.5203	21.2254	21.2254
Standard ...		702.0101	135.4337	0.9654	0.9654
Minimum		3999.2154	1228.8280	20.6258	20.6258
Maximum		5558.7303	1529.7639	22.6244	22.6244
Range		1559.5149	300.9359	1.9986	1.9986
Sum		44895.3474	13245.2033	212.2539	212.2539
Index of Min		69.0000	37.0000	23.0000	30.0000
Index of ...		61.0000	61.0000	15.0000	1.0000
Shown ob...		10.0000	10.0000	10.0000	10.0000
Total obj...		75.0000	75.0000	75.0000	75.0000

The **Volume Measurements Data Table** toolbar includes the following buttons:



**Save table:** Clicking this button saves the **Volume measurements data table** in tab-delimited or comma-delimited text format, or as an HTML page.



**Send table to Excel:** Clicking this button sends the data table to an active Excel worksheet (via DDE).



**Volume measurements options:** Clicking this button shows the **Volume Measurement Options** window.



**Show measurements statistics:** Clicking this button shows or hides the statistics pane of the **Volume Measurements data table**. Note that the statistics are calculated based on only visible objects. So, to get statistics of some sub-set of the objects, the unwanted objects have to be hidden in the table.



**Histogram:** Clicking this button shows the **Histogram** window. You can display either volume measurements or manual measurements in the histogram.



**Select objects:** Clicking this button activates the selection mode, which allows you to select objects on the image. Double clicking on the image activates the color editing dialog, where you can

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change an object's color and transparency. To select more than one object, hold down the < Ctrl> key and click the mouse on the various objects.



**Split objects manually:** Click this button to activate the split mode. To split a 3D object into 2 objects you have to click twice on the imaginary line that splits the objects. The splitting plane passes through the two selected points and through the position of the viewing camera, so the object is always split with a plane perpendicular to the screen plane.



**Merge selected objects:** Clicking this button merges selected objects into one. It can be used if segmentation does not allow you to connect all the pieces of one object.



**Undo split/merge:** Clicking this button undoes the last split or merge operation. The number of undo levels is practically unlimited.



**Get center of the object:** Clicking this button creates a point measurement of the center of the selected objects.



**Measure distance between centers:** Clicking this button creates a line that connects centers of two selected objects.



**Measure distance between surfaces:** Clicking this button creates a line between the surfaces of two selected objects. This line represents the shortest distance between two objects.



**Show all objects:** Clicking this button shows all objects (undoes all hide operations).



**Hide unselected objects:** Clicking this button hides unselected objects and shows selected objects only.



**Hide selected objects:** Clicking this button hides selected objects.




**Split polyline into lines:** Clicking this button creates a new line measurement from every segment of the selected poly-lines or tracks. Analyzing the length of every line provides information related to the velocity of the object moving along the track.



**Extract points from lines/polylines:** Clicking this button creates a new point measurement for every coordinate of the selected poly-lines or tracks. It can provide information about the object coordinates on different time points (the same information can be obtained in the Volume measurements data table).

The values in the data table can be sorted by clicking on the column header.

Double clicking on the color icon  opens the **Edit Color** dialog that allows you to change the object's color and transparency.

The **Name** of any object can be modified or changed by double clicking on the cell with that name and editing the text.

## 4D Tracking

*4D Tracking* has been added to the **Volume Measurements Data Table** in *3D Constructor*. You will see these buttons:



**4D Tracking options.** Clicking this button opens the **4D Tracking Options** dialog. In this dialog, you can select measurements, select motion type and set the speed.. See the **Tracking Options** section (below) for more information.



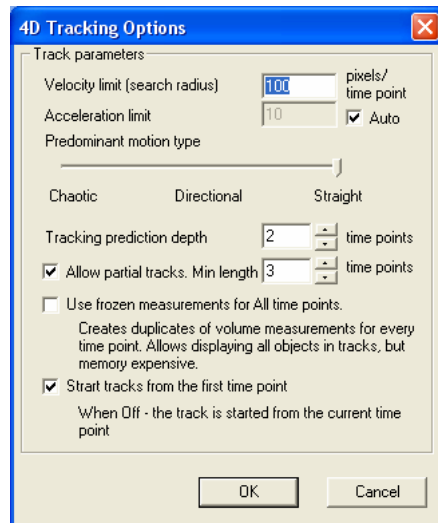
**Add track manually:** Clicking this button allows you to add volume tracking measurements for every time point using existing parameters. More information about adding a track manually appears later in this section.



**Add track automatically:** Clicking this button creates volume measurements automatically for every time point using existing parameters. More information about adding a track automatically appears later in this section.

### 4D Tracking Options

Clicking this button displays the following dialog:



- **Velocity limit (Search radius)** : The velocity limit (search radius) defines the maximum distance in calibrated units between object positions on the neighboring time points. The parameter is used with automatic tracking to find tracks on the first two time points (on all time points when using the *Chaotic* movement type). If no object is found in the search radius, you will be prompted to click on the expected position manually.
- **Acceleration limit** : The acceleration limit defines the maximum change in velocity of objects between the time points. The value is in calibrated units. Starting from the third time point, the estimated position of an object is calculated based on the movement vector on the first two frames. The search volume is defined as a sphere with center in the estimated position and radius of the *acceleration limit*. If no object is found within the search volume user is prompted to click on the expected position manually. If **Auto** option is *on*, the acceleration limit is taken as half of the *velocity limit*.
- The **Predominant motion type** defines the type of movement of the objects  
Select **Chaotic** if there is no directional movement of the objects. The direction of the movement can be changed abruptly. In this case the search volume on all time points is defined only by the *speed limit*, as the *acceleration limit* is ignored. The center of the

search volume (estimated object position) is at the last object position.

Select **Directional** if the object's movement is predominantly directional, but not always straight. In this case the estimated position of the object on a new time point is calculated based on the last velocity vector.

If the objects move in relatively straight trajectories, choose **Straight**. With this type of motion, the estimated object position is calculated based on the average velocity vector for the last **N** time points. The **N** is defined by **Tracking Prediction Depth**, but not less than 2. Averaging vectors helps to find correct objects even if different tracks merged for **N-1** time points.

- **Tracking Prediction Depth** defines the number of time points ahead of the current time point, which are used to find most suitable track for the given object (default is 2). This parameter also defines the maximum number of consecutive *occlusions* on the track. The number of occlusions is less than the **Prediction Depth** by 1.

For example, if the **Prediction Depth** is 3, the maximum number of consecutive object occlusions is 2. This means that if the tracking object in a 4D sequence disappears (or is hidden) for 1 or 2 consecutive time points, it will still be detected as a part of the track. The estimated position will be used for missed objects. The tracking points of missed objects do not have associated volume objects. High prediction depth values increase computation time significantly.

If the track length can be less than the total time points, it can be taken into account by activating the **Allow partial tracks** option. The tracks that shorter than the **Minimum track length** are ignored with automatic tracking.

- When the **Use frozen measurements for All time points** option is *on*, (box is checked ) the duplicate volume measurements for each time point are created before tracking begins. (The same as **Duplicate iso-surfaces** button on *Time page*). This allows 3D Constructor to display all the objects in the tracks, but requires additional memory. When the option is *off*, no duplicates are generated. This mode can be used when memory requirements are critical. In this case the volume measurements are created anew changing the time point.
- If **Start track from the first time point** is checked, the time position of the 4D track is set to the first time point, before

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creating a new track. If the option is *Off*, the track is created starting from the current time point

### Adding Tracks Manually



Clicking this button prompts you to click on one of the objects in the volume displayed in the viewer, as shown here:



After clicking on the object, the coordinate for its center of mass is added to the track. The point is incremented by 1 and another prompt is shown. You may continue adding tracks in this manner.

Clicking the **Exit** button closes the track and discards the results.

The **Close track** button closes the track at the current time point and adds a partial track to the measurements data table. If the object is hidden on some time points, click the *Skip point* button. In that case the previous object coordinate will be used for the track and no object will be associated with the time point.

### Adding Tracks Automatically



Clicking the this button prompts you to select the first object, as shown here:

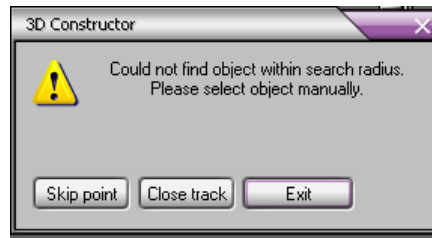


When user clicks on the object, the program tries to create a track starting from that object. At the next time point, all objects within the search radius are considered as track point candidates. The point which can create a track with the lowest cost is selected

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from the available candidates. The program uses multi-frame forward tracking for every tracking point candidate (the number of analyzed time points is defined by **Tracking Prediction Depth**). Change in direction and magnitude of velocity vector are penalized with higher cost.

If there are no objects found within search radius from the predicted place user is prompted to locate the object manually.

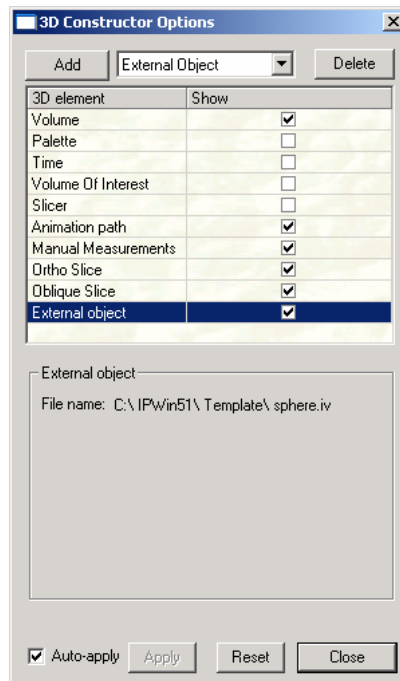


To cancel the track, click the **Exit** button.

To close the track, click the **Close track** button. A partial track will be added to the list. If the object is hidden on some time points, click the **Skip point** button. In that case the previous object coordinate will be used for the track, and no object will be associated with the time point.

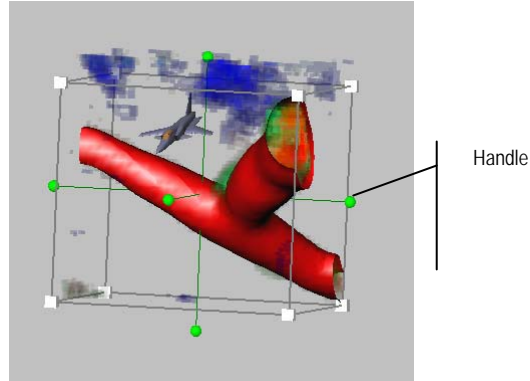
## External Objects

**External objects** are iso-surface coordinate links that can be imported into the *3D Constructor Viewer*. Multiple external objects can be loaded from files selecting **External object** from drop-down list box on the **Options** dialog, and clicking the **Add button**.



The position, scale and orientation of any external objects can be edited. To perform this operation, the *3D Viewer* and the **Manual Measurements** must be in **Selection** mode. Clicking on the object will invoke a transformer around the object.





Use the handles at the corners of the transformer to change the size, position and orientation of the object. Hold down the **<Ctrl> + <Shift>** keys allows you to adjust each dimension independently.

Click and drag any face to move the dragger within the plane of that face. The face you selected will be highlighted in yellow. While you drag, yellow feedback arrows display the two directions of motion.

Press the **<Shift>** key and the arrows turn orange; you may now pick between these two directions to constrain the motion. The direction you move the cursor in will determine which direction is chosen.

Press the **<Control>** key and the dragger will move perpendicular to that plane. The volume display is modified as the face is dragged. Click a corner to scale the dragger. The corner you selected will turn yellow and radial lines will indicate that you may move toward and away from the center of the box. Drag radially and you will perform uniform scale.

Press the **<Control>** key to scale about the opposite corner instead of the center of the box. To stretch the dragger non-uniformly, press **<Shift>** when you drag the corner cube. Now you will see three orange arrows indicating that your gesture will determine which direction to choose. Move the cursor and the selected arrow will turn yellow while the others disappear. Now the dragger will stretch only in the direction you selected.

Pressing **<Control>** at the same time as **<Shift>** allows you to stretch the dragger while keeping the opposite side pinned in place. When you drag a corner, this modifies the *scaleFactor* and possibly the translation field of the dragger.

Click one of the spherical knobs to rotate the dragger. When you first click, you'll see two orange lines and two purple circles. The purple

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circles indicate the two ways you can rotate. The orange lines are, once again, the two choices for your mouse movements. Each line begins you moving around one of the two circles. Once you move the cursor far enough, the selected line turns yellow and you begin rotating the dragger about the selected circle. The other circle and line will disappear.


To perform unconstrained (free) rotation, just press **<Shift>** while you drag the spherical knob. There will be no choices; instead all three purple circles will be displayed, forming a ball that you can roll around. Regardless of how you rotate, dragging the spherical knobs will modify the rotation field of the dragger. Depending on how it is centered and what transforms precede it in the scene graph, rotation is likely to affect the translation and scaleFactor fields as well.

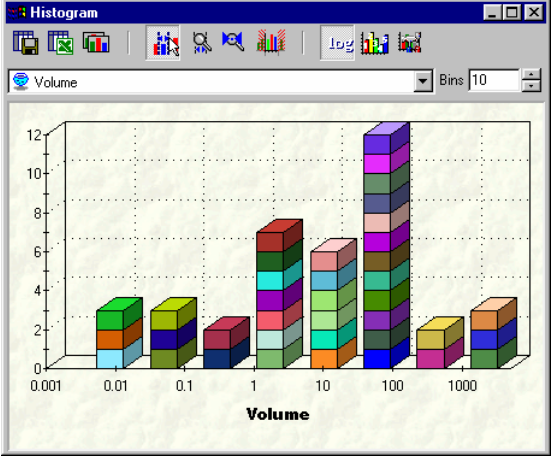
Pressing the **<Control>** key will change the center of rotation in both the constrained and unconstrained (**<Shift>**) case. By default, rotation occurs about the center of the bounding box. With **<Control>** depressed, rotation occurs about the middle of the opposite side of the bounding box. The purple feedback will change to illustrate this; the feedback circles increase in size and purple crosshairs sprout at the new rotational center.<sup>1</sup>

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<sup>1</sup> From the *TGS/OpenInventor User Guide*  
Page 2-470

### Histogram

Open the **Histogram** window by clicking on the **Histogram** button  in the **Volume Measurements or Manual Measurements data table** toolbar. A sample **Histogram** is shown here:



The **Histogram** window displays the frequency distribution of objects according to the selected **Volume** measurement selected from the drop down list box below the toolbar. The number of bins is defined by the **Bins** control, shown above.

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The **Histogram** toolbar contains the following buttons:



**Save histogram data to file:** Clicking this button saves **Histogram** data in text, tab-delimited, comma-delimited, or HTML format.



**Send histogram data to Excel:** Clicking this button sends histogram data to the active page of **Excel** in the following format (via DDE):

Bin #	Volume		Objects	Selected
	From...	...To		
1	0.0001	0.000501	1	0
2	0.000501	0.002511	1	0
3	0.002511	0.012582	2	0
4	0.012582	0.063044	3	0
5	0.063044	0.315904	1	0
6	0.315904	1.582948	7	0
7	1.582948	7.931909	4	0
8	7.931909	39.74558	5	0
9	39.745581	199.159	13	0
10	199.15901	997.9552	2	1
11	997.955247	5000.601	2	0



**Copy graph to clipboard:** Clicking this button copies the **Histogram** graph to the *Windows* clipboard (in enhanced metafile format \* .emf) .



**Select Objects:** Clicking this button activates the selection mode on the Histogram. In this mode, objects on the **Histogram** can be selected by clicking and dragging a selected zone, or just by clicking on the objects. Selected objects are shown with a textured fill style.



**Zoom:** Clicking this button enlarge all or part of the **Histogram** display.



**Reset zoom:** Clicking this button resets zoom and restores the **Histogram** display to its original dimensions.



**Set histogram range:** Clicking this button sets the current histogram range as the new measurement filter range.



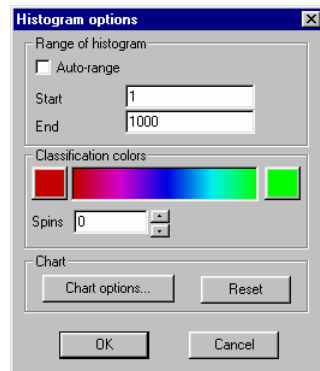
**Log scale:** Clicking this button creates a logarithmic scale for the X axis of the histogram.



**Classification:** Clicking this button classifies the objects according to the current binning. Classification colors can be defined in the *Histogram options* window. After classification, objects will have **Class** measurement value set to the corresponding bin number.



**Histogram options:** Clicking this button opens the **Histogram options** window:



- **Range of histogram:** This group box defines the **Histogram** range.
  - **Start:** Enter the number for the low end of the **Histogram**.
  - **End:** Enter the number for the high end of the **Histogram**.
  - **Auto-range:** Select this check box to have 3D Constructor automatically define the Histogram range.

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The **Start** and **End** numbers for the histogram range are used if the **Auto-range** box is not checked. If the box is checked, the ranges are automatically determined from the data values of the corresponding measurements.

- **Classification colors:** This group box sets the color palette for the **Histogram**.
- **Spins:** The **Spins** list box defines the number of iterations of the color wheel used to create the classification palette.
- **Reset:** The **Reset** button resets all the histogram parameters to their original state.

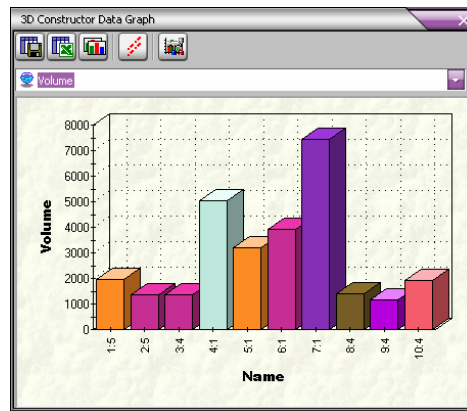
## Data Graph

The **Data Graph** displays information about manual, volume, or tracking measurements. Only visible (not hidden) measurements may be displayed graphically.

The **Data Graph** is accessible from either the **Manual Measurements Data Table** or the **Volume Measurements Data Table** by clicking the

**Data Graph** button. 

A sample **Data Graph** is shown here:



The Data Graph toolbar contains the following buttons:



**Save Data Graph data to file** saves the data in text tab-delimited, comma-delimited or HTML format.



**Send Graph's data to Excel** sends the data to the active page of an Excel worksheet.



**Copy graph to clipboard** copies the graph to the Windows clipboard in enhanced metafile format.



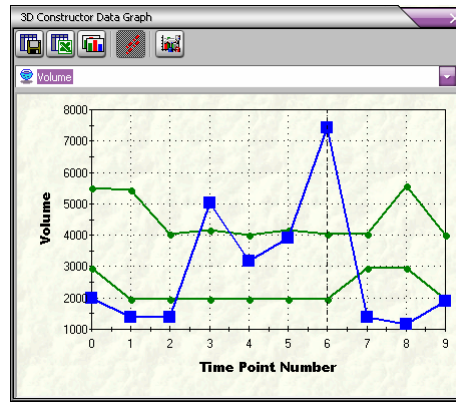
**Data Graph Options** opens the **Data Graph Options** dialog.

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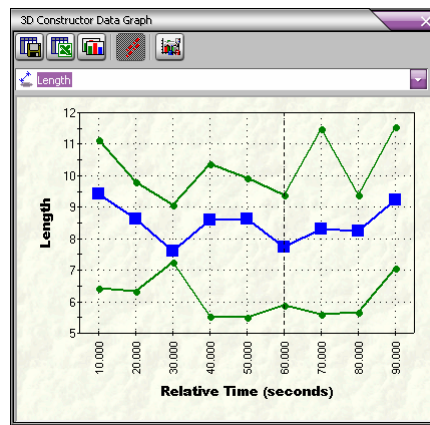
**Tracking mode:** This button toggles the tracking mode of the graph on or off. When the option is on, the graph displays only tracking data (if any can be found). The X-axis in that case represents **Time**.

A sample graph displaying tracking data appears here:



Clicking on the graph selects the track closest to the click point and sets the corresponded time point (only in the *Tracking* mode). The size of the data points and line width on the graph depend on the **Element Size** (see **Manual Measurements Options**). The values change at element size of 5 and 10.

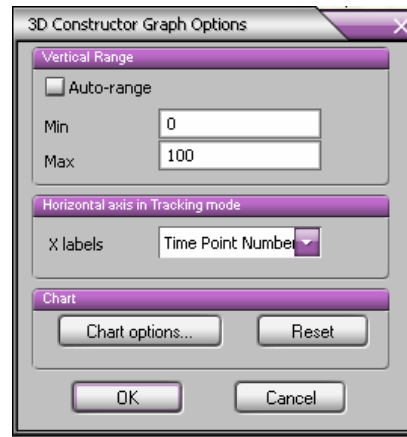
When the **Length** measurement is selected, the graph displays the distance traveled between time points.





## Graph Options

Clicking the **Graph Options** button displays the following dialog:



- **Vertical Range:** The **Vertical range** group box defines the **Start** and **End** of the graph ranges if the **Auto-range** box is not checked (*off*). If the box is checked (*on*), the ranges are defined from the data values of the corresponding measurements.
- **Min:** Enter the lowest value for the range of the graph.
- **Max:** Enter the highest value for the range of the graph.
- **X Labels:** The **X Labels** defines the type of labels along the X axis in *tracking* mode. The choices are:
  - **Time Point Number** indicates the time point number
  - **Relative Time** indicates the time (in seconds) relative to the first time point
  - **Absolute Time** displays an absolute time string (for example: 2003/06/03 09:48:21.035 AM)  
The time (absolute and relative) corresponds to the **Time of Capture** property of the set. The time can be set through the **Set info** dialog (see **The Set Manager** in your *Image-Pro Plus Reference*).
- **Chart Options:** Click this button to display settings for the Data Graph display options.

## Shortcuts and Mouse Controls

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*3D Constructor* uses some special mouse controls and keyboard shortcuts to help you manipulate the volume on the screen:

**<Left>** Click the left mouse button to rotate the virtual trackball.

**<Ctrl>+<Left> or <Shift>+<Left>** (or the middle mouse button)  
Use one of these combinations to pan across the image in the viewer.

**<Ctrl>+<Shift>+<Left>** Use this combination to move back and forth (dolly) toward or away from the image.

**Right Mouse:** Open the popup menu.

**<ALT>:** When the viewer is in *Select/Pick* mode, pressing and holding the **<ALT>** key temporarily switches to *View* mode. When the **<ALT>** key is released, the viewer returns to selection mode. Note that if any of the mouse buttons are currently pressed, the **<ALT>** key has no effect.

**<ALT> + <Left>** = Zoom in and out

**<ALT>+ <CTRL> + Left** or

**<ALT>+<SHIFT> + Left** = Pan (move up/down/left/right)

**<CTRL> + <s>:** invokes the **seek to point** function, which works only when an iso-surface exists and one clicks on the iso-surface

**<CTRL> + <x>:** show x-plane face on

**<CTRL> + <y>:** show y-plane face on

**<CTRL> + <z> :** show z-plane face on

**<CTRL> + <u> :** auto-update on/off

**<CTRL> + <e>:** save iso-surface as object

**<CTRL> + <c>:** stereo mode on/off

## **The Sequence Menu**

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The *Sequence* menu contains those features associated with sets of images (*\*.ips*) or image sequence files (*\*.seq*).

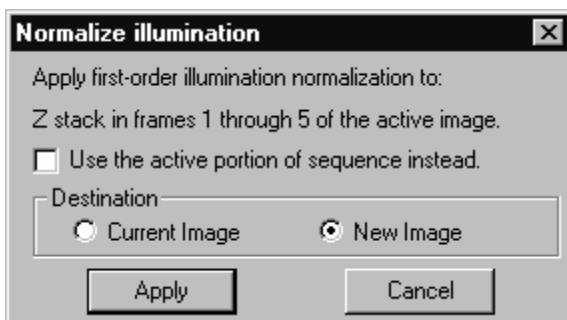
## Normalize Illumination

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You would use this option when the illumination or intensity of emitted light will change in the time that it takes to acquire all of the planes. Basically, this option corrects for uneven illumination as the Z plane changes.

An example would be a sample that uses a fluorescent dye that degrades with exposure to light. The first captured plane will have a higher intensity than the last plane captured. In this case all captured planes will be normalized before being processed into the final image.

When you select *Normalize Illumination* from the *Sequence menu*, you will see the following dialog:



**Apply first-order illumination to....the active image** indicates the type of Z stack that the correction will be applied to when the **Apply** button is clicked.

**Use active portion of sequence:** Check this box to apply the correction to only the active portion of the image sequence.

**Destination:** The **Destination** group box allows you to apply the correction to the existing workspace, or to create a new image containing the corrected stack.

**Current Image:** Clicking this button applies the correction to the current workspace. This option will be disabled if the Z stack comes from a set, is only a portion of a sequence containing other Z stacks, or if the active portion of the sequence is not the entire sequence.

**New Image:** Clicking this button creates a new image containing the corrected stack.

*Sequence:Normalize Illumination*

**Apply:** Click this button to apply the correction.

**Cancel:** Click this button to cancel the normalization. After applying the normalization, the **Cancel** button will change to read **Done**.

## Background Correction

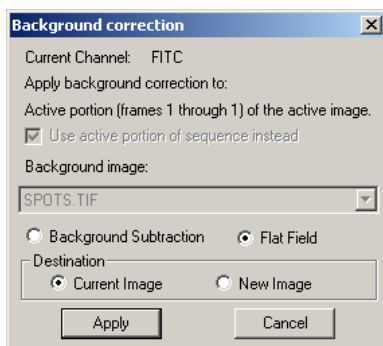
---

Use the *Background Correction* command to make adjustments to the background of your image set. This tool is used to better distinguish image background from image objects, making it easier to extract the objects during a counting or measurement operation. The *Background Correction* command can correct uneven background intensities, and compensate for irregularities due to uneven lighting, nonuniform camera response or minor optic imperfections. It might be used to remove evidence of dust on the lens, or to correct for bright spots caused by the light beneath the microscope's stage.

*Background Correction* can only be performed if at least two frames in the set are open. The active image must be the one you want to perform the background operation upon. You must also have a "background" image open. A background image contains only the background. You can produce a background image by acquiring an image with the sample removed (e.g., remove the slide from the microscope's stage) or with the optics defocused entirely.

Two types of background correction are provided in the **Set Manager**: **Background Subtraction** should be used for all images except those of transmitted light experiments which will be used to measure optical density. For optical density applications, **Flat Field** correction option should be used instead. **Flat Field** correction accounts for the fact that optical density is not a linear function of the gray scale.

When you select *Background Correction* from the *Sequence menu*, you will see the following dialog:



**Current Channel:** This line of text indicates which channel is being corrected.

*Sequence:Background Correction*

**Apply background correction to...the active image** indicates the which portion of the active set that the correction will be applied to when the **Apply** button is clicked.

**Use active portion of sequence:** Check this box to apply the correction to only the active portion of the image sequence.

**Background Image:** This drop-down list box lists all single-frame image workspaces that are the right image class and size to be used as a background image for the active image.

**Background Subtraction:** Click this option button if you want to subtract one of the open images from the active image. **Background Subtraction** will compare the active image to the background image, and pixels deemed to be part of the background will be replaced with a value close to the mean background intensity

**Flat Field:** Click this option button if you want to correct the background of an image measuring optical density via transmitted light. **Flat Field** is similar to **Background Subtraction**, but uses division instead of subtraction, to account for the fact that optical density is not a linear function of the gray scale.

**Destination:** The **Destination** group box allows you to apply the correction to the existing workspace, or to create a new image containing the corrected stack.

**Current Image:** Clicking this button applies the correction to the current workspace.

**New Image:** Clicking this button creates a new image containing the corrected stack.

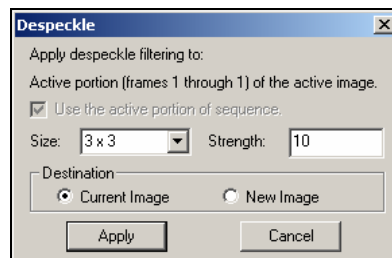
**Apply:** Click this button to apply the correction.

**Cancel:** Click this button to cancel the background correction. After applying the normalization, the **Cancel** button will change to read **Done**.

## Despeckle

---

The **Despeckle** filter removes extraneous “noise” from the frame or frames in the image set. When you select *Despeckle* from the *Sequence menu*, you will see the following dialog:



**Apply despeckle filtering to ...the active image** indicates the which portion of the active set that the filter will be applied to when the **Apply** button is clicked.

**Use active portion of sequence:** Check this box to apply the filter to only the active portion of the image sequence.

**Size:** Use the drop-down list box to select the filter kernel size for the despeckling operation.

**Strength:** Enter a value from 1 - 10 that reflects how much of the filtering effect you want applied to the image. A value of 10 specifies that you want the full strength (100%) of the filtered result applied to each pixel. Values less than 10 cut the full weight of the filter - a value of 1 indicates that only 10% of the *difference* between the filtered pixel value and the original pixel value should be applied, a value of 2 indicates that 20% of the difference should be applied, and so forth.

**Destination:** The **Destination** group box allows you to apply the correction to the existing workspace, or to create a new image containing the corrected stack.

**Current Image:** Clicking this button applies the filter to the current workspace.

**New Image:** Clicking this button creates a new image containing the filtered stack.

**Apply:** Click this button to apply the correction.



*Sequence:Despeckle*

**Cancel:** Click this button to cancel the background correction. After applying the normalization, the **Cancel** button will change to read **Done**.

## **The Set Manager**

---

Sets are collections of images containing more than one variable dimension. A time lapse movie is a simple example of the type of image sets that *Set Manager* is designed to handle. A more complex set may contain time lapse movies at several different positions on the slide, with a Z-series at each time and spatial position. It is possible to create image sets that have multiple channels, stage positions (user defined or arrayed), Z slices, images per stage position (tiles), and time points.

Whether you are using *AFA* or another application to acquire images, or using *SharpStack* and/or *3D Constructor* to analyze your images, *Set Manager* provides the tools you need for creating, editing, and viewing these image sets.

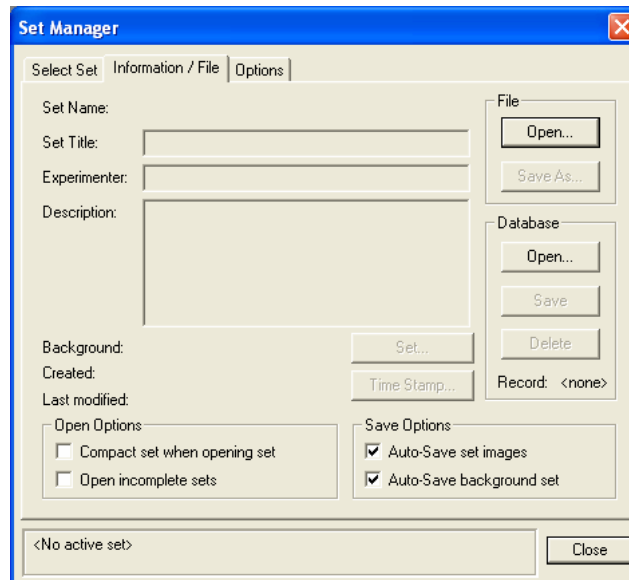
Without using *Set Manager*, it would be difficult to determine the organization and meaning of a multi-dimensional image sequence, or to distinguish between time series and Z-stack sequences, or to associate multiple image workspaces that are related to the same subject.

*Set Manager* will track images that are members of any active set. If an image that is a set member is closed, a dialog will ask you whether to save the image, remove the image from the set or cancel closing the image.

Note that a set can be managed without all of the images being actively open in the application (but it cannot be navigated).

## The Information/File Tab

The **Information/File** tab allows you to display and edit set information, and load or save the current sets. The first time that you choose *Set Manager* from the *Sequence menu*, you will see this tab page:



The controls on this page, except **File:Open** and **Database:Open**, are disabled until there is an active set. The **Select Set** tab is disabled until there is an active set or an open image.

**Set Name:** This field displays the name of the image set that is currently active.

**Set Title:** Enter a new title for this set in this field. This field is limited to 255 characters.

**Experimenter:** Enter the name of the experimenter here. This field is limited to 255 characters.

**Description:** Enter a brief description of the image set here. This field is limited to 511 characters.

*Sequence: The Set Manager*

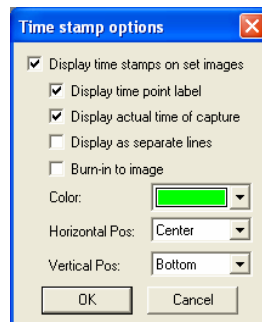
**Background:** The current background image is displayed here. An image or a sequence can be set as the background, using the **Set** button.

**Created:** This field displays the date that the set was originally created.

**Last Modified:** This field displays the date that the set was last modified and saved.

**Set:** Clicking the **Set** button allows you to specify an image or sequence as the background image for the set.

**Time Stamp:** The **Time Stamp** allows you to save the date and time with your image. Clicking the **Time Stamp** button opens the following dialog:



Check the appropriate box. If **Display time stamps on set images** is checked, the following options become available:

- Display time point label
- Display actual time of capture
- Display as separate lines
- Burn-in to image
- Color
- Horizontal position
- Vertical position

When you have made your selection, click **OK** to save it, or **Cancel** to return to the **Set Manager** dialog without saving a time stamp.

**Open Options:** Check the box next to one of the following options:

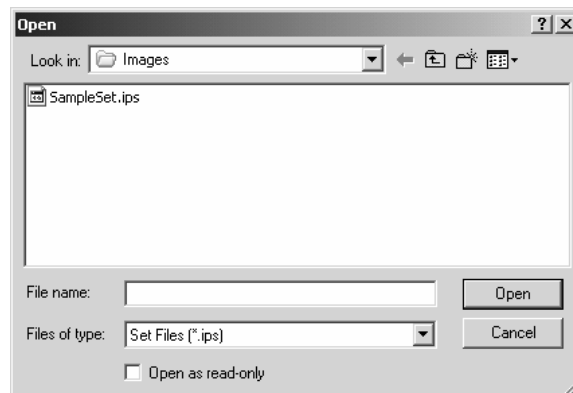
- **Compact Set** when opening set: This option combines all the separate images into sequences, one per channel. This will help insure that *Image-Pro Plus* can open the images.
- **Open Incomplete Sets:** This option allows you to load sets even when some of the images that should be included cannot be found.

**Save Options:** Check the box next to one of the following options, indicating what to do to save your set:

- Auto-save set images
- Auto-save background set

**Close:** Click this button to close **Set Manager**.

**File: Open:** Clicking this button takes you to the **File:Open** dialog where you can select a new set to open, as shown here:



*Image-Pro* sets can also be opened using *File:Open* on the *Image-Pro Plus* menu. All image sets have the extension **\*.ips** (*Image-Pro* set).

**File:Save As:** Clicking this button takes you to the **File:Save** dialog where you can save your new or modified image set as a **\*.ips** file.

**Database:Open:** Clicking this button allows you to open an image set from the database. You will see the *Image Database Search* dialog.

*Sequence: The Set Manager*

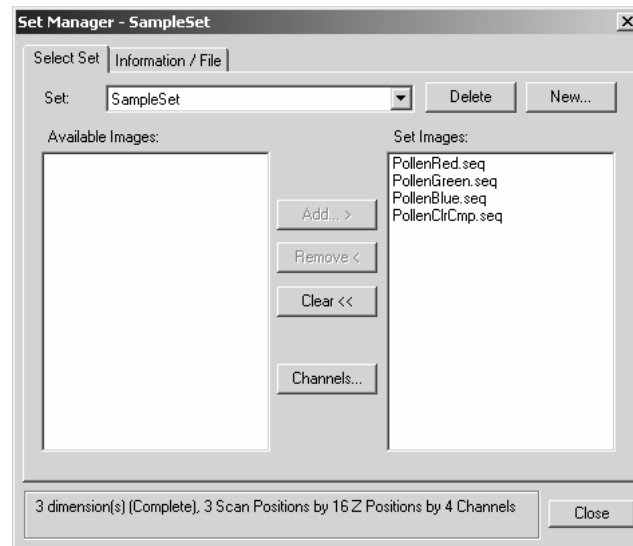
**Database: Save As:** Clicking this button allows you to save the image set in the database. Using the **Save As** command creates a new record in the database, containing the set definition and images.

**Database: Delete:** Clicking this button removes the image set from the database.

**Record:** This field shows the record number for this set as saved in the *Image Database*.

## The Select Set Tab

When you have one or more images or image sets open, the *Select Set* tab will be enabled.



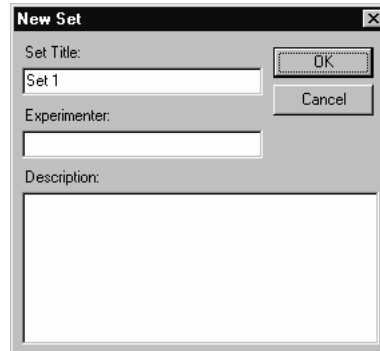
This page is used to build sets from open images.

**Set:** This drop-down list displays all the active sets. The set you want to use can be selected from this list. Changing the active set will activate one of the images in the set, visible in the *Image-Pro* workspace.

**Delete:** Click this button to remove the active set from the list. You must have at least one active set to enable the **Delete** button.

**New:** Click this button to create a new set. You will see the following dialog:

*Sequence: The Set Manager*



**Set Title:** Enter the name of your new image set here. The default name is **Set 1**. The title is limited to 255 characters.

**Experimenter:** Enter the name of the experimenter here. The name most recently used will appear here automatically. This field is limited to 255 characters.

**Description:** Enter a brief description of the new image set here. This field is limited to 511 characters.

**OK:** Click **OK** to create the new image set.

**Cancel:** Click **Cancel** to return to the **Set Manager** dialog. A new set will not be created.

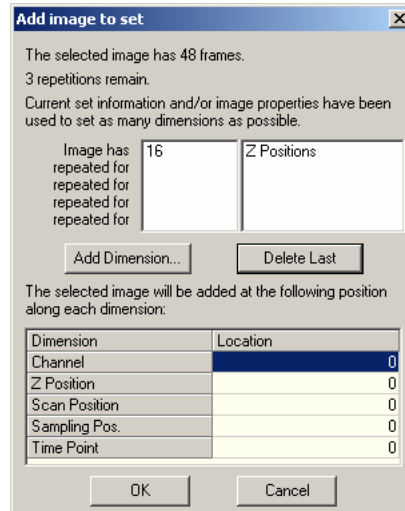
**Available Images:** This is a list of all the open workspaces that are not already part of an active image set, and therefore available to be included in an active set. Use your cursor to highlight the image that you wish to add to the current set.

**Set Images:** This is a list of images in the current set. You may select one or more images in this list to be deleted from the set by using the **Remove** button.

**Add...:** Click this button to add the highlighted image from the **Available Images** list to the current set. You may only add one image at a time.

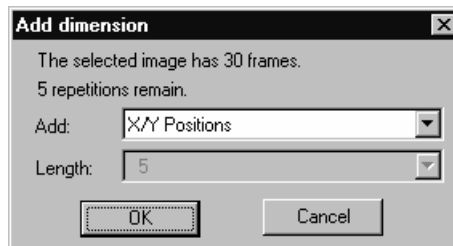
When you click the **Add** button, you will see the following dialog:





The **Add image to set** dialog enables you to set the number of dimensions and the location of each dimension. When first opened, this dialog will contain whatever information is available from the selected workspace.

**Add Dimension..:** Use the **Add Dimension** button to specify the additional dimensions. Clicking Add brings up the **Add dimension** dialog:



Select the dimension that you wish to add from the drop-down list box, and click **OK**.

**Tip:** What you are trying to accomplish here is to inform **Set Manager** of the organization of the frames in the images that you are adding to the set. For example, assume that your image was captured first in one channel, and again in another channel. Then the stage was moved to a new Z position, and both channels were captured again. The images were captured a third time at yet another Z position. Then, the XY stage was moved to different positions, and the images captured again,

*Sequence:The Set Manager*

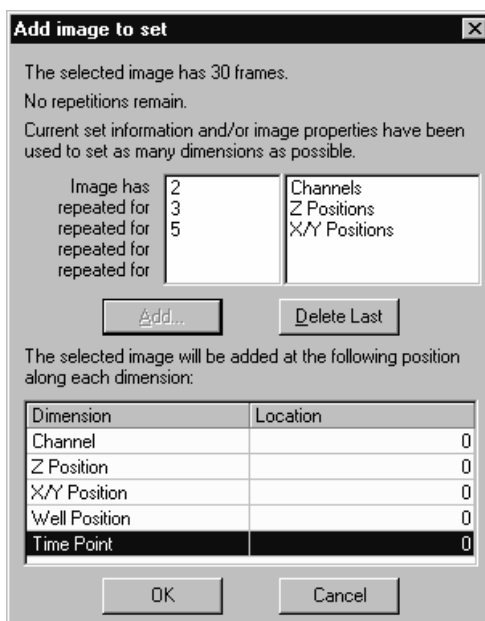
for a total of 3 Z positions and 5 XY positions. The image sequence would be 30 frames long:

2 channels \* 3 Z positions \* 5 XY positions

This example is used in the picture below, after defining the images' organization and just prior to adding it to the active set.

Note that for single-frame images, you will still have to add one dimension. Specify the dimension that you will be incrementing as you add the other single-frame images to the set.

After all the dimensions have been added, your **Add image to set** dialog will look something like this:



This dialog displays the number of frames in the selected image, and the number of frames or repetitions that remain. The dialog will be initialized with whatever information can be gleaned from the image's properties (e.g. if the Z position and/or Channel properties are set as shown above).

**Delete Last:** Click this button to remove the last dimension added to the set.

*Sequence: The Set Manager*

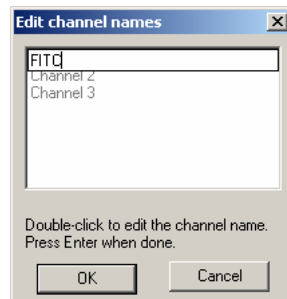
**OK:** Click **OK** when you are satisfied with the number of dimensions that you've added to the set.

**Cancel:** Click **Cancel** to return to **Set Manager** dialog without adding any dimensions.

**Remove...:** Click this button to delete the highlighted image(s) from the **Set Images** list. You may delete multiple images at one time. This button is enabled whenever at least one image in the **Set Images** list is selected.

**Clear:** Click this button to remove all the images from the **Set Images** list.

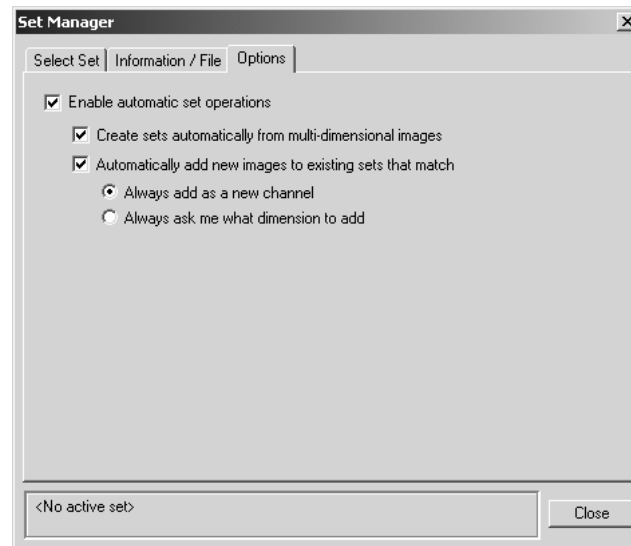
**Channels:** This button is enabled when the set has more than one channel. Click it to see the **Edit Channel Names** dialog:



You can edit the names of the channels by double-clicking on it. Click **OK** when you've finished, or **Cancel** to return to the **Select Set** tab.

## The Set Options Tab

The *Set Options* tab allows you to add new images to existing sets automatically by selecting some or all of the following options:



**Enable automatic set operations:** Check this box to enable the rest of the selections on this page. When automatic set operations are disabled, the remaining controls on this page are disabled and not selected.

**Create sets automatically from multi-dimensional images:** Check this box to enable the auto-set capability.

**Automatically add new images to existing sets:** Check this box to add newly opened or created images to a matching existing set (an operation that takes precedence over creating a new set from the image).

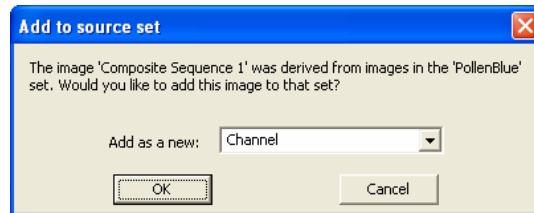
When the image is added, there are further options:

- **Always add as a new channel**
- **Always ask me what dimension to add.**

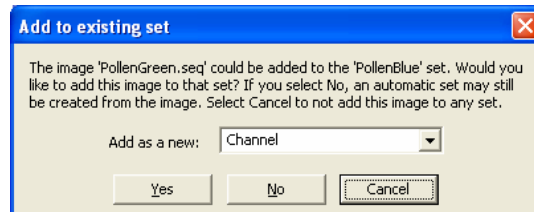
These options are disabled and not selected if **Automatically add...** is *not* selected.

### Sequence: The Set Manager

If you choose to **Always ask me...**, you will see the following dialog, which will vary slightly depending on how the destination set is determined. If the image was created as the result of operations on images from an existing set, the following is displayed:



This dialog will have the following appearance if the new image (opened or created) seems to match the characteristics of an existing set:



**Yes** or **OK** will add the image as the selected dimension.

**Cancel** will prevent the image from being add to a set or auto-creating a set.

**No** will cause a new set to be created from the image.

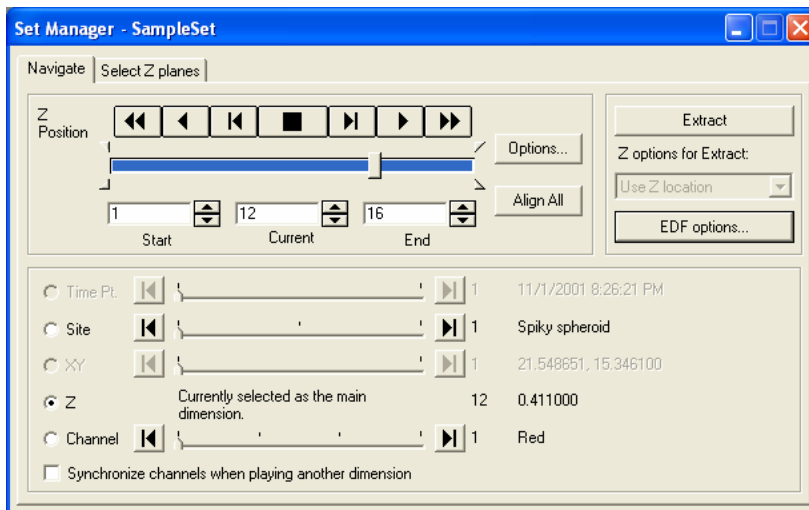
## The Set Navigator

The *Set Navigator* allows you to select any one of the dimensions available in a set to be played as a sequence. You also have the ability to specify the position along all other dimensions that will be displayed when the selected dimension is played. So, for a set with 3 channels, 10 Z slices, 4 stage positions and 20 time points, it is possible to play a time lapse movie of the fifth Z slice through the third stage position in the first channel. Or to play a Z movie through the second stage position at time point 12 in the third channel.

Since an image set is stored in sequence files ( \*.SEQ ), one per channel, there may be times when it is necessary to work with a movie showing changes along a single axis, without having to worry about also altering the other images in the set. For this reason, the Extract button allows you to create a new sequence that contains only the current channels at the positions selected for each of the other channels in the set.

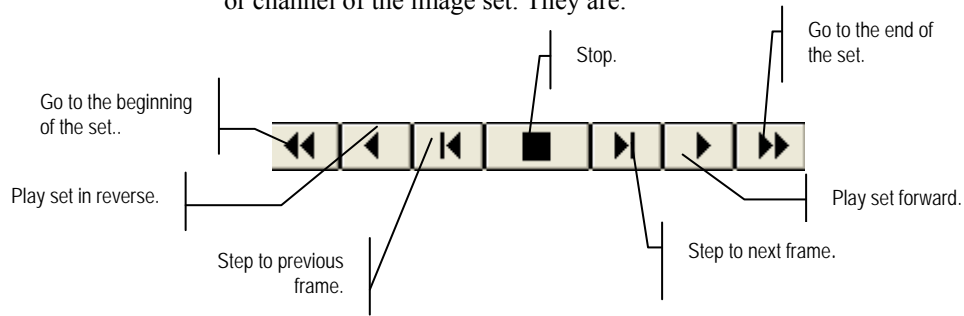
## The Navigate Tab

When you select *Set Navigator* from the *Sequence menu*, you will see the *Navigate* tabbed page:



## Sequence: The Set Navigator

The top left group of controls is used to play the a selected dimension or channel of the image set. They are:



**Start:** This box indicates the number of the starting frame.

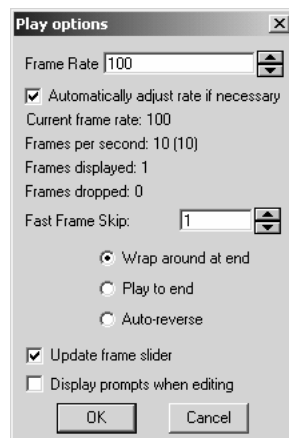
**Current:** This box indicates the number of the frame currently in view.

**End:** This box indicates the number of the last frame.

**Z** in the screen above indicates which dimension is currently selected.

The slider under the navigation controls can be used in place of the navigation buttons. The edit controls under the slider can be used to set the active range of the currently selected dimension.

**Options:** Clicking this button displays the **Play Options** dialog:



## Sequence: The Set Navigator

**Frame rate:** Allows the user to define the time per frame during playback. Specifying 0 will result in maximum speed playback with no delay between frames; however this makes it more difficult to stop playing the set.

**Automatically adjust rate if necessary:** Checking this box allows *Image-Pro* to adjust the frame rate if the system is unable to play the sequence at the original rate.

**Frames per second:** Displays the rate at which frames in the sequence are played.

**Frames displayed:** Indicates the number of frames displayed in the active sequence.

**Frames dropped:** Indicates the number of frames removed from the active sequence

**Wrap around at end:** When selected, playback will wrap around. For instance, when playing forward and the end of the sequence is reached, the playback will continue at the beginning of the sequence. Playback continues until the **Stop** button is pressed. This is the current form of playback.

**Play to end:** When selected, playback will occur once, from the start frame to the end frame if playing forward, from the end frame to the start frame if playing backward, and then stop.

**Auto-reverse:** When selected, playback will reverse directions when an end stop is reached. Playback continues until the Stop button is pressed.

**Update Frame Slider:** When selected, the current frame slider and edit box will be updated as the sequence plays. When not selected, these controls will not update until the sequence is stopped.

**Display Prompts When Editing:** When checked, this box allows the Navigator to display editing prompts when you perform one of the sequence editing commands (discussed in the following pages). These prompts describe what will happen when you use one of the sequence editing operations. To hide the prompts, uncheck this box.

**Align All** is used to align all the workspaces in the active set with the currently active workspace.

The extraction controls are all contained in the top-right group box.

**Extract :** Click this button to extract a new sequence along the active dimension.



The **Z options for Extract** list allows you to choose to extract planes as follows:

**Use Z location:** Extracts the selected dimension using the current Z location for each position along the selected dimension.

**Use selected planes:** Extracts the selected dimension using the Z planes selected on the Select Z planes page.

**Use best-focus plane:** Extracts the selected dimension using the best-focus plane as determined by the Extended Depth of Field (EDF) feature.

**Use composite best-focus:** Extracts the selected dimension using a composite best-focus plane created by the EDF feature.

**EDF Options:** Clicking this button takes you to the *Extended Depth of Field* feature, where you can set the EDF parameters on the **Focus Settings** page.

The bottom set of controls is used to select the active dimension (which can be played or extracted) and the current location along the other available dimensions. Dimensions that are not present in the active set will be disabled.

The active dimension is selected by the radio buttons (e.g. **Channel** in the example). The location controls for the active dimension are replaced by text indicating that the dimension is active. The right two columns of the bottom set of controls indicate the location along each dimension, and the value of that location (e.g. physical position, channel name, etc.).

**Time Point:** Indicates the time point of the active dimension.

**Site:** Indicates the well position of the active dimension.

**XY:** Indicates the XY position of the active dimension.

**Z :** Indicates the Z position of the active dimension.

**Channel:** Indicates the current channel of the active dimension.

**Synchronize channels when playing another dimension.**

Checking this box will “lock” channels together. When a set contains multiple dimensions, you might find it useful to scan through one of the dimensions while observing the results in each of the channels. As long as each channel is contained in a separate workspace from the other channels (as it will be if the set is captured by *AFA*) you can use this control to keep the channels in synch. As you play or step through the another dimension (e.g. Z position, or time point) each

*Sequence: The Set Navigator*

of the channel workspaces will be updated to display the correct frame for that location in each channel.

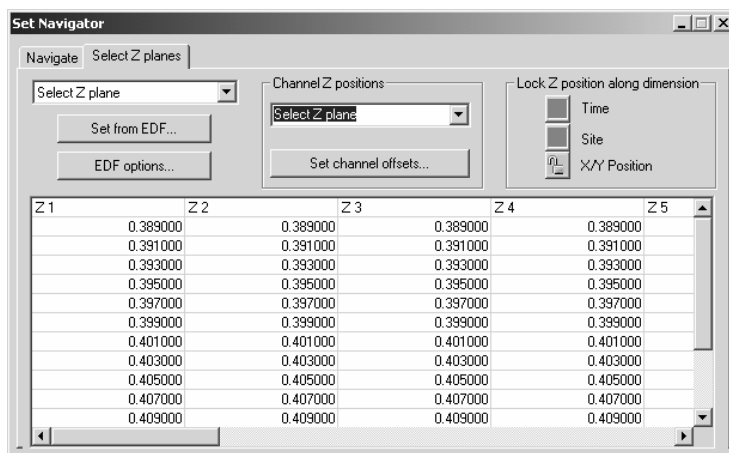
---

**Note:** In version 7.0 of *Image-Pro Plus*, you can now synchronize any of the non-active set dimensions that are displayed in separate workspaces. For example, in a set with Z-stacks taken at multiple time points, where there is one image workspace per stack, you could synchronize all the time points and play through the Z dimension.

---

## The Select Z Planes Tab

The *Select Z Planes* tab provides improved control over the Z plane that is displayed as you navigate through the image stack. You will see this tab page:



**Select Z plane:** This option controls how the Z plane is determined when any other dimension is played. Choose one of the following from the drop-down list:

**Use Z Location:** Plays the selected dimension using the current Z location for each position along the selected dimension.

**Select Z planes:** Plays the selected dimension using the Z planes selected on this page.

**Set From EDF:** Clicking this button allows you to select the best-focus Z plane for each position along the active dimension, using the current EDF settings. The selected plane is indicated by a darker background.

**EDF Options:** Clicking this button takes you to the *EDF* feature to allow you to adjust the options on the **Focus Settings** page.

**Channel Z Positions:** The Channel Z positions group determines whether the selected Z positions are replicated along the other channels in the set. Your choices are:

**Lock all channels:** The Z plane selected on this page will be used for each of the channels in the set.

**Use fixed offset:** The Z plane selected on this page will be combined with a channel-specific offset to determine the Z plane displayed.

**Select Z planes:** Different Z planes can be selected for each channel.

**Set Channel Offsets:** Clicking this button displays the Set channel offsets dialog. This dialog allows you to set the number of planes above or below the selected plane the other channels will be offset when displayed. For instance, if the selected plane in the current location is plane 15, an offset of -1 for one channel would result in the channel displaying plane 14 while an offset of 2 will cause another channel to display plane 16.

**Lock Z Positions:** The **Lock Z position along dimension** group determines whether the selected Z positions are replicated along the other positions of the other dimensions. For each of the indicated dimensions, the **Lock** button will be disabled if the dimension does not exist in the current set. When **Lock** is active, the selected Z position will be used for all the other positions along the associated dimension.

You may navigate along the dimensions of the set that are present but are not locked, or along the active dimension, selecting a Z plane at each position along the active dimension. For instance, if all dimensions are absent or locked, Z planes can be selected for the entire set by one visit to the Select Z planes page. In a 4 dimensional set where no dimension is locked, you should select Z planes for each position along the 2 dimensions not represented on this page.

The Z-plane grid can be used to view and adjust the selected Z planes. The columns are labeled by a simple (and space-saving) abbreviation of the position along the active dimension, e.g. “Ch 1-N”, “TP 1-N”, “SP 1-N” or “XY 1-N”. The Z positions themselves will be labeled by frame number (if the Z position and EDF confidence are not known), by Z position (if EDF confidence is not known), or by Z position and EDF confidence if both are known.

*Sequence: The Set Navigator*

Clicking on the first row position indicator will display that position without changing the selected Z plane. Clicking on the Z position selects that plane and simultaneously displays the selected plane, so the best Z plane can be selected visually.

## **Set Information**

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The *Set Information* feature reports information about the currently active image and the set in which it belongs. You will find a complete description of this feature under *Edit:Info* earlier in this manual.

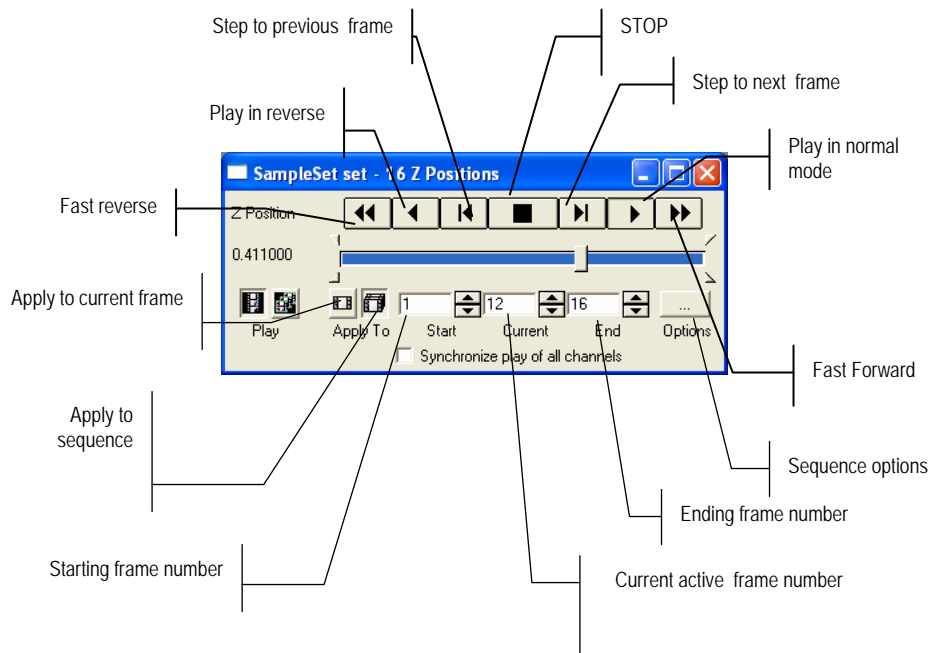
## Sequence Toolbar

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The *Sequence Toolbar* command displays the **Sequencer Toolbar** and performs various operations on an image sequence. This toolbar also appears when you minimize the Set Navigator tabbed dialog. These features are all described below.












### The Sequencer Toolbar

The buttons on the **Sequencer Toolbar** are very similar to the buttons used to control your VCR or CD player. You can scroll forward and back through the group of images, using the buttons for next image, previous image, last image, etc. You can select the starting and ending frames to play only part of the sequence, edit the sequence, and save it.



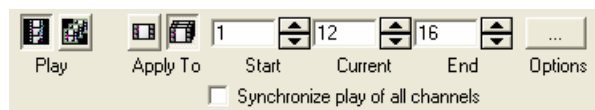
## Sequence: Sequence Toolbar

The following controls are used to step through or play the active portion of the sequence (i.e. bounded by the Start and End frame controls to be described later):

-  Plays the sequence backward in fast mode.
-  Plays the sequence backward in normal mode.
-  Steps the sequence to the previous frame. This will not wrap around when the beginning of the sequence is reached (i.e. frame 1).
-  Stops sequence play so that the stepping controls can be used.
-  Steps the sequence to the next frame. This will not wrap around when the beginning of the sequence is reached.
-  Plays the sequence backward in normal mode.
-  Plays the sequence forward in fast mode.
-  Plays the selected channel (as selected in the Set Navigator)
-  Plays the selected sequence.
-  Apply to active frame
-  Apply to sequence

The active portion of the sequence is defined by the starting and ending frames. You can apply the different sequence operations to just the active portion of the sequence, or the entire set of images.

The following controls are used to set the active portion of the sequence, the active frame of the sequence, and various other options:



The start and end frame controls illustrated above set the active portion of the sequence, which is represented by the colored portion of the slider. The active portion is used for the 4 continuous playback types

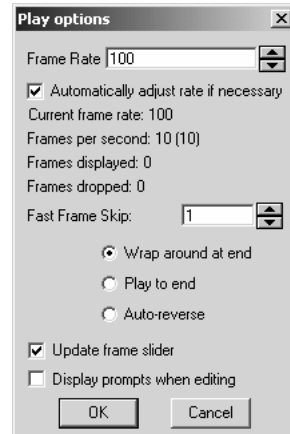
## Sequence:Sequence Toolbar

described above, and for determining the portion of the sequence to which operations will be applied (when “Apply To Sequence” is selected). It is possible to step or scroll through the entire sequence regardless of the setting of these controls.

**Synchronize play of all Channels:** If this box is checked, all open channels will play simultaneously using the current play options, and the common denominator will be the length of the shortest channel.

**Synchronize all Sequences:** If you have more than one sequence open, and this box is checked, all open sequences will play simultaneously using the current play options, and the common denominator will be the length of the shortest sequence.

**Sequence Options** set the various playing controls:



**Frame rate (ms):** Allows the user to define the time per frame during playback. Specifying 0 will result in maximum speed playback with no delay between frames.

**Automatically adjust rate if necessary:** Checking this box allows *Image-Pro* to adjust the rate at which the sequence is played, if the system cannot play the sequence at the original rate.

**Frames per second:** Displays the rate at which frames in the sequence are played.

**Frames Displayed:** Indicates the number of frames in the active portion of the sequence.

**Frames Dropped:** Indicates the number of frames excluded from the active portion of the sequence.



**Fast Frame Skip (for FF/FR):** Allows the user to define the number of frames to skip during “fast mode” playback. Specifying 1 will result in other frame being skipped (which will double playback speed), specifying 2 will result in 2 frames being skipped for every frame displayed, etc.

**Wrap around at end:** When selected, playback will wrap around. For instance, when playing forward and the end of the sequence is reached, the playback will continue at the beginning of the sequence. Playback continues until the **Stop** button is pressed. This is the current form of playback.

**Play to end:** When selected, playback will occur once, from the start frame to the end frame if playing forward, from the end frame to the start frame if playing backward, and then stop.

**Auto-reverse:** When selected, playback will reverse directions when an end stop is reached. Playback continues until the Stop button is pressed.

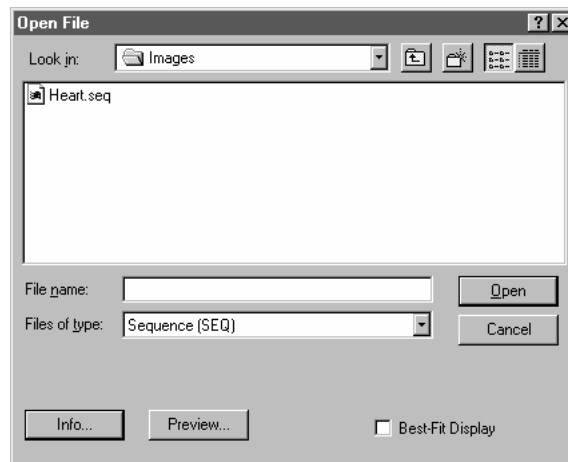
**Update Frame Slider:** When selected, the current frame slider and edit box will be updated as the sequence plays. When not selected, these controls will not update until the sequence is stopped.

**Display Prompts When Editing:** When checked, this box allows the Sequencer to display editing prompts when you perform one of the sequence editing commands (discussed in the following pages). These prompts describe what will happen when you use one of the sequence editing operations. To hide the prompts, uncheck this box.

## Merge Files

---

**Merge Files** allows you to combine two sequence files into one file, or to add a new image to an open sequence file. *The Merge command always places the new sequence file or image at the end of the open one (append).* Selecting this option displays the **Open File** dialog box:



If you have an image or sequence open, the files that you select will be merged with the active image; otherwise a new workspace will be opened for the sequence. Select the name of the file(s) you want to merge. If any of the files are sequences also, you can select the starting frame of the sequence being opened, and number of frames that you want to combine.

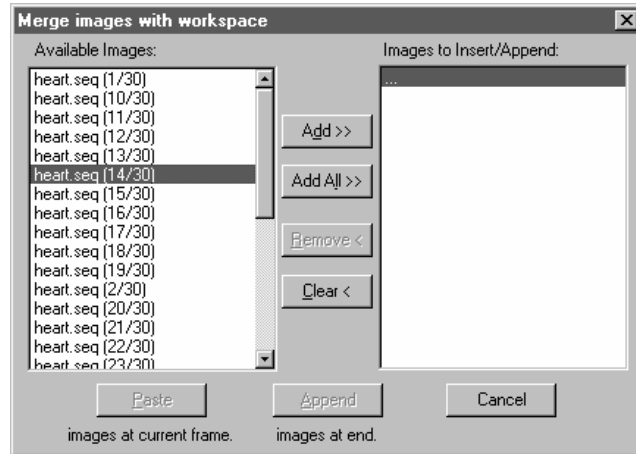
## Sequence Editing Commands

---

The *Sequence Editing* functions in *Image-Pro Plus* allows you to edit and work with multiple-frame image workspaces, called sequences. Each sequence is made up of image frames, stored together in one workspace. By clicking on one of the sequence editing commands, you may modify the sequence in several different ways. These commands assume that a sequence file is open in the *Image-Pro* application area, and that *Apply to Sequence* is activated (see *Sequencer Tools*). When you click on the command, you will see the following options:

- ◆ **Cut Frames** will remove the selected portion of the sequence and place it on the clipboard.
- ◆ **Copy Frames** will copy the selected portion of the sequence to the clipboard.
- ◆ **Delete Frames** will permanently erase selected portion of the sequence.
- ◆ **Paste Frames** is only available when there is sequence data (from a **Cut** or **Copy** operation) on the clipboard. The clipboard data will inserted into the current sequence, just before the active frame.
- ◆ **Append Frames** is only available when there is sequence data (from a **Cut** or **Copy** operation) on the clipboard. The clipboard data will be appended after the last frame in the active sequence.
- ◆ **Extract Frames** copies the selected frames from the sequence in the workspace into individual single-frame workspaces (if you are working with a single-frame workspace, this is identical to the *Duplicate* Command).
- ◆ **Merge Images** allows you to combine frames from two or more workspaces into one file. Selecting this option displays the **Merge Images** dialog box:

## Sequence: Sequence Editing Commands



**Available Images** lists all of the frames from all of the open workspaces (excluding the active workspace). Note that open sequences are expanded to list each of the frames.

**Images to Insert/Append** lists all of the frames that have been selected for the merge. The frames will be merged with the active workspace in the order in which they are listed in the *Insert/Append* list.

There will always be one and only one item highlighted in the *Insert/Append* list, which defines the point at which frames will be added to the list. The “...” item will always remain at the end of the list, allowing a frame to be appended to the end of the list (select “...” and add the frame before it).

**Add:** The **Add** button will be enabled only when one or more frames are selected in the *Available* list. Add will add the selected frame(s) in the *Available Images* list to the *Insert/Append* list, before the currently highlighted item.

**Add All:** The **Add All** button will always be enabled and will add all of the available frame(s) in the *Available Images* list to the *Insert/Append* list, before the currently highlighted item.

**Remove:** The **Remove** button will be enabled only if the selected item in the *Insert/Append* list is not the “...” end marker. **Remove** will remove the selected frame from the *Insert/Append* list. The frame after the removed frame (or the end marker) will become the selected item.

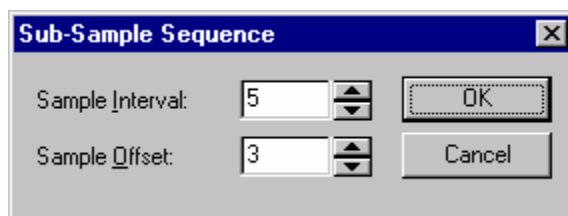
### Sequence:Sequence Editing Commands

**Clear:** The **Clear** button will always be enabled and will remove all of the selected frame(s) from the *Insert/Append* list.

**Paste:** The **Paste** button will be enabled only when one or more frames are selected. **Paste** will insert the selected frames in order (top to bottom) at the current frame position of the active workspace (effectively before that frame).

**Append:** The **Append** button will be enabled only when one or more frames are selected. **Append** will append the selected frames in order to the end of the current workspace sequence, regardless of the currently selected frame or active portion.

- ◆ **Sub-Sample Sequence** allows you to create a new sequence from a portion of the original sequence. This is especially useful when working with the *Mosaic* tool. Sub-sampling builds a new sequence from copies of the selected frames.



**Sample Interval:** This control allows the user to specify the interval that will be used for the sub-sampling. In the example above, the sub-sampled sequence will be composed of every 5<sup>th</sup> frame of the original sequence.

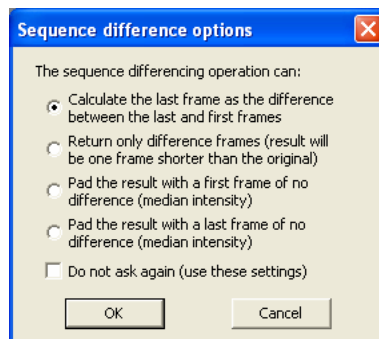
**Sample Offset:** This control allows the user to specify the offset to first frame sampled. If **Apply to Sequence** is selected, the first frame sampled will be offset from the start of the active portion of the sequence, otherwise the first frame will be offset from the beginning of the sequence. In the example above with **Apply to Sequence** not selected, the sub-sampled sequence will be composed of frames 3, 8, 13, etc.

When you click **OK**, a new workspace will be created with a sub-sampled copy of the original sequence. If **Apply to Sequence** is selected, the active portion of the sequence will be sampled, otherwise the entire sequence will be sampled.

## Sequence Operations

The remaining three commands affect sequence operations:

- ◆ Difference of Frames
  - ◆ Average
  - ◆ Running Average.
- ◆ **Difference of Frames:** Clicking this button tells *Image-Pro* to look at the differences between specified frames. It finds the elements that are different and creates a new sequence based on the differences between each of the frames. You will see the following dialog:



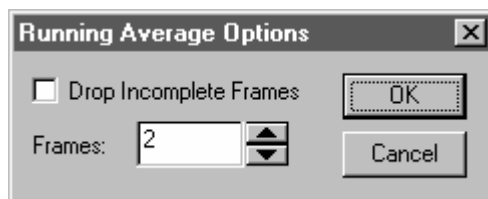
Click the radio button indicating how you would like the resulting sequence calculated. **Do not ask again (use these settings)** will suppress this options dialog for future sequence difference operations, with the operation using the last-selected option

- ◆ **Average Sequence:** Clicking this button creates a new image based on the average of corresponding pixel values in each of the collected images. This is usually done for noise-reduction purposes. When you perform an averaging operation, *Image-Pro* accumulates the specified number of frames (i.e., adds the corresponding pixel values), then divides the result by the specified divisor. The resulting image is placed into a new, untitled image window within *Image-Pro*.

### Sequence:Sequence Editing Commands

For example, if you have a sequence of 3 frames, the averaging operation will look at the pixel in position  $x1, y1$  in each of the three frames. The value of the pixel in position  $x1, y1$  of the new image will be the average of those pixel values.

- ◆ **Running Average:** Clicking this button creates a new sequence where each frame is composed of the average values in  $n$  number of frames. ( $N$  is specified in the **Frames** field.) You will see the following dialog box:



**Drop Incomplete Frames:** Clicking the button instructs *Image-Pro* to remove any partial or blank frames from the running average. If this option *is* checked, the new sequence created by the running average will contain fewer frames than the original sequence, because partial frames where a full average is not available will be dropped.

For example, in a sequence of 4 frames, where  $N$  equals 2, (and **Drop Incomplete Frames** is *not* checked) the first frame in the sequence created by the running average will be the average of frame 0 and itself. The second frame will be the average of frame 0 and frame 1, the second frame will be the average of frame 1 and frame 2, etc. If **Drop Incomplete Frames** is *not* checked, the new sequence will have the same number of frames as the original sequence

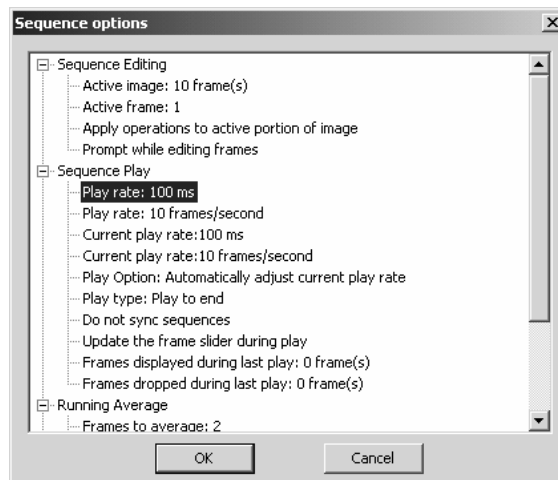
**Frames:** Indicates the number of frames to average for the **Running Average**.

Click **Apply** to modify the sequence, or **Cancel** to leave the sequence unchanged.

## Sequence Options

---

The *Sequence Options* menu item displays information about the active sequence.



Some of the settings, such as the **Play Rate**, are editable by double-clicking on that particular item. Other items are for information only.



## **The Enhance Menu**

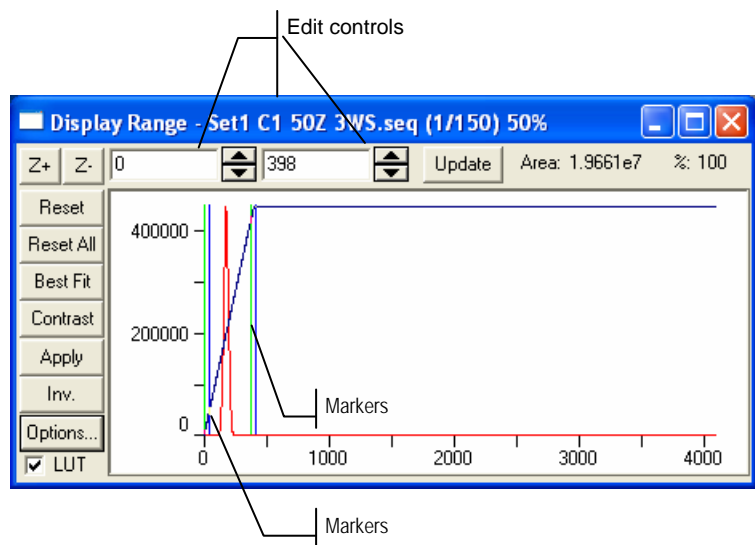
---

*Enhance* menu commands are used to adjust the contrast of your active image. You may adjust the lookup table to increase the contrast and enhance the display of images in low-light situations.

## Display Range

The *Display Range* command allows you to set the intensity levels of your images to increase contrast and enhance display in low-light situations. Although it can be used with all image classes (except Palette), it works best on 12-bit and 16-bit images. Up to 65535 gray levels can be displayed.

Selecting the *Display Range* command opens the following dialog:



This dialog shows the histogram of the active image. Two vertical markers show the upper and lower limits of the intensity levels. These markers can be moved with your cursor. If you are looking at a color image, the histogram will reflect the red, green, and blue values with lines of the same color.

Two edit controls indicate the values for the intensity levels. You can use the spin buttons to increase or decrease these values. All values between 0 and your lower limit will be black; all values between your upper limit and the upper end of the scale will be white.

The **Z+** and **Z-** controls allow you to zoom in and out on the area between the markers.

The **Reset** button restores both the lookup table and the Display Range window to their original settings.

## Enhance:Display Range

**Reset All** resets both the LUT and the Display Range to their original settings.

The **Best Fit** button applies a “best fit” display range to high-precision images, and a “best fit” contrast equalization to 8-bit images. Best fit instructs *Image-Pro* to optimize the *Brightness* and *Contrast* values for your particular image. If you are working with a floating point image, the Best Fit histogram automatically applies a best-fit display range to the image before modifying the LUT.

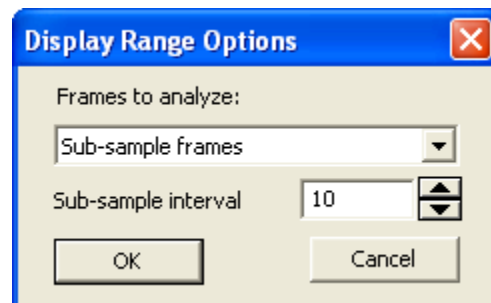
**Contrast** opens the Contrast Enhancement dialog (please see the next section for more information about Contrast Enhancement).

**Apply** applies the current intensity response curve to the image.

The **Inv** button reverses the display.

The **LUT** checkbox turns the Look-Up Table on or off.

**Options:** The options dialog allows you to control which frames will be analyzed when the histogram is calculated; the histogram is displayed in the profile, and used to calculate best-fit etc.



Select one of the following options from the drop-down list:

**All image frames:** All frames of the active image are analyzed. This can cause performance issues when you are using on-demand loading since only one or a few frames will be opened when the image is opened. It can also cause severe performance issues if the entire sequence will not fit in memory.

**All frames in memory:** This option will use all of the frames of the active image that have already been loaded. This may be just a single frame with on-demand loading of sequences.

**Sub-sample frames:** This option will use the Sub-sample interval to sample every Nth frame of the sequence. So if the Sub-sample interval is 10, every 10<sup>th</sup> frame of the sequence will be analyzed.

*Enhance:Display Range*

**First, last and middle frames:** This option will use the first frame of the sequence, the last frame of the sequence, and a frame in the middle of the sequence. Since it will only use a maximum of 3 frames, this option will always be quite fast even if very few of the image frames are in memory.

**Sub-sample interval:** Indicate the number of frames to sample. The sub-sample interval can be set to any number between 1 (which is the same as the All image frames option, or no sub-sampling) and 100.

---

**Note:** The *All Image Frames* option will respect the **Apply to Sequence** settings on the **Sequence** toolbar and *IPP* status bar.

---

## Contrast Enhancement

---

Use the *Contrast Enhancement* command to adjust the current Brightness, Contrast and Gamma settings. You can also make adjustments to the Luminance, Red, Blue, and Green color channel settings.

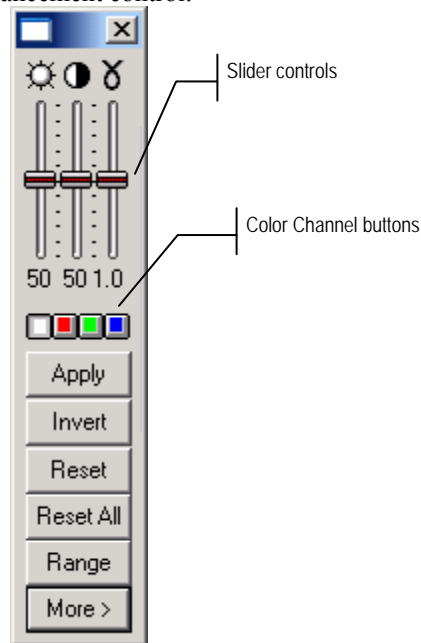
When the *Contrast Enhancement* controls are adjusted, the pixel values in your image bitmap are not actually modified; the controls modify a Lookup Table (LUT) through which your image is interpreted. For example, when you increase brightness in an image, all of the intensity values in your image are increased by a certain amount. The pixel values in your image are not actually changed; the changes you see on the screen are calculated by reading the image's original value and processing it through the Lookup Table. The LUT dictates how each pixel value is interpreted. In our brightness example, the table might convert a *Gray Scale* pixel value of 120 to 140, and a value of 50 to 70.

*Image-Pro* uses a LUT for processing intensity enhancements because 1) it is the fastest way to apply multiple adjustments to an image, and 2) it lets you easily reverse adjustments you have made.




When contrast modifications are performed, all changes are placed into the LUT. If you were to copy, resize or otherwise perform an operation that produces a new window from your bitmap, the results would be derived from the *unenhanced* values. To make the LUT enhancements permanent, you must select the *Apply Contrast* command to write the modified pixel values to your image bitmap, or click the **Apply** button on the Contrast Enhancement window. If an AOI is active when the *Apply Contrast* command is performed, the LUT values will be written to just the pixels within the AOI.

## Enhance: Contrast Enhancement

When you select the *Contrast Enhancement* command, the **Contrast Enhancement window** appears, in either the “simple” or the “expanded” mode. This is the “simple” mode of the contrast enhancement control:



**Contrast Enhancement Controls:** These controls are used to adjust the visual qualities of your image. There is an independent slider control for each characteristic:

-  **Brightness:** Move this slider up to increase, or down to decrease, the image brightness. A value of 50 represents no change to the image values.
-  **Contrast:** Move this slider up to increase, or down to decrease, the image contrast. A value of 50 represents no change to the image values.
-  **Gamma:** Move this slider up to increase the Gamma value (increase dark area contrast) or down to decrease the Gamma value (increase bright area contrast). A value of 1 represents no change to the image values.

## Enhance: Contrast Enhancement

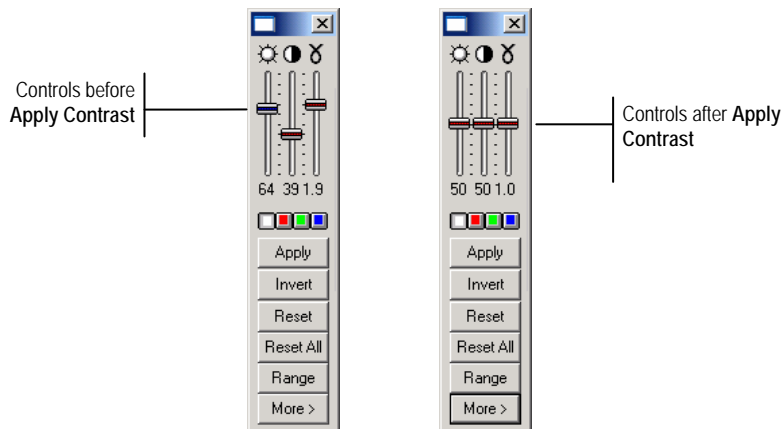


**Color Channel:** Select the Luminance, Red, Green or Blue channel button corresponding to the color in your image. If your image is not *True Color* or *Palette*, the **Luminance** channel will be your only option here. The **Luminance** channel is the intensity produced by the combined RGB channels. Click on the color button, and move the slider controls until the new color values are reflected in your image.

Clicking **Apply** writes the modified pixel values to your image bitmap.

**Important** - If you want to save the LUT so it can be applied to similar images at a later date, you must do this before you perform the **Apply Contrast** command. **Apply Contrast** clears the LUT; if you save the LUT after the **Apply Contrast** is performed, your LUT will be empty.

Once the LUT has been applied, you will see the Contrast Enhancement controls return to their initial, flat positions.



**Invert** reverses the values in the lookup table.

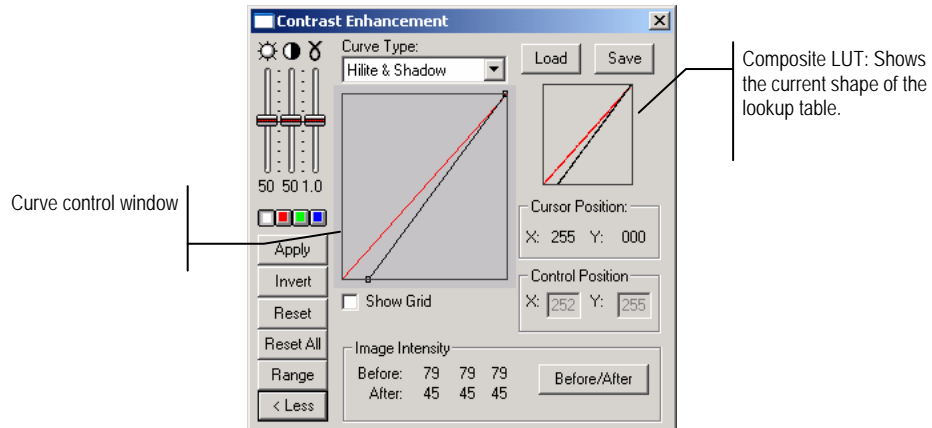
**Reset** restores both the lookup table and the **Display Range** window to their original settings.

**Reset All** restores both the lookup table and the **Display Range** window to their original settings.

The **Range** button opens the **Display Range** window

*Enhance: Contrast Enhancement*

**More** expands the **Contrast Enhancement window** to display the expanded (advanced) mode:



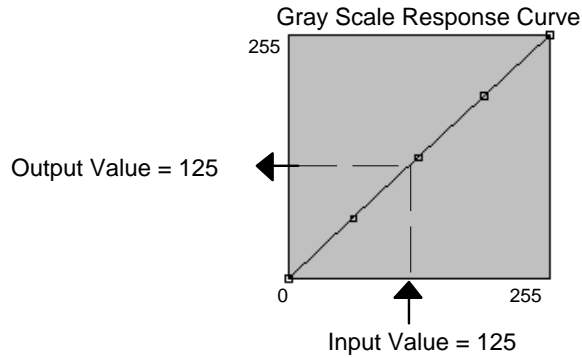
The expanded mode of the **Contrast Enhancement** window lets you modify the Lookup Table (LUT) using the advanced controls, giving you more control over the results. For example, you might want to modify the contrast to interpret all values above 200 as white (255), or boost the blue channel between values 0 and 128.

It contains controls that allow you to select the curve type, load and save Lookup Table settings, and track your cursor position. The controls are scaled to use values ranging from 0 to 255.

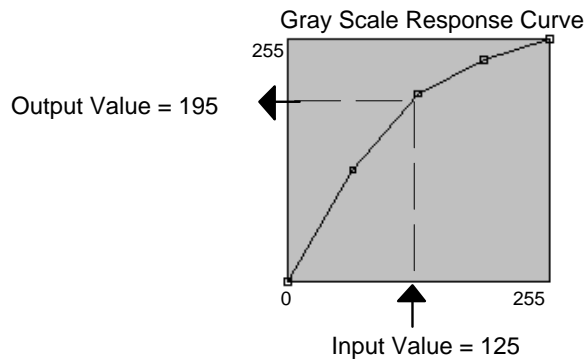
The *Contrast Enhancement* operates by letting you reshape the curve that describes your intensity scale. Initially, the values on your scale are linearly distributed from its smallest value to its largest. As shown in the example below, this produces an identity response curve, describing a function where the input and output values are identical (e.g., an intensity value of 125 is interpreted as 125).



*Enhance: Contrast Enhancement*



The *Contrast Enhancement* command lets you view this curve, and change its shape by changing the output value of certain control points along the curve. *Image-Pro* gives you the ability to control intensity values at 2, 5, 9 or 256 points along the curve. When you change the value of a control point, the values between it and the preceding/ succeeding control point are linearly derived as shown in the 5 point example below. As you can see, our example value of 125 no longer generates a response of 125. Instead, 125 is interpreted as 195. With this map, any pixels with a value of 125 would be rendered as if they possessed a value of 195. Other pixel values would be similarly adjusted, according to what output value is described by this map.



**Curve Type:** In this combo box, select the number of control points you want to be given. When you select a **Curve Type**, the appropriate curve will be displayed in the curve window. You can modify the curve's shape by dragging a control point to a new position. When you drag a control point, its initial and modified values are displayed in the x and y fields in the **Control Position** group box.

## Enhance: Contrast Enhancement

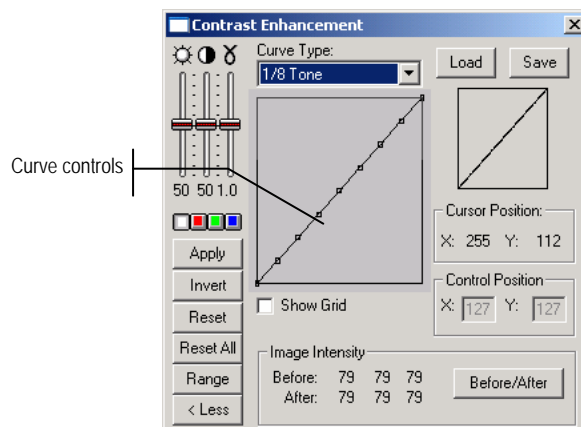
You will also see the results of a change immediately in your image. When you are happy with the changes you have made, select *Apply* to write the changes permanently to your image. If you want to clear the curve changes you have made, use the *Reset* command.

The following curve types are provided:

**Highlight and Shadow:** This option lets you modify two control points: the **Highlight** point (the largest value on the scale) and the **Shadow** point (the smallest value on the scale). **Highlight and Shadow** differs from all other control point options in that it allows you to move the control points in a horizontal direction only. Moving the highlight point to the left specifies the point at which you want the scale to become white -- all values from this point on are set to the highest value on your scale (255 if you are working with a *Gray Scale* image). Moving the shadow point towards the right sets the point up to which you want the scale set to black -- all values less than or equal to this point are considered black (0).

**1/4 Tone:** This option lets you modify the curve using 5 control points, allowing you to change the shape of the curve in 4 segments. Moving a control point up increases the intensity value of that point and points along the segments it touches. Moving a control point down decreases the intensity value of that point and points along the segments it touches.

**1/8 Tone:** This option lets you modify the curve using 9 control points, allowing you to change the shape of the curve in 8 segments. Moving a control point up increases the intensity value of that point and points along the segments it touches. Moving a control point down decreases the intensity value of that point and points along the segments it touches.



## Enhance: Contrast Enhancement

**Composite:** The **Composite** option in the *Contrast Enhancement* allows the user to directly specify the 256 element LUT used to modify image contrast. This can be set by drawing on the window containing the response curve using the mouse.

A pixel with a particular intensity value (X axis) will receive a certain output value (Y axis) as a result. Note that, unlike the other options in the **Curve Type** combo box, the Composite window shows the effects of the BCG settings in both windows - the rightmost always shows the final LUT, and in Composite mode so does the central window. The Composite mode allows the most detailed control over the Contrast Enhancement LUT.

**Show Grid:** Check this option if you want a grid displayed within the curve window. Each square on the grid is 16 pixels x 16 pixels.

**Load/Save:** These options allow you to load and save the Lookup Table settings in \*.LUT files. For more information about Loading and Saving, please refer to the section, *Load and Save Dialog Boxes* at the beginning of this manual.

**Image Intensity:** This informational field shows you before and after intensity settings of the pixels under your cursor in the Red, Green, and Blue value of the active image before and after the LUT is applied.

**Before/After:** Click this button if you want to view your image in its original state without *any* interpretation through the Lookup Table. When you click **Before/After**, *Image-Pro* opens



the **Display** window, allowing you to view your document in its original or enhanced form. This window allows you to toggle between views of the two versions of your image. When you have finished viewing your image, you must close the window (click on the **Display** window's close button). You will not be able to proceed within *Image-Pro* until you do so. When you close the window, *Image-Pro* will automatically revert the image to its enhanced form.

**Cursor position:** This group box shows the input (x) and output (y) values of the cursor in the **curve control** window.

**Control position:** This group box shows the input (x) and output (y) values of the selected controls on the line in the **curve control** window.

## Equalize

---

Use the *Equalize* command to use one of *Image-Pro's* advanced histogram manipulation functions to enhance the contrast and dynamic range of an active image.

When you click on the *Equalize* command, you will have several options:

- ◆ Best Fit
- ◆ Linear
- ◆ Bell
- ◆ Logarithmic
- ◆ Exponential.

When you select one of the options, *Image-Pro* will analyze your image's accumulated histogram and redistribute it into the shape you have specified (see the Histogram and Intensity Analysis discussions in Section 1 for more information about Histograms).

---

**Note:** When you apply an **Equalize** option, *Image-Pro* implements the modifications through the Lookup Table (LUT). Your original image bitmap is not updated. If you want to make the enhancements permanent, you must write them to the image with the **Apply Contrast** command.

---

**Best Fit:** Use the *Best Fit* command to instruct *Image-Pro* to optimize the values for your particular image. The results are achieved by stretching the histogram to achieve the best possible contrast distribution of pixel values in your image in this way: Best fit assigns the bottom 3% of the values to the shadow point, (0) and the top 3% of the values to the highlight point (255). The rest of the values are distributed evenly across the scale).

**Linear:** This option distributes the histogram equally across the intensity scale. This function produces a high contrast image with the highest possible dynamic range.

**Bell:** This option distributes the histogram evenly around the center of the intensity scale. This function produces a high contrast image with less dynamic range than the **Linear** distribution.

**Logarithmic:** This option concentrates the histogram at the low end of the scale. This function produces a high contrast image with little dynamic image. It will tend to darken the image overall. Useful for increasing the contrast in a very light image.

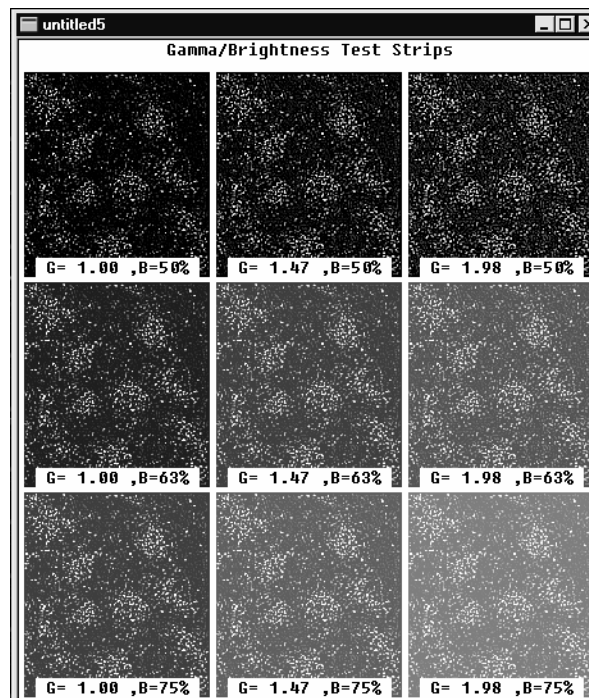
**Exponential:** This option concentrates the histogram at the high end of the scale. This function produces a high contrast image with little dynamic image. It will tend to lighten the image overall. Useful for increasing the contrast in a very dark image.

## Test Strips

---

Use the *Test Strips* command to create a single page of several image miniatures, each with various Brightness, Contrast and Gamma settings.

Test strips let you quickly identify which BCG or halftone options will give you the best image results on your particular printer or system. Once you have generated and evaluated a test strip, you can make the appropriate BCG corrections with the BCG Controls, or select the most suitable halftoning options for your image. The example below shows a test strip of an image rendered with a range of Gamma and Brightness values.



If you want to create a test strip from just a portion of your image, define an AOI before selecting the *Test Strips* command.

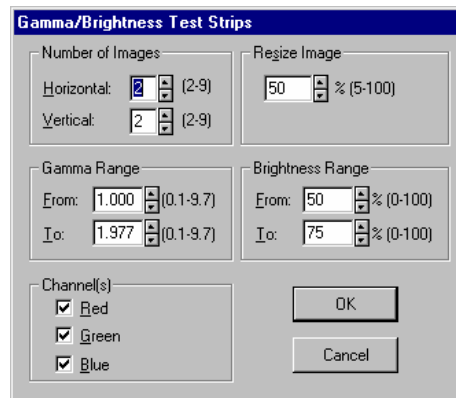
## Enhance:Test Strips

The *Test Strips* command presents a menu from which you must first select the type of strip you want to create. For intensity settings, you can generate test strips for Brightness, Contrast, Gamma, or combinations of any two. The choices are:

- ◆ Gamma
- ◆ Brightness
- ◆ Contrast
- ◆ Gamma\*Brightness
- ◆ Gamma\*Contrast
- ◆ Brightness\*Contrast

Select the type of test strip that you want to create.

- 
- ◆ **When you select one of the intensity menu options** (Brightness, Contrast, Gamma or combination), *Image-Pro* will present a **Test Strips** dialog box. With this box you control the number and size of the images generated on your strip, and the range of values over which the samples are spread.



Once you have made your selections in this box, click **OK** to create the strip. *Image-Pro* will generate the specified number of images in a single image window. You can view, print, store and manipulate this image just as you would any other image within *Image-Pro*.

## *Enhance:Test Strips*

**Number of Images:** Within this box, select the number of test images you want to create. The test images will be arranged in an array of the dimensions that you specify here. You might want to create an arrangement that is compatible with your output device, if you plan on printing the strip -- e.g., one that is wider than long, if you will be printing on a landscape oriented page, longer than wide if printing in portrait.

**Resize Images:** Within this box, set the size you want each test image created at. Keep in mind that the entire set of examples in the strip (as defined by the values you have set in **Number of Images**) will be placed into a single image window. So, reduce them to a size that will allow you to easily view or print the strip as a group.

**Brightness / Contrast / Gamma Range:** Depending upon the type of strip you selected from the *Test Strips* menu, you will have one or two boxes of range values to set. The **From** and **To** values in these boxes determine the intensity settings that will be assigned to the first and last test images (respectively). The remaining images will be distributed at equal intervals within the range (if a combination of intensities has been selected, *Image-Pro* will generate equally distributed permutations).

The **From** and **To** values are expressed as percentages for Brightness and Contrast operations, and in absolute value for Gamma operations. This is consistent with the way these functions are adjusted with the **BCG Controls**. When you set a value below 50 for Brightness or Contrast, you are reducing the level. When you set a Gamma value below 1, you are reducing Gamma.

**Channel(s):** Within this box, select the color channel or channels (more than one can be simultaneously selected), you want adjusted when it produces the sample images. If you want it to operate upon the combined intensity, select all three.

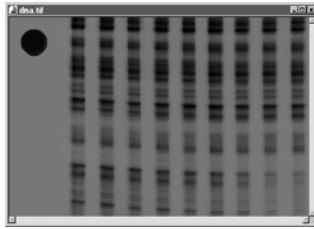
The **Channel(s)** option box will only be active if you are creating a test strip for a *True Color* or *Palette* image.



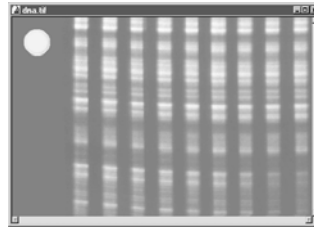
## Invert Contrast

---

Use the *Invert Contrast* command to instruct *Image-Pro* to reverse the values in the lookup table for your active image. The example below shows the original image, and the image with the inverted contrast.



*Original Image*



*Image with Inverted Contrast*

If you are happy with the contrast adjustments, and want to make them permanent, use the *Apply Contrast* command to write the modified values to your image.

## **Reset Contrast**

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Use the *Reset Contrast* command to undo any Lookup Table modifications that have not yet been applied to your image, and reset the Lookup Table to its linear state.

Notice that when *Reset Contrast* is performed, your image returns to its unaltered state, and the Contrast Enhancement controls are reset to their initial, flat positions.

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*Note:* The **Reset Contrast** command can also be performed by clicking the **Reset** button located in the **Contrast Enhancement** window, or by clicking on the **Reset** button in the toolbar.

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## Apply Contrast

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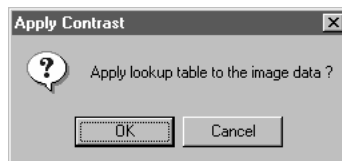
Use the *Apply Contrast* command to make the current Lookup Table adjustments permanent.

When the **Contrast Enhancement** controls are adjusted, the pixel values in your image bitmap are not actually modified; the controls modify a Lookup Table (LUT) through which your image is interpreted. For example, when you increase brightness in an image, all of the intensity values in your image are increased by a certain amount. The pixel values in your image are not actually changed; the changes you see on the screen are calculated by reading the image's original value and processing it through the Lookup Table. The LUT dictates how each pixel value is interpreted. In our brightness example, the table might convert a *Gray Scale* pixel value of 120 to 140, and a value of 50 to 70.

*Image-Pro* uses a LUT for processing intensity enhancements because 1) it is the fastest way to apply multiple adjustments to an image, and 2) it lets you easily reverse adjustments you have made. The following intensity operations modify the LUT for the image:

- ◆ the *Brightness*, *Contrast*, and *Gamma* controls
- ◆ the *Contrast Enhancement* command options
- ◆ the *Equalize* command options, or
- ◆ the *Invert Contrast* command.

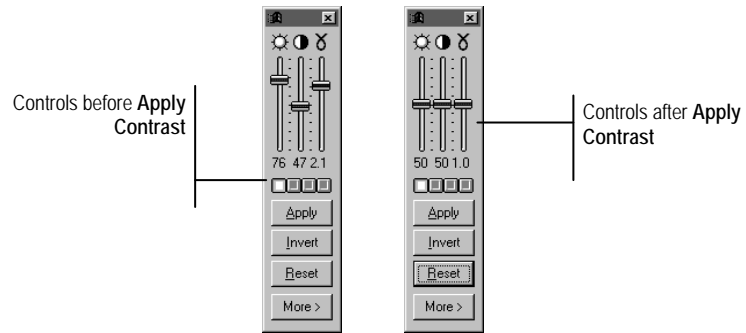
When you select *Apply Contrast* you will be prompted to confirm your request.



**Important** - If you want to save the LUT so it can be applied to similar images at a later date, or you want to save the LUT to a printer or scanner calibration file, you must do this before you perform the **Apply Contrast** command. **Apply Contrast** clears the LUT; if you save the LUT after the **Apply Contrast** is performed, your LUT will be empty. See the **Contrast Enhancement** command for information about saving a Lookup Table.

*Enhance:Apply Contrast*

Once the contrast has been applied, you will see the Contrast Enhancement controls return to their initial, flat positions.



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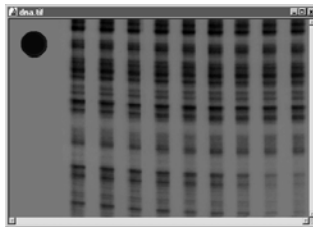
**Note:** The **Apply Contrast** command can also be performed by clicking the **Apply** button located beneath the BCG sliders.

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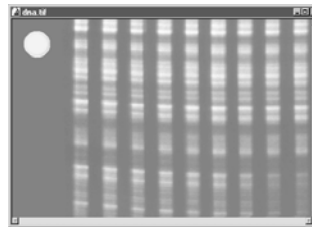
## Invert Image

---

Use the *Invert Image* command to instruct *Image-Pro* to reverse the pixel values for your active. Unlike *Invert Contrast*, the modifications are made directly to the image data, rather than going through the Lookup Table. The example below shows the original image, and the inverted image.



*Original Image*



*Inverted Image*

*Enhance:Invert Image*

## **The Process Menu**

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*Process* menu commands are used to manipulate your active image. You may adjust the background of the image, apply filters, and combine images in several ways.

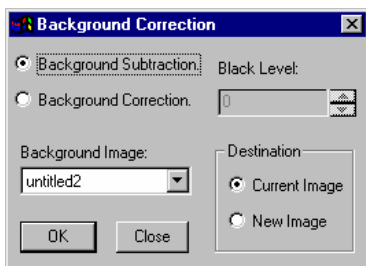
## Background Correction

---

Use the *Background Correction* command to make adjustments to the background of your image. This tool is used to better distinguish image background from image objects, making it easier to extract the objects during a counting or measurement operation. The *Background Correction* command can correct uneven background intensities, and compensate for irregularities due to uneven lighting, nonuniform camera response or minor optic imperfections. It might be used to remove evidence of dust on the lens, or to correct for bright spots caused by the light beneath the microscope's stage.

*Background Correction* can only be performed if at least two images are open. The active image must be the one you want to perform the background operation upon. You must also have a "background" image open. A background image contains only the background. You can produce a background image by acquiring an image with the sample removed (e.g., remove the slide from the microscope's stage) or with the optics defocused entirely.

Two types of background correction are provided by *Image-Pro*: **Background Subtraction** should be used for all images except those of transmitted light experiments which will be used to measure optical density. For optical density applications, the **Background Correction** option should be used instead. **Background Correction** accounts for the fact that optical density is not a linear function of the gray scale.





**Background Subtraction:** Click this option button if you want to subtract one of the open images from the active image. **Background Subtraction** will compare the active image to the background image, and pixels deemed to be part of the background, will be replaced with a value close to the mean background intensity.

The algorithm is based on the following formula:

$$CI_{x,y} = I_{x,y} - BI_{x,y} + M$$

where

$I_{x,y}$  is a pixel value of the original image at location (x,y);

$BI_{x,y}$  is a pixel value of the background image at location (x,y);

M is the average pixel value of the background image; and

$CI_{x,y}$  is the new pixel value in the corrected image.

*Note - Do not use **Background Subtraction** on transmitted light experiments where optical density will be measured; use **Background Correction** instead.*

**Background Correction:** Click this option button if you want to correct the background of an image measuring optical density via transmitted light. **Background Correction** is similar to **Background Subtraction**, but uses division instead of subtraction, to account for the fact that optical density is not a linear function of the gray scale.

The algorithm is based on the following formula:

$$CI_{x,y} = \left( \frac{I_{x,y} - BL}{BI_{x,y} - BL} * (M - BL) \right) + BL$$

where

$I_{x,y}$  is a pixel value of the original image at location (x,y);

$BI_{x,y}$  is a pixel value of the background image at location (x,y);

M is the average pixel value of the background image;

BL is the level of black; and

$CI_{x,y}$  is the new pixel value in the corrected image.

*Process:Background Correction*

**Background Image:** From this list box, select the open image that represents the background. The background image must be the same class as the image for which it will be used to correct.

**Black Level :** This field will contain the black level as specified by the current Intensity Calibration.

**Destination Image:** Select the window into which you want the corrected image written.

**Current Image:** Select this option if you want the corrected image written to the active image window.

**New Image:** Select this option if you want the corrected image written to a new image window.

## Filters

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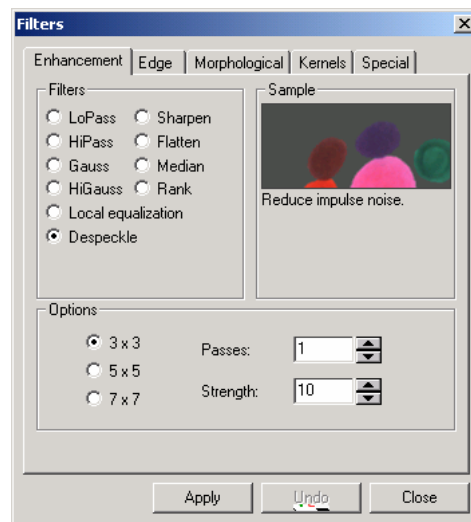
Use the *Filters* command to apply one of *Image-Pro's* numerous filters to the AOI or active image. Filtering operations reduce or boost the rate of brightness change in an image. If you are not familiar with the process and effects of filtering, you might want to review the *Spatial Filtering* discussion in the *Image-Pro Plus Start-Up Guide* for additional discussion about this command.

*Image-Pro* provides an extensive set of convolution and nonconvolution (morphological) filters. You can also create your own filtering kernels and apply them with the *Filters* command.

When you select the *Filters* command, the **Filter** tab dialog is opened. Each group of filters has its own property sheet or tab, where you select the type and size of filter you want to use. Filtered results are almost always written to the active image. You may use the *Undo* command if you want to remove the effects of a filter you have applied.

The **Filter** window is a “common access” window. That is, once it is activated, it can be utilized with any open image window (it is not linked to a specific image like a **Histogram** window).

Clicking on the **Filters** command displays the following dialog:



*Process:Filters*

**Sample:** This group box contains a **Preview** window where you can see the effects of a filter before it is applied to the active image. A brief description of the filter appears beneath the **Preview** checkbox.

To preview a filter, click your the left mouse button inside the preview window . You will see the effect of that filter on a small portion of the image. You can scroll the cursor around inside the preview window to see different areas of the image. To update the preview, click the left mouse button in the preview window. To end the preview, click on a different filter tab, or close the **Filter** dialog box.

**Options:** The choices in this group box will vary depending upon the kind of filter you have selected. The options for each set of filters will be described in the following sections.

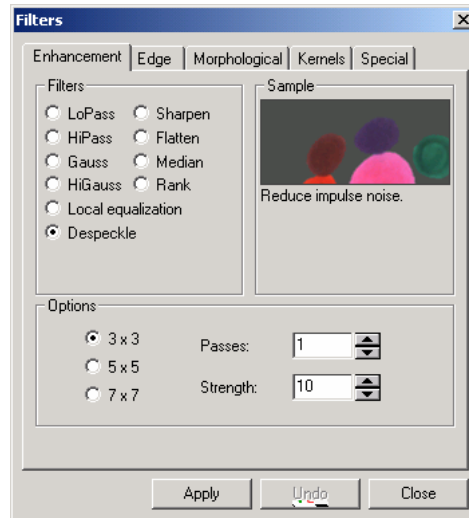
**Apply:** Click this button to apply the specified filter. Before clicking this button, be sure you have selected the filter you want to use (e.g., **LoPass, Median, HiPass**), and set the **Options** that you need. Be sure to select the filter type first, because your choices are determined by the type of filter selected.

**Close:** Click this button when you have finished working with the filters.

**Undo:** Click this button to reverse the effects of the filter you just applied.

## Enhancement Filters

Select enhancement (also known as convolution) filters from this tab.



**LoPass:** Select this filter if you want to soften an image by eliminating high-frequency information (this has the effect of blurring sharp edges). The **LoPass** filter replaces the center pixel with the mean value in its neighborhood. The **LoPass** filter can also be used to remove noise.

**HiPass:** Select this filter if you want to enhance high-frequency information. The **HiPass** filter replaces the center pixel with a convolved value that significantly increases its contrast from its neighbors. The **HiPass** filter leaves only elements of high contrast.

**Gauss:** Use this filter to soften an image by eliminating high-frequency information using a Gauss function. This has the effect of blurring sharp edges. The operation of the Gauss filter is similar to the LoPass filter, but it degrades the image less than the LoPass filter.

**Higauss:** Use this filter when you want to enhance fine details. Its operation is similar to the unsharp masking technique (see the **Sharpen** filter), but it introduces less noise in the process. It uses a Gaussian curve type of kernel. Available in 7x7 and 9x9 kernel sizes.

**Local Equalization:** This filter is used to enhance pixel contrast based on the histogram of the local neighborhood.

**Despeckle :**This filter reduces impulse noise.

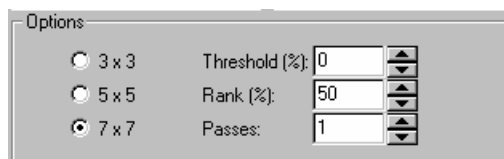
**Sharpen:** Select this filter if you want to enhance fine detail, or refocus an image that is blurred. The **Sharpen** filter sharpens the image using the “unsharp masking technique.”

**Flatten:** Use the **Flatten** filter to even out background variations. This is often done to prepare an image for a count/size operation if its objects are difficult to isolate because the background contains pixels of the same intensity as the objects of interest. **Flatten** reduces the intensity variations in the background pixels.

**Median:** Select this filter if you want to remove impulse noise from an image. The **Median** filter replaces the center pixel with the median value in its neighborhood. It will also blur the image.

**Rank:** Select this filter to remove impulse noise from an image. The pixels in the kernel are ranked by order of intensity, and the pixel in that range at the **Rank** percentage is chosen for comparison. For example, in a 5x5 kernel, there are 25 pixels. A rank percentage of 95% would choose the second-brightest pixel for comparison. If the differences between the selected pixel and the center pixel is greater than the threshold value, the Rank filter replaces the center pixel with the selected pixel.

**Options:** If you have selected one of the enhancement filters, the options will relate to kernel size and filtering strength. The following options will be presented:



**3 x 3:** Select this button to use the 3 x 3 kernel size. A small kernel produces a more subtle filtering effect.

**5 x 5:** Select this button to use the 5 x 5 kernel size.

**7 x 7:** Select this button to use the 7 x 7 kernel size. A large kernel produces a more extreme filtering effect.

**Passes:** Enter the number of times you want the filter applied to your image. When a filter is applied multiple times, its effect is amplified by each pass. An image that has been softened by one pass of the LoPass filter, will be softened further by a second pass.

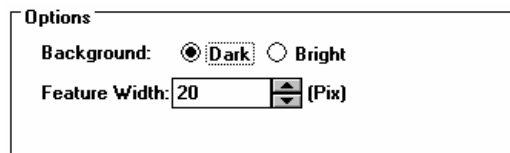
**Strength:** Enter a value from 1 - 10 that reflects how much of the filtering effect you want applied to the image. A value of 10

specifies that you want the full strength (100%) of the filtered result applied to each pixel. Values less than 10 cut the full weight of the filter - a value of 1 indicates that only 10% of the *difference* between the filtered pixel value and the original pixel value should be applied, a value of 2 indicates that 20% of the difference should be applied, and so forth.

**Threshold:** Enter a percent value to specify the amount of absolute difference between the value of the pixel at the center and the value of the replacement pixel. A threshold value of 0 means the Median Filter will always replace the center pixel. Since the dynamic range of image classes are different, the threshold value will be specified in terms of a percentage of the dynamic range. A 10 % threshold for IMC\_GRAY16 image class translates to  $10 / 100 * 65535 = 6554$ .

**Rank:** This value specifies which pixel in the sorted array will be used to replace the center pixel. Pixels in the array will be sorted in ascending order. The pixels are indexed from 0 to  $KernelSize \times KernelSize - 1$ . Pixel index 0 corresponds to the lowest pixel value. The Rank will be specified in terms of a percentage of the indexes ( $KernelSize \times KernelSize - 1$ ). A 50% Rank means the middle of the array. 0% rank means the lowest index (lowest gray value), and 100% rank means the highest index (highest gray value).

**If you have selected Flatten**, the options will relate to background intensity and object size:



The image shows a dialog box titled "Options" with two rows of controls. The first row is labeled "Background:" and contains two radio buttons: "Dark" (which is selected) and "Bright". The second row is labeled "Feature Width:" and contains a numeric spinner box with the value "20" and a label "(Pix)".

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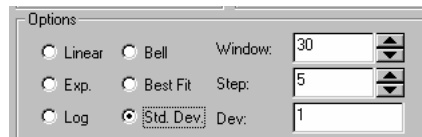
**Note:** The **Flatten** filter will not work with images smaller than 19 x 19 pixels.

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**Background:** Click the option button that describes the intensity of your background relative to the foreground objects. If your image contains dark objects on a bright field, select **Bright**; if your image contains light objects on a dark field, select **Dark**.

**Feature Width:** Enter a value that defines the diameter (in pixels) of the largest object in your image.

If you have selected **Local Equalization**, the options will relate to the histogram equalization. Local Histogram Equalization modifies the contrast of an image based on the pixel values in a small window surrounding each pixel.



**Linear:** This option distributes the histogram equally across the intensity scale. This function produces a high contrast image with the highest possible dynamic range.

**Bell:** This option distributes the histogram evenly around the center of the intensity scale. This function produces an image with somewhat less contrast than the **Linear** distribution.

**Logarithmic:** This option concentrates the histogram at the low end of the scale. This function produces a high contrast image with little dynamic image. It will tend to darken the image overall. Useful for increasing the contrast in a very light image.

**Exponential:** This option concentrates the histogram at the high end of the scale. This function produces a high contrast image with little dynamic image. It will tend to lighten the image overall. Useful for increasing the contrast in a very dark image.

**Best Fit:** Use the *Best Fit* command to instruct *Image-Pro* to optimize the values for your particular image. The results are achieved by stretching the local histogram to maximize contrast between the brightest and darkest pixels in the local window region.

**Standard Deviation:** This method will find the statistical mean or average, and standard deviation of the window used for local histogram equalization. It stretches the contrast so that black is set to the mean *minus* the desired number of standard deviations; and that full intensity is set to the mean *plus* that number of standard deviations. Using the statistical behavior of the image in this fashion prevents *outlier* pixels (extremely bright or extremely dark pixels) from unduly influencing contrast modifications.

By comparison, the **Best Fit** method stretches local contrast to the extrema of all the pixels in the window, and may produce areas where a single bright or dark pixel throws off the contrast. Use the **Standard Deviation** method in cases where the **Best Fit** method produces overly contrasted or jumpy results.



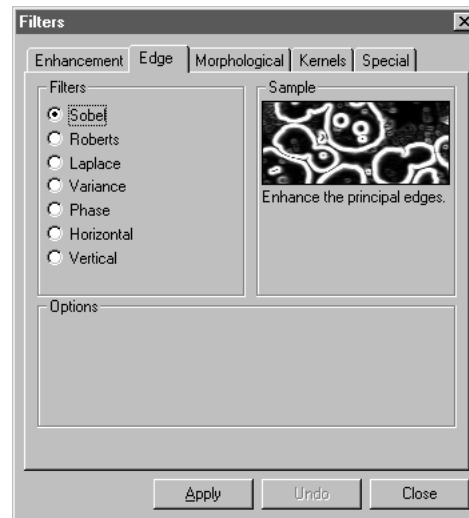
**Window:** Image pixel statistics (min, max, histogram, mean, standard deviation, etc.) will be calculated on a small window of the image. These measurements are then used to derive the local contrast for that area of the image. In short, an area of *Window x Window* around each pixel is all that is considered when modifying the intensities in the image. Larger windows produce smoother results, while small windows more closely track small details.

**Step:** Calculation of the local image statistics is time consuming, so the statistics are calculated for an area, and then applied over a space of *Step x Step*. Decreasing this number causes the algorithm to track the image more closely, at a cost in computation time. **Step** is always less than **Window**.

**+/- Dev:** This is the range of standard deviations plus and minus that will be scaled into the image contrast range. As an example, if the pixel values were distributed in a Gaussian fashion, their mean, +/- (plus or minus) one standard deviation, would include roughly 65% of the pixel values. Two standard deviations would include 95% of the pixel values, and three standard deviations would include 99% of the pixel values. This parameter is used only by the **Standard Deviation** method.

## Edge Filters

Select edge filters from this tab dialog.



**Sobel:** Select this filter if you want to enhance just the principal edges in an image. The **Sobel** applies a mathematical formula to a 3 x 3 neighborhood to locate and highlight its edges.

**Roberts:** Select this filter if you want to enhance fine edges in an image. The **Roberts** filter is not a convolution filter. It applies a mathematical formula upon a 4 x 4 neighborhood to produce its effect. The upper left pixel in the neighborhood is the one replaced.

**Laplace:** Select this filter if you want to enhance all the edges in an image.

**Variance:** Select this filter if you want to detect and emphasize edges and textures. The **Variance** filter substitutes a pixel with the standard deviation for its neighborhood.

**Phase:** Select this filter if you want to enhance edges in a manner that also indicates the direction of the intensity change. The **Phase** filter is the complement of the **Sobel** filter.

**Horizontal:** User this filter to detect and emphasize horizontal edges.

**Vertical:** Use this filter to detect and emphasize vertical edges.

**Options:** If you have selected the edge filters, the options will relate to kernel size and filtering strength. The following options will be presented:

The image shows a dialog box titled "Options" with a close button in the top-left corner. On the left side, there are three radio buttons labeled "3 x 3", "5 x 5", and "7 x 7". The "3 x 3" radio button is selected. To the right of these buttons are two spinners. The first spinner is labeled "Passes:" and has the value "1". The second spinner is labeled "Strength:" and has the value "1".

**3 x 3:** Select this button to use the 3 x 3 kernel size. A small kernel produces a more subtle filtering effect.

**5 x 5:** Select this button to use the 5 x 5 kernel size.

**7 x 7:** Select this button to use the 7 x 7 kernel size. A large kernel produces a more extreme filtering effect.

**Passes:** Enter the number of times you want the filter applied to your image. When a filter is applied multiple times, its effect is amplified by each pass. An image that has been softened by one pass of the LoPass filter, will be softened further by a second pass.

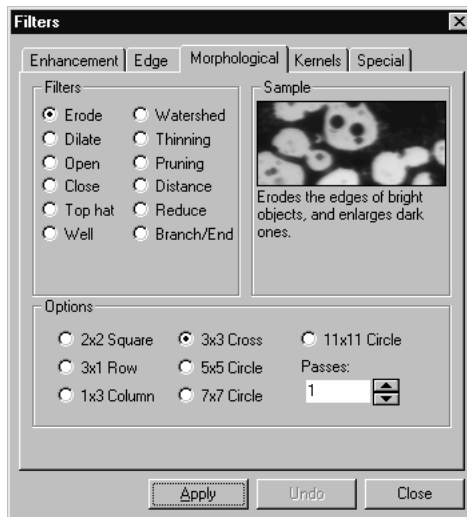
**Strength:** Enter a value from 1 - 10 that reflects how much of the filtering effect you want applied to the image. A value of 10 specifies that you want the full strength (100%) of the filtered result applied to each pixel. Values less than 10 cut the full weight of the filter - a value of 1 indicates that only 10% of the *difference* between the filtered pixel value and the original pixel value should be applied, a value of 2 indicates that 20% of the difference should be applied, and so forth.

**If you have selected Sobel, Phase or Roberts,** no options are available.

**If you have selected Variance,** the **Passes** and **Strength** options are not available

## Morphological Filters

Select morphological filters from this page.



**Erode:** Select this morphological filter if you want to modify the size of objects in your image. The **Erode** filter erodes the edges of bright objects, and enlarges dark ones.

**Dilate:** Select this morphological filter if you want to modify the size of objects in your image. The **Dilation** filter dilates bright objects, and erodes dark ones.

**Open:** Select this morphological filter if you want to modify the shape of objects in your image. Assuming your image contains bright objects on a dark field, the **Open** filter will smooth object contours, separate narrowly connected objects, and remove small dark holes.

**Close:** Select this morphological filter if you want to modify the shape of objects in your image. Assuming your image contains bright objects on a dark field, the **Close** filter will fill gaps and enlarge protrusions to connect objects that are close together.

**Tophat:** Use this filter to detect and emphasize points, or grains, that are brighter than the background. Available in 3 kernel sizes; click the radio button indicating the kernel size that most closely matches the size of the grains you want to detect.

**Well:** Use this filter to detect and emphasize points, or grains, that are darker than the background. Available in 3 kernel sizes; click the radio button indicating the kernel size that most closely matches the size of the grains you want to detect.

**Watershed:** Select this filter to separate objects that are touching. The **Watershed** filter erodes objects until they disappear, then dilates them again, but will not allow them to touch. The **Watershed** filter will not operate upon *True Color* images. If you want to separate objects in a *True Color* image, you must first convert it to *Gray Scale*.

**Thinning:** Select this filter to reduce an image to its skeleton. When you choose this filter, you must set the threshold that determines whether a pixel is part of the subject, or part of the background (see **Options** below). The **Thinning** filter will not operate upon *True Color* images. If you want to thin a *True Color* image, you must first convert it to *Gray Scale*. The following images show the source image and the image resulting from two passes of the **Thinning** filter.



**Pruning:** Select this filter to eliminate projecting arms from an object. The following images show the source image and the image resulting from three passes of the **Pruning** filter.



**Distance:** The distance filter is used for showing the distances of pixels within blobs to the outer boundaries of those blobs. After applying the distance filter, the background will be black (i.e., pixels with value 0). Only the area within the blobs will have non-zero values (will be white). The values of each pixel within the blob will be a count of the shortest distance from that pixel to the edge of the blob. Thus, all pixels along the blob's border will have a value of 1 (since they are one pixel away from the edge of the blob), pixels that are a distance of 2 from the border will have the value 2, and so on. This creates a distance map of the image.

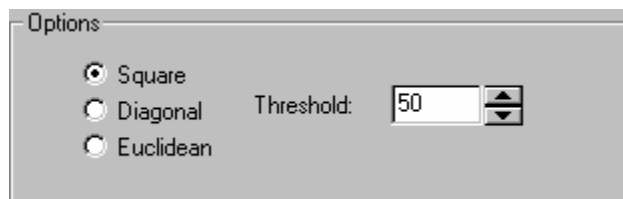
The **Distance** filter may not operate accurately upon *True Color* images. If you want to use the **Distance** filter with a *True Color* image, you must first convert it to *Gray Scale*.

**Reduce:** Select this filter to reduce the objects in an image to a single point or group of points. The **Reduce** filter will not operate upon

*True Color* images. If you want to use the **Reduce** filter with a *True Color* image, you must first convert it to *Gray Scale*.

**Branch/Endpoints:** Use this filter to identify morphological branch and endpoints in an image.

**If you have selected the Distance or Reduce filter** the options will relate to filter direction and thresholding. The following options will be presented:



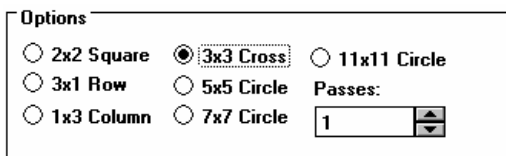
**Square:** Click this radio button if you want the **Reduce** or **Distance** filters applied to the image in a square pattern. Only pixels adjacent horizontally or vertically are included.

**Diagonal:** Click this radio button if you want the **Reduce** or **Distance** filters slanted across the image. All neighboring pixels, including ones diagonally adjacent, are included.

**Euclidian:** Click this radio button if you want the **Reduce** or **Distance** filters applied to the image in a larger pattern than either square or diagonal. Filters the image using Euclidian distances and a larger neighborhood that includes the “knight’s move” pixels.

**Threshold:** Enter a percentage value from 1 - 100 that specifies the intensity value you want *Image-Pro* to use for binarizing the image. For example, a Threshold of 50% on a *Gray Scale* image would set all values  $\leq 127$  to 0 (black) and all values  $\geq 128$  to the maximum value for that image class (white).

**If you have selected Erode, Dilate, Open, or Close filters,** the options will relate to kernel size and shape. The following options will be presented:



**2 x 2 Square:** Select this button to use the 2 x 2 square kernel configuration.



**3 x 1 Row:** Select this button to use the 3 x 1 row kernel configuration.



**1 x 3 Column:** Select this button to use the 1 x 3 column kernel configuration.



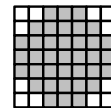
**3 x 3 Cross:** Select this button to use the 3 x 3 cross kernel configuration.



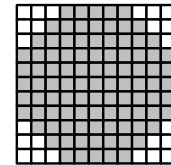
**5 x 5 Circle:** Select this button to use the 5 x 5 circular kernel configuration.



**7 x 7 Circle:** Select this button to use the 7 x 7 circular kernel configuration. This is a two-pass filter, accomplished using a 5 x 5 circle followed by a 3 x 3 cross.



**11 x 11 Circle:** Select this button to use the 11 x 11 circular kernel configuration. This is a three-pass filter, accomplished using a 5 x 5 circle followed by another 5 x 5 circle, followed by a 3 x 3 cross.



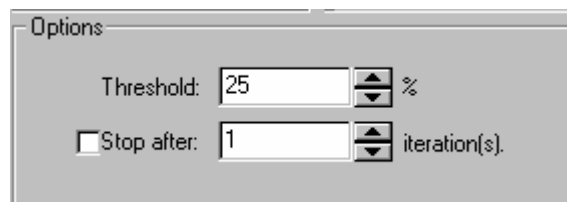
**Passes:** Select the number of times you want *Image-Pro* to iterate the filter.

---

**Note:** The circular kernels are especially effective on round objects (cells, grains and so on) because their circular configuration preserves the circular shape of your objects better than square configurations.

---

**If you have selected the Watershed, Thinning, or Pruning filter** the options will relate to thresholding. The following options will be presented:

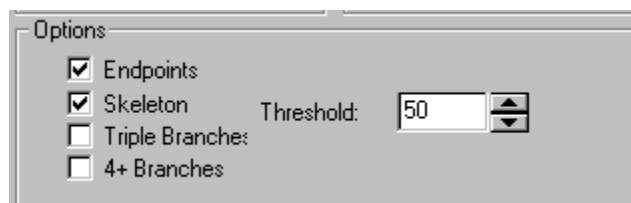


**Threshold:** Enter a percentage value from 1 - 100 that specifies the intensity value you want *Image-Pro* to use for binarizing the image. For example, a Threshold of 50% on a *Gray Scale* image would set

all values  $\leq 127$  to 0 (black) and all values  $\geq 128$  to the maximum value for that image class (white).

**Stop After:** Enter the number of times you want *Image-Pro* to iterate the filter. Depending on the filter, successive iterations will strengthen the effect of the filter. Bear in mind that successive passes also require additional time for execution. If this box is not checked, *Image-Pro* will continue to iterate until the image has been completely “thinned” or “pruned.”

**If you have selected the Branch/Endpoints filter**, the options will relate to the number of branches and endpoint that you want the filter to reveal:




Check the box next to the desired selection:

**Threshold:** Enter a percentage value from 1 - 100 that specifies the intensity value you want *Image-Pro* to use for binarizing the image. For example, a Threshold of 50% on a *Gray Scale* image would set all values  $\leq 127$  to 0 (black) and all values  $\geq 128$  to the maximum value for that image class (white). This option allows you to apply this function to images that have not yet been skeletonized.

**End Points** will mark one and zero connectivity pixels with a value of 32.

**Skeleton** will mark all two connectivity pixels with a value of 16. These values allow in-place filtering for all image classes.

**Triple Branches** will mark all three-connectivity pixels with a value of 64.

**4+ Branches** will mark all pixels with four or more connections with a value of 128. An example of a 4-way branch might be a + or an  in a skeleton.

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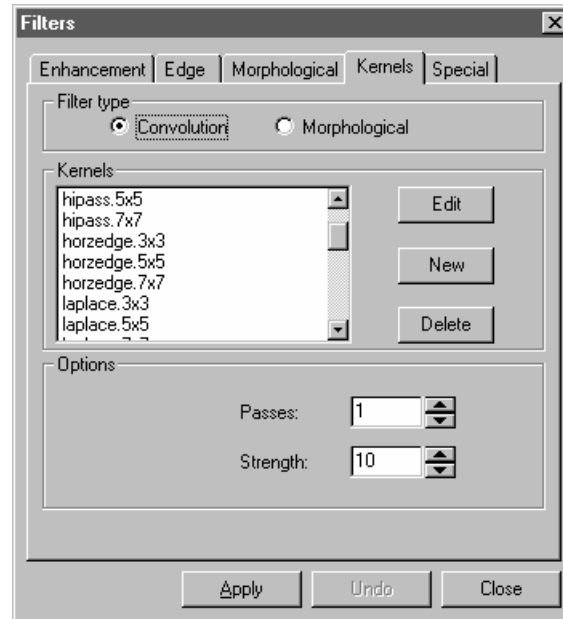
**Note:** The results of a branch/endpoint filter will probably be too dark to view. To increase visibility, try increasing the contrast using the **Brightness/Contrast** tool or applying a pseudocolor map.

---



## Kernels

The **Kernel** page allows you to edit the kernel files for the morphological and convolution filters. Clicking this tab displays the following dialog:



See *Spatial Filtering* discussion in *Section 1* for more information about filters of this kind.

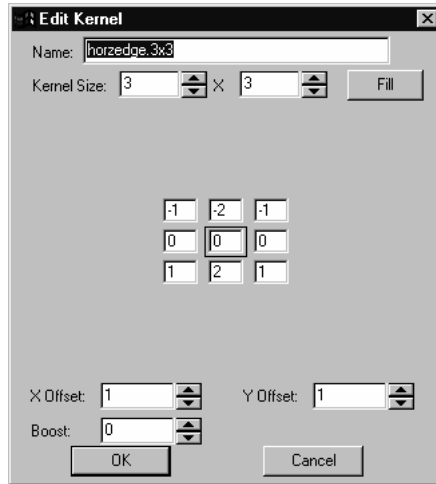
---

**Note:** The HiPass, LoPass, Laplace and Unsharp kernel files are used by the HiPass, LoPass, Laplacian and Sharpen options listed in the Filter window (i.e., there is no difference between selecting one of these kernel files and selecting its option button in the **Filter** window -- the two methods ultimately do the same thing). Because these kernel files are essential to the operation of these filtering options, they must not be deleted or renamed.

---

**Filter Type:** Click this button to modify the kernel for a selected filter type, either **convolution** or **morphological** filters.

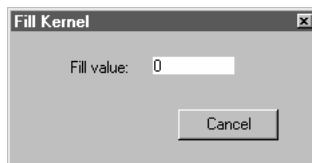
**Edit:** Click this button to modify the selected filter kernel. The Edit Kernel dialog box appears.



**Name:** This list box contains the name of the selected kernel file. If you want to save the modified kernel file to the same file, leave it as is. If you want to save the file to a new location, enter the new filename here.

**Kernel Size:** Click the spin buttons or enter the number to change the size of the kernel. Either direction may take into account from one to nine pixels. As you modify the **Kernel Size**, the shape of the kernel representation changes accordingly. In the center of the dialog box, there are white boxes containing coefficients that will be multiplied with each pixel that will be taken into account by the filter kernel. You can change any coefficient by clicking on it to select it, then changing it as you will. In addition, it is possible to create a custom filter (up to 21 x 21 pixels) by editing a text file, but we do not recommend this as performance deteriorates as the filter size increases.

**Fill:** Click this button to fill every element of the kernel with a particular value. The **Fill Kernel** dialog box appears. You may enter a value between -128 and +128. Using the **Fill** button is useful for setting all coefficients to the same value. You may then change the coefficients that require a different value.

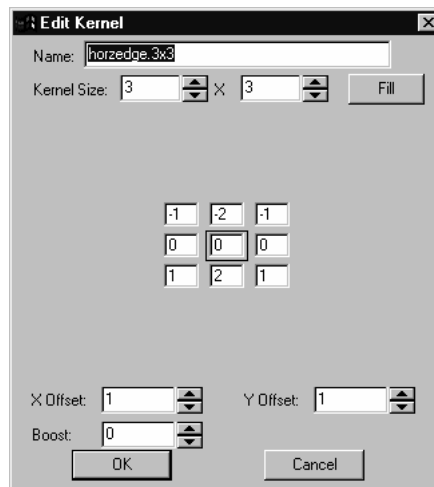


**Offset :** The pixel whose value is being changed is usually the center-most pixel. You may, however, designate any pixel you want. *Image-Pro* signals the pixel to be changed by putting a box around it. Use the **X** and **Y Offset** spin buttons to do so.

**Boost :** Enter here the constant value to be added to the result after the value of the modification has been calculated.

*Note - If you are editing or creating a morphological filter kernel, the **Boost** option is not available.*

**New:** Click this button to create a new filter kernel. The **Edit Kernel** dialog box appears.



This dialog box functions in the same way as the dialog box for editing, with the exception that you must provide the file name for the new kernel file.

---

**Note:** You may choose a different path for your kernel file, but *Image-Pro* will not list it when you invoke the **Filter** dialog box.

---

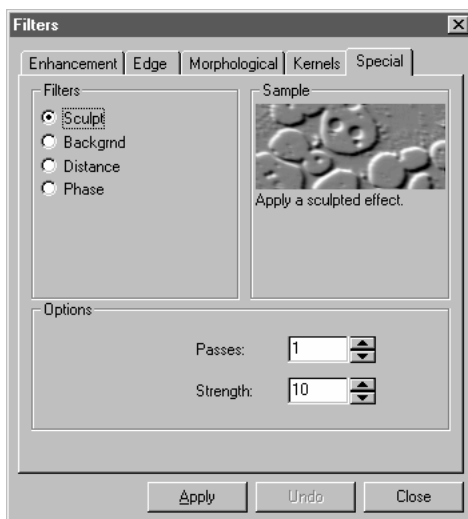
Observe that the above dialog box represents a configuration for a new morphological filter kernel (**Boost** and **Kernel Sum** are not represented).

**Delete:** Click this button to delete the selected filter kernel file.

**Options:** The choices in this group box will vary depending upon the kind of filter you have selected.

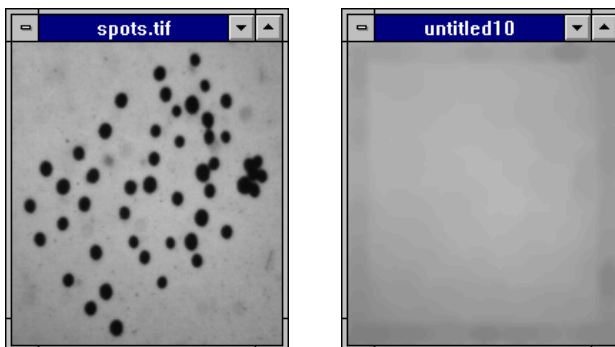
## Special Filters

You can select the following **Special** filters from this page:



**Sculpt:** Use this filter to apply a sculpted effect to the image

**Background:** Select this filter to extract the background from an image. The **Background** filter works by filtering out objects using a very large filtering kernel. The background image, with objects removed, is placed into a new, untitled image window, as shown in the example below.



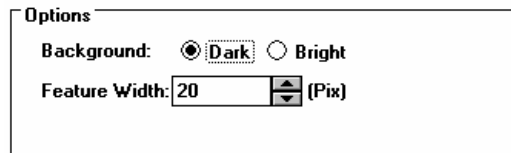
When performing a background extraction, it is very important to specify your background intensity and object size in the **Options** group box. This tells *Image-Pro* how to distinguish objects from background, and what kernel size to use during the extraction

process. To achieve the best results, specify a **Feature** size that describes the largest object in your image. The minimum size is 19 x 19 pixels.

**Distance:** Use this filter to create a distance map of the image. The **Distance** filter may not operate accurately upon *True Color* images. If you want to use the **Distance** filter with a *True Color* image, you must first convert it to *Gray Scale*.

**Phase:** Select this filter if you want to enhance edges in a manner that also indicates the direction of the intensity change.

**Options:** If you have selected **Background**, the options will relate to background intensity and object size:



Options

Background:  Dark  Bright

Feature Width: 20 [spin button] (Pix)

**Background:** Click the option button that describes the intensity of your background relative to the foreground objects. If your image contains dark objects on a bright field, select **Bright**; if your image contains light objects on a dark field, select **Dark**.

**Bright:** Select this option button if your object is bright and the background dark.

**Dark:** Select this option button if your object is dark and the background bright.

**Feature Width:** Enter a value that defines the diameter (in pixels) of the largest object in your image.

**If you have selected Distance,** the options will relate to the shape and threshold of the distance map:

**Square:** Click this radio button if you want the **Distance** filter applied to the image in a square pattern. Only pixels adjacent horizontally or vertically are included. This is known as a “city-block” distance.

**Diagonal:** Click this radio button if you want the **Distance** filter slanted across the image. All neighboring pixels, including ones diagonally adjacent, are included. This is known as a “chessboard” distance.

*Process:Filters*

**Euclidian:** Click this radio button if you want the **Distance** filter applied to the image in a larger pattern than either square or diagonal. Filters the image using Euclidian distances and a larger neighborhood that includes the “knight’s move” pixels.

**Threshold:** Enter a percentage value from 1 - 100 that specifies the intensity value you want *Image-Pro* to use for binarizing the image. For example, a Threshold of 50% on a *Gray Scale* image would set all values  $\leq 127$  to 0 (black) and all values  $\geq 128$  to the maximum value for that image class (white).

**If you have selected the Sculpt filter,** the options will relate to filtering strength. The following options will be presented:

**Passes:** Enter the number of times you want the filter applied to your image. When a filter is applied multiple times, its effect is amplified by each pass. An image that has been softened by one pass of the LoPass filter, will be softened further by a second pass.

**Strength:** Enter a value from 1 - 10 that reflects how much of the filtering effect you want applied to the image. A value of 10 specifies that you want the full strength (100%) of the filtered result applied to each pixel. Values less than 10 cut the full weight of the filter - a value of 1 indicates that only 10% of the *difference* between the filtered pixel value and the original pixel value should be applied, a value of 2 indicates that 20% of the difference should be applied, and so forth.

**If you have selected Phase,** no options are available

## 3D Filters

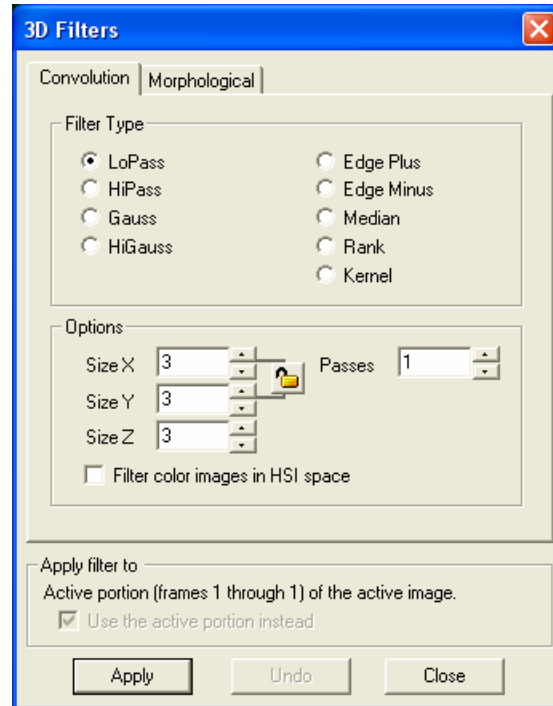
---

*Image-Pro Plus* version 7.0 includes a set of 3-dimensional filters. Use the *3D Filters* command to apply one of the filters to the object in the Image-Pro workspace. Filtering operations reduce or boost the rate of brightness change in an image. If you are not familiar with the process and effects of filtering, you might want to review the *Spatial Filtering* discussion in the *Image-Pro Plus Start-Up Guide* for additional discussion about this command.

This version contains an extensive set of convolution and morphological filters. You can also create your own filtering kernels and apply them with the *3D Filters* command.

When you select the *3D Filters* command, the **Filter** tab dialog is opened. Each group of filters has its own property sheet or tab, where you select the type and size of filter you want to use. Filtered results are almost always written to the active image. You may use the **Undo** command if you want to remove the effects of a filter you have applied.

Clicking on the *3D Filters* command displays the following dialog:

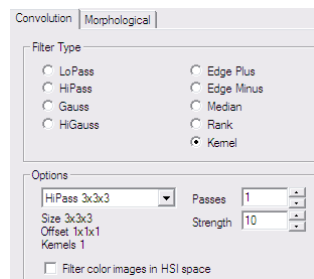



## Convolution Filters

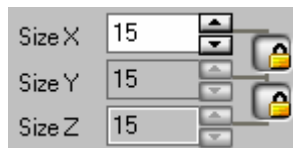
- **LoPass:** Select this filter if you want to soften an image by eliminating high-frequency information (this has the effect of blurring sharp edges). The **LoPass** filter replaces the center pixel with the mean value in its neighborhood. The **LoPass** filter can also be used to remove noise.
- **HiPass:** Select this filter if you want to enhance high-frequency information. The **HiPass** filter replaces the center pixel with a convolved value that significantly increases its contrast from its neighbors. The **HiPass** filter leaves only elements of high contrast.
- **Gauss:** Use this filter to soften an image by eliminating high-frequency information using a *Gauss* function. This has the effect of blurring sharp edges. The operation of the Gauss filter is similar to the LoPass filter, but it degrades the image less than the LoPass filter.



- **Higauss:** Use this filter when you want to enhance fine details. Its operation is similar to the unsharp masking technique but it introduces less noise in the process. It uses a Gaussian curve type of kernel. Available in 7x7 and 9x9 kernel sizes.
- **Edge Plus:** Use this filter to extract and enhance positive edges, with bright features on a dark background.
- **Edge Minus:** Use this filter to extract and enhance negative edges, with dark features on a bright background.
- **Median:** Select this filter if you want to remove impulse noise from an image. The **Median** filter replaces the center pixel with the median value in its neighborhood. It will also blur the image.
- **Rank:** Select this filter to remove impulse noise from an image. The pixels in the kernel are ranked by order of intensity, and the pixel in that range at the **Rank** percentage is chosen for comparison. For example, in a 5x5 kernel, there are 25 pixels. A rank percentage of 95% would choose the second-brightest pixel for comparison. If the differences between the selected pixel and the center pixel is greater than the threshold value, the **Rank** filter replaces the center pixel with the selected pixel.
- **Kernel:** This is a convolution filter where you can control the size, strength, and passes. You will see the following dialog:



- **SizeX**, **SizeY**, and **SizeZ** define filter size along X, Y and Z axes correspondingly. The sizes can only be odd numbers from 1 to 999. **SizeX**, **SizeY**, and also **SizeZ** can be linked checking the  button, so adjusting **SizeX** will also change values of **SizeY** and **SizeZ**:

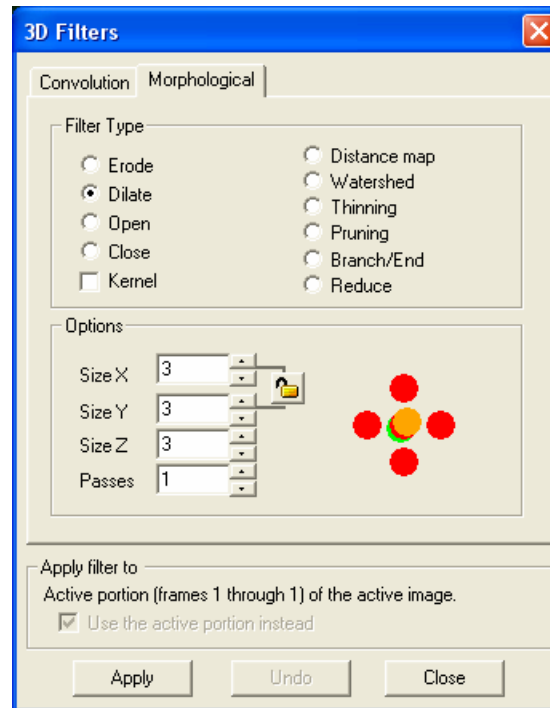


- **Passes:** Enter the number of times you want the filter applied to your image. When a filter is applied multiple times, its effect is amplified by each pass. An image that has been softened by one pass of the LoPass filter, will be softened further by a second pass.
- **Strength:** Defines the strength of the filter.
- **Rank (%):** This value specifies which pixel in the sorted array will be used to replace the center pixel. Pixels in the array will be sorted in ascending order. Pixel index 0 corresponds to the lowest pixel value. A 50% Rank means the middle of the array. 0% rank means the lowest index (lowest gray value), and 100% rank means the highest index (highest gray value).
- **Filter Color Images in HSI Space:** Check this box to control the way color images are filtered. If the box is not checked (*off*) every channel in a color image (RGB) is filtered independently. The computation time in this case will be 3 times higher than for a gray image. When the box is checked (*on*) the RGB color image is converted to HSI color space, and only the Intensity channel is filtered. After filtering, the HSI image is converted back to an RGB image.

This type of filtering can be used with naturally acquired color images. The method does not introduce color artifacts and reduces filtering time. Images where color channels are acquired separately should use RGB 3-channel filtering (default).
- **Use the active portion instead** allows you to apply the filter only to a portion of the active sequence, instead of the current Z stack of set (see the Set Manager chapter for more information) .

## Morphological Filters

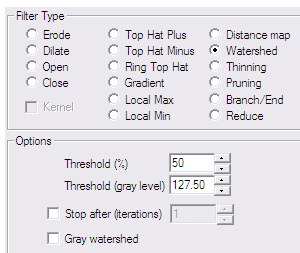
Select morphological filters from this page.



- **Erode:** Select this morphological filter if you want to modify the size of objects in your image. The **Erode** filter erodes the edges of bright objects, and enlarges dark ones.
- **Dilate:** Select this morphological filter if you want to modify the size of objects in your image. The **Dilation** filter dilates bright objects, and erodes dark ones.
- **Open:** Select this morphological filter if you want to modify the shape of objects in your image. Assuming your image contains bright objects on a dark field, the **Open** filter will smooth object contours, separate narrowly connected objects, and remove small dark holes.
- **Close:** Select this morphological filter if you want to modify the shape of objects in your image. Assuming your image contains bright objects on a dark field, the **Close** filter will fill gaps and enlarge protrusions to connect objects that are close together.

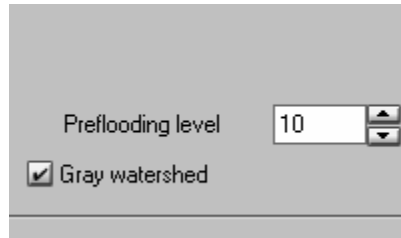
Process:3D Filters

- **Top Hat Plus:** Extracts bright objects on a dark background. It is equal to the difference of the input and a grey-scale opening.
- **Top Hat Minus:** Extracts dark objects on a bright background. It is equal to the difference of a grey-scale closing and the input.
- **Ring Top Hat:-** Extracts pixels that are brighter than the pixels in the ring neighborhood. It is equal to the difference of the input and a grey-scale dilation with a ring kernel.
- **Gradient:** Morphological gradient. It is equal to the difference of a grey-scale dilation and a grey-scale erosion.
- **Local Max:** Locates pixels with maximum intensity in the filter neighborhood. Extreme pixels are preserved, other pixels are replaced with minimum image value.
- **Local Min:** Locates pixels with minimum intensity in the filter neighborhood. Extreme pixels are preserved, other pixels are replaced with maximum image value.
- **Distance Map:** This filter creates a distance map of the current sequence. The distance map is created as a new floating point image, where the amplitude of every pixel corresponds to the shortest Euclidean distance to the edge of binary object. The binarization is based on the **Threshold** value, which is defined in percentage of the maximum pixel value of the image class. For floating point images the minimum and maximum are defined by the dynamic range.
- **Watershed:** Select this filter to separate objects that are touching. The Watershed filter erodes objects until they disappear, then dilates them again, but will not allow them to touch. The **Gray Watershed** option defines whether to separate objects using the gray levels of the objects on the original image (*on*) or using the distance map of the binarized image (*off*).



The **preflooding level** is in gray levels. The points of local maxima are filled until their basins meet, creating a watershed separating line. If the **Stop after** option is on the distance map is limited to the **Iteration**

value. This option can be used to separate only slightly touching objects, avoiding separation of large objects.



- **Thinning:** Select this filter to reduce an image to its skeleton. The thinning is based on the distance map of the volume and produces a 26-connected medial axis of the objects above the **threshold**. If the **Stop after** option is on the pixels farther than the **Iterations** distance from the object's edge are not skeletonized

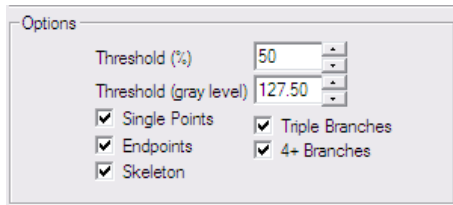


- **Pruning:** Select this filter to eliminate projecting arms from a skeleton input image. The number of iterations defines the branch length to be deleted. If the **Preserve Long Branches** option is checked, branches longer than the number of iterations will not be pruned. The following images show the source image and the image resulting from three passes of the pruning filter.



- **Reduce:** Select this filter to reduce the objects in an image to a single point or group of points. The filter is based on the thinning filter and if the object has holes the output will be a ring.
- **Branch/Endpoints:** Use this filter to identify morphological branches and endpoints in a skeleton image.

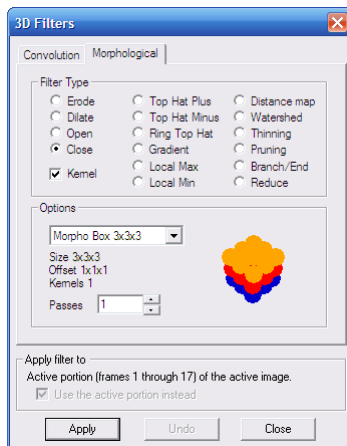
Process:3D Filters



The check-boxes: **Single point, Endpoints, Skeleton, Triple Branches** and **4+ branches** define the morphological elements to be included into the output image. The gray level of the output points is defined by the type of element according to the following table:

Single point	10
Endpoints	20
Skeleton	30
Triple Branches	40
4+ branches	50 and above

- Kernel:** The option defines whether to use free size or file kernel. When the box is not checked (option is *off*) the kernel is defined by setting the **SizeX**, **SizeY** and **SizeZ** dimensions of the kernel. When the box is checked (option is *on*) the kernel is defined by the kernel file name.



The combo-box contains a list of 3D morphological kernels from the *Image-Pro Plus Documents and Settings* folder. Morphological filter files have the **FilterType** field set to 1 (0 - for convolution filters).

Non-zero kernel values define the shape of the filter.

The **kernel preview image** can be rotated by clicking and dragging.

The following morphological kernels are installed:

Morpho Box 3x3x3.k3d

Morpho Cross 3x3x3.k3d

Morpho Line 1x1x3.k3d

Morpho Line 1x3x1.k3d

Morpho Line 3x1x1.k3d

Morpho Sphere 5x5x5.k3d

Morpho Sphere 7x7x7.k3d

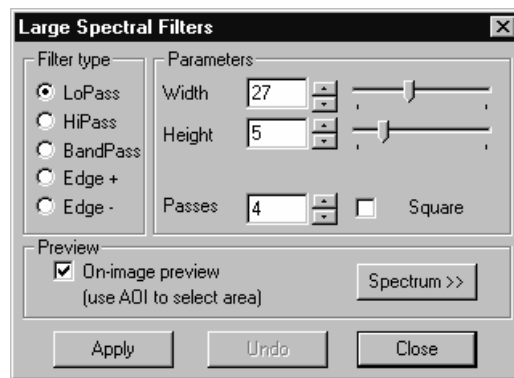
- **Passes:** Enter the number of times you want the filter applied to your image.

## Large Spectral Filters

---

The *large spectral filters* are filters of large aperture that can be used for various purposes in image processing. These filters can be applied to an AOI, active image, or active sequence. The size of the large spectral filters is not limited. All the large spectral filters are very fast regardless of the size.

When you select the **Large Spectral Filters** command, you will see the following dialog:



The main problem with large kernel filters is the slower filtering speed. Usually, the filtering process is based on the convolution of a kernel and an image. Convolution filters process image neighborhoods by multiplying the values within a neighborhood by a matrix of filtering coefficients called a kernel. The result replaces the center pixel in the image neighborhood (see *Convolution filters* in the previous section). The time for this filtering method increases proportionally to the size of the kernel. For kernels with sizes larger than 20-30, the process is unacceptably slow.

All filters in the **Large Spectral Filters** group use an algorithm that eliminates most of the multiplication and sum operations, which increases the filtering speed significantly. In theory, the size of the filters is not limited. However, *Image-Pro Plus* limits the size of the **Large Spectral Filters** to 4Kx 4K.



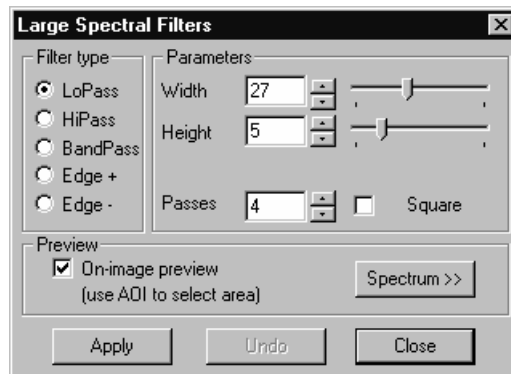
## Filter Types and Parameters

There are 5 types of large spectral filters: **LoPass**, **HiPass**, **BandPass** and two **Edge** filters. Each filter type has its own set of related parameters.

The main problem with large kernel filters is the speed of filtration. The traditional way of filtration is based on convolution of a kernel and an image. Convolution filters process image neighborhoods by multiplying the values within a neighborhood by a matrix of filtering coefficients called kernel. The result replaces the center pixel in the image neighborhood (see Convolution filters). The time of this filtration increases proportionally to the size of a kernel and for kernels with size large than 20-30 it becomes unacceptable.

All filters in the large filters group use an algorithm that eliminates most of multiplication and sum operations increasing the speed of filtration significantly.

**LoPass:** A **LoPass** filter can be used with both small and large apertures. With a small aperture, it removes image noise and smoothes an image. With a large aperture, it extracts the background .



**Width:** Indicates the width of the filter being applied.

**Height:** Indicates the height of the filter being applied.

**Passes:** Indicates the number of times the filter should be applied to the image. For example, an image that has been softened by one pass of the **LoPass** filter, will be softened further by a second pass.

**Square:** When this box is checked, the filter uses a square matrix.

*Process:Large Spectral Filters*

From mathematical point of view the filters can be presented as convolution filters. The following matrix shows LoPass filter kernel for a sample 3x5 filter.

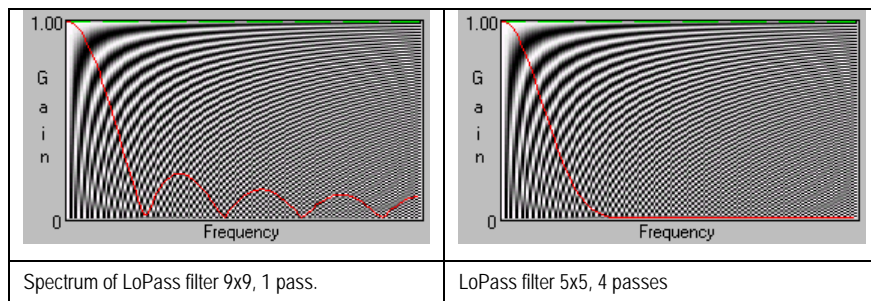
where N – is the total number of elements in the filter. In this example  $N=3*5=15$

<b>1/N</b>	1	1 1 1			1
	1 1		1	1 1	
	1	1 1 1			1

**Passes** – shows the number of times the filter is applied to an image.

*Tip – to achieve better image smoothing and clearer spectrum function use smaller filter size and more passes.*

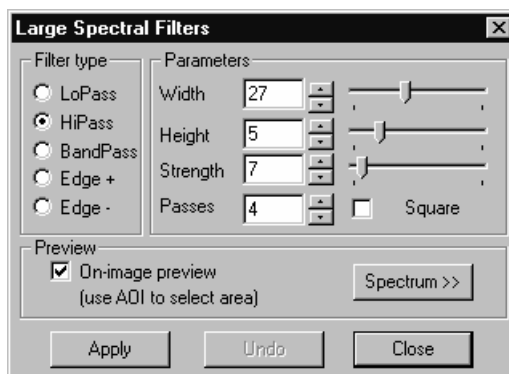
Here is an example of a LoPass filtered image:



Spectrum of LoPass filter 9x9, 1 pass.

LoPass filter 5x5, 4 passes

**HiPass:** The **HiPass** filter increases image sharpness and contrast settings. When used with a large aperture and high strength settings, this filter can perform automatic image binarization.



**Width:** Indicates the width of the filter being applied.

**Height:** Indicates the height of the filter being applied.

**Strength:** Enter a value from 1 - 100 that reflects how much of the filtering effect you want applied to the image. A value of 100 specifies that you want the full strength (100%) of the filtered result applied to each pixel. Values less than 100 cut the full weight of the filter - a value of 10 indicates that only 10% of the *difference* between the filtered pixel value and the original pixel value should be applied, a value of 20 indicates that 20% of the difference should be applied, and so forth.

**Passes:** Enter the number of times you want the filter applied to your image. When a filter is applied multiple times, its effect is amplified by each pass.

**Square:** When this box is checked, the filter has a square matrix.

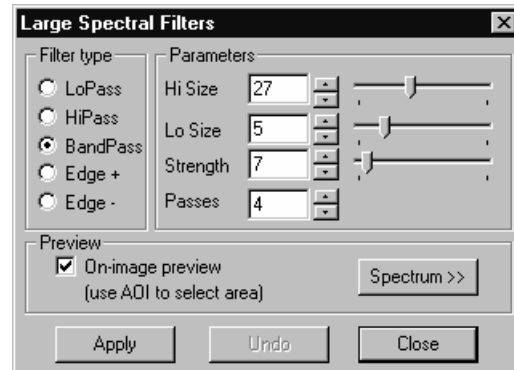
The matrix for a 3x5 HiPass filter is shown here, where St is the strength of the filter. The sum of this filter is always 1 assuming that the nil frequency of the image spectrum is preserved.

St/N

-1 -1		-1	-1	-1
-1 -1		<b>N-1+N/St</b>	-1 -1	
-1 -1		-1	-1	-1

## Process:Large Spectral Filters

**BandPass:** The **BandPass** filter can simultaneously reduce noise and increase image contrast, which can be very useful on low-contrast, noisy images.



The **BandPass** filter is a *combination* of the **LoPass** and **HiPass** filters. First an image is processed by a **LoPass** filter with a given number of passes, and then by one pass of the **HiPass** filter. For example, the dialog shown above defines the **BandPass** filter with 3 passes of a 5x5 **LoPass** filter, and one pass of a 27x27 **HiPass** filter. The shape of a **BandPass** filter is always a square.

Filter combinations such as these allow you to suppress hi-frequency components of the image spectrum, which generally represent image noise. You may also amplify middle components of the spectrum, corresponding to the edges and fine details of various objects, and increase the contrast in these areas.

**Hi Size:** Indicates the size of the **HiPass** filter to be used for a **BandPass** filter.

**Lo Size:** Indicates the size of the **LoPass** filter to be used for a **BandPass** filter.

**Strength:** Enter a value from 1 - 100 that reflects how much of the filtering effect you want applied to the image. A value of 100 specifies that you want the full strength (100%) of the filtered result applied to each pixel. Values less than 100 cut the full weight of the filter - a value of 10 indicates that only 10% of the *difference* between the filtered pixel value and the original pixel value should be applied, a value of 20 indicates that 20% of the difference should be applied, and so forth.

**Passes:** Enter the number of times you want the filter applied to your image. When a filter is applied multiple times, its effect is amplified by each pass. An image that has been softened by one

pass of the **BandPass** filter, will be softened further by a second pass.

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**Note:** **BandPass** filters provide clearer images with smaller **LoSize** and more passes.

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An example of a bandpass filter is shown here:

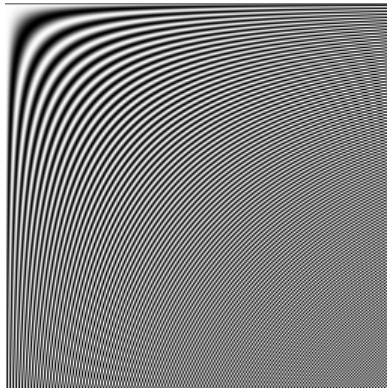


Image before bandpass filter is applied

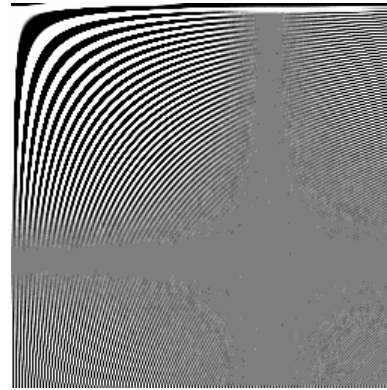
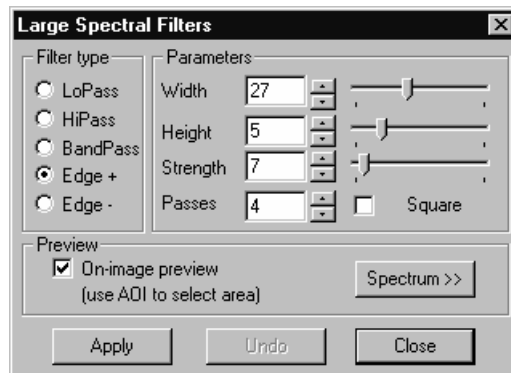


Image after bandpass filtering

**Edge:** The **Edge** filters extract and enhance positive or negative edges. The **Edge +** filter extracts positive edges (bright features on a dark background) from an image; the **Edge -** filter enhances negative edges (dark features on a bright background).



Using non-square **Edge** filters, you can create filters that will enhance either vertical or horizontal edges on the image.

**Width:** Indicates the width of the filter being applied.

**Height:** Indicates the height of the filter being applied.

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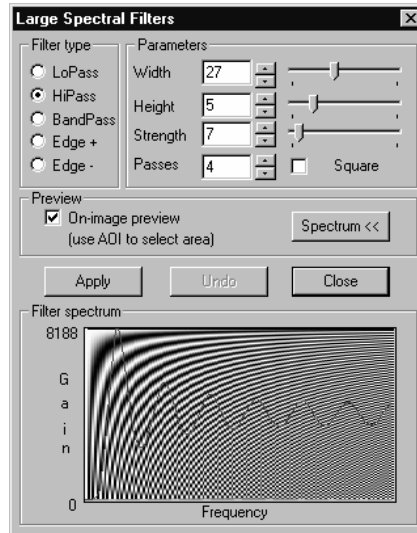
**Strength:** Enter a value from 1 - 100 that reflects how much of the filtering effect you want applied to the image. A value of 100 specifies that you want the full strength (100%) of the filtered result applied to each pixel. Values less than 100 cut the full weight of the filter - a value of 10 indicates that only 10% of the *difference* between the filtered pixel value and the original pixel value should be applied, a value of 20 indicates that 20% of the difference should be applied, and so forth.

**Passes:** Enter the number of times you want the filter applied to your image. When a filter is applied multiple times, its effect is amplified by each pass. An image that has been modified by one pass of the selected filter, will be modified further by a second pass.

### **Preview and Spectrum**

**On-image Preview.** Check this box to see results of the filtering process before the filter is actually applied to the image. The active image or an AOI is used as the preview area.

**Spectrum:** Clicking this button displays a window that contains a spectrum of the current filter. This graph helps to set the correct filter parameters to achieve the desired results. The background of the **Spectrum** window shows a range of spatial harmonics for corresponding frequencies. The spatial harmonics are vertically oriented and their scale is equal to image scale with 100% zoom. The background is used to evaluate the size of objects, which will be enhanced or filtered out.



**Apply:** Click this button to apply the specified filter. Before clicking this button, be sure you have selected the filter you want to use (e.g., **LoPass, Edge**), and set the parameters that you need. Be sure to select the filter type first, because your choices are determined by the type of filter selected.

**Undo:** Click this button to reverse the effects of the filter you just applied.

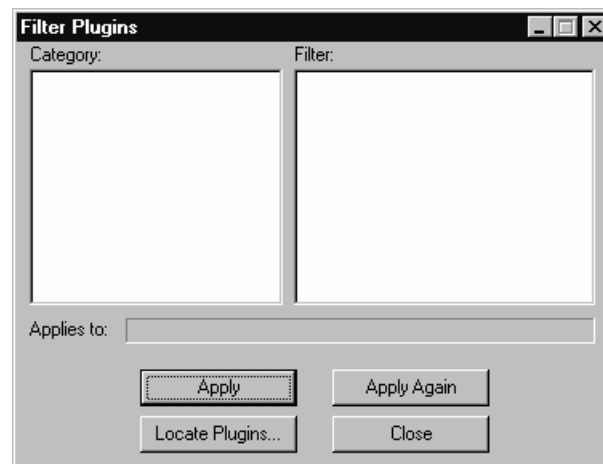
**Close:** Click this button when you have finished working with the filters.

## Filter Plugins

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The **Filter Plugins** command allows you to use existing third-party filters with *Image-Pro Plus v. 7.0*.

When you select this command, you will see the **Filter Plugins** dialog:



**Category:** This list box contains all the categories of third-party plug-in filters. Filters are specified by a category and a filter name.

**Filter:** This list box contains the names of all loaded filters in the selected category. Note that a category may include filters from a number of different plug-ins.

**Applies To:** This static text box displays the formats that the filter supports. This list is limited to only those image formats supported by *Image-Pro Plus*, with “No supported formats” displayed if the filter cannot work with any native formats.

**Apply:** Apply the filter to the active image. This button will be disabled if the filter does not apply to the active image pixel class.

**Apply Again:** Re-applies the selected filter to the active image.

**Locate Plugin:** Use this button to browse your system folders for the plug-in files.

**Close:** Closes the dialog box.



## Threshold/Segmentation

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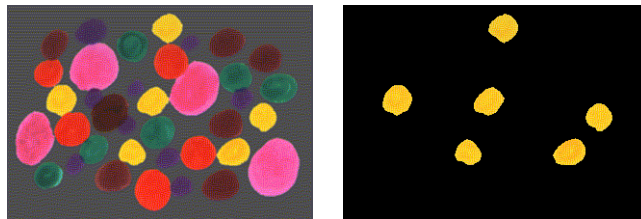
In *Image-Pro Plus 7.0*, the *Threshold* and *Segmentation* tools have been combined. The *Threshold* tool can be thought of as color segmentation applied to a black and white image, which is exactly what *Image-Pro Plus 7.0* does. However, to retain familiarity and backward compatibility, both the *Threshold* and *Segmentation* commands still appear under the *Process* menu.

*Segmentation* is a process by which certain colors (or gray levels) in an image can be visually identified then isolated from the image as a whole. Areas identified by segmentation (classes) can be either removed from the image or kept, while discarding the remainder of the image. Therefore, this process can be used for separating items or objects of interest from the “background noise” that naturally occurs in most acquired images. Further, segmented areas can be either kept in their original color or turned into a single color (masking). Masking an image facilitates or improves the performance of other operations in *Image-Pro Plus 7.0*. In particular, the *Watershed* and *Thinning* filters generally produce better results on masked images.

Use the *Segmentation* command to separate objects or features from the background, based upon their color characteristics. You might do this to extract just the objects of interest from an image, modify them, then return them to the image using the *Operations : Add* command.

The *Segmentation* command extracts objects by locating all objects of the specified color(s) and setting everything else to black. You can also do the reverse; and remove (set to black) objects of the specified colors, and keep everything else. You may write the final segmented image using its remaining original colors, or convert it to black and white.

In the example below, an image containing spots of various colors was segmented so only the yellow spots remained in the image. Notice that the rest of the image is set to black.



The *Segmentation* command works with all image classes except Bilevel (bilevel images are not supported in *Image-Pro Plus v. 7.0*).

### *Process: Threshold/Segmentation*

The *Segmentation* command operates upon the entire active image. It is not constrained by an AOI.

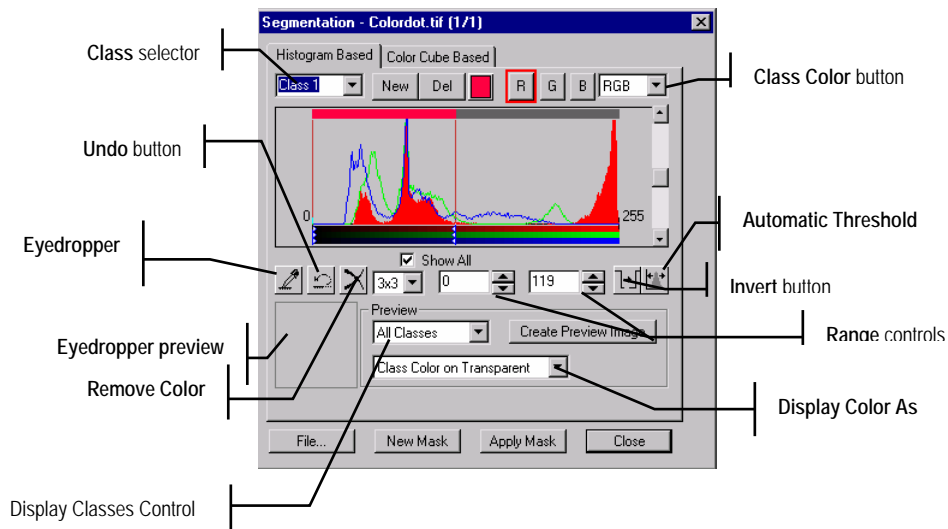
When the *Segmentation* command is selected, the **Segmentation** tab dialogs are presented.

## **Segmentation Models**

As discussed previously, the process of identifying colors is key to the operation of *Segmentation*. Because of the vast differences possible in images, and therefore the color composition of the object(s) to be identified, *Image-Pro Plus 7.0* provides two models for identifying the segmented area: **Histogram-based** and **Color Cube-based**. When the *Segmentation* command is activated on an RGB class image, a dialog with tabs for each of these color models will appear. When using a greyscale image, and/or whenever *Threshold* is used, only the **Histogram** based model is available. To process a gray image using the **Color Cube** model, first convert it to an RGB image class. The results from applying each of these segmentation models are kept separate by *Image-Pro Plus 7.0*, allowing an image to be simultaneously processed by both methods. You can then choose the model providing the best results.

### **Histogram-Based Model**

This color selection model separates an image into RGB (Red, Green, and Blue) or HSI (Hue, Saturation, and Intensity) components (channels). Each channel is represented by a range of the component's "presence" (e.g. how much Red there is in a particular pixel). The reason for providing two color representation models is, once again, to facilitate color selection. The RGB model is typically more familiar to users and intuitive. However, there is a strong correlation between the RGB components of many naturally occurring colors. For example, yellow and brown, while very different to the eye, produce very similar RGB patterns, since yellow is really just "bright brown". Therefore, separating yellow from brown objects can be a laborious task using the RGB model. However, colors that correlate in such a manner typically have very different HSI channels. Going back to the previous example, yellow and brown can be separated in the HSI model solely by their intensity channel (once again, because yellow is "bright brown"). The Histogram-based model of segmentation is associated with the following property sheet on the segmentation dialog:



**Class Selector:** This drop-down list identifies the current class (i.e. which class is represented in and will be changed via the **Range** controls). Changing the selected item in this drop-down list will change the current class.

**New:** This button will add a new class. On RGB images, the new class will encompass the entire range of all channels, but will not be displayed until the range value in one or more of its channels is changed. On grayscale images, the new class will encompass only the range defined from the lowest unsegmented value to the lowest segmented value. This difference is caused by the condition that classes may not overlap in grayscale images, although they may in RGB images. Pixels in an image that are encompassed by more than one class are represented visually by the class that is highest in the **Class Selector** (i.e. the class created first containing the pixel).

**Delete:** This button removes the current class.

**Class Color Selector:** This button opens a color selection dialog. The color chosen in this dialog will become the class color, which can represent pixels contained in the current class depending on the preview options.

**Channel Selectors:** These buttons change and indicate the current channel in the histogram. Press the appropriate button to select the corresponding channel. These buttons are not present on grayscale images.

*Process: Threshold/Segmentation*

**Color Model Selector:** This drop-down list identifies the color representation model the histogram uses. Changing the selection in this control will change color model and reset segmentation (delete all classes and erase all range values).

**Zoom Tool:** Moving the cursor over the image histogram activates the zoom tool. The cursor will change to a magnifying glass to represent this tool's activation. Clicking the left mouse button will zoom into (increase the resolution) of the portion of the histogram under the cursor. Alternately, holding down the shift key while clicking will zoom out (decrease the resolution) of the displayed histogram. The magnifying glass will contain a plus (+) when zoom in is active and a minus (-) when zoom out is active.

**Range Markers:** These lines change and visually represent the values of the current channel's segmented range. Click and drag the appropriate marker to change the range's upper or lower value. Remember that ranges may not overlap on grayscale images.

**Show All:** When checked, the dialog will display the histograms of all channels, the current being displayed as a solid color, the others as outlines. Uncheck the box to display only the current channel's histogram. This check box is not present on grayscale images.

**Scale Adjuster:** This scroll bar will change the scale (maximum amplitude) represented at the top of the histogram. This control will cause colors represented by a small number of pixels to appear in the histogram.

**Eyedropper:** This button will activate the eyedropper color selection tool and remain depressed while the tool is active. While the eyedropper is active, the eyedropper zoom frame will display an enlargement of the area under the eyedropper. Clicking the left mouse button will cause the current class' range values to be expanded, if possible, to contain all colors under the eyedropper. Press this button again to deactivate the eyedropper tool.

**Undo:** This button will reverse the last change to the current class' range values.

**Remove Color:** This button will erase the current class' range values and treat it like a new class. As such, the current class' color will be removed from the preview. This button is disabled on Gray images when there is more than one class present.

**Eyedropper Size Control:** The drop-down list changes the size of the area covered by the eyedropper tool, measured in pixels. The eyedropper tool can only be a square with an odd-numbered side length, from one to seven pixels.

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**Range Controls:** These text boxes and spinners change and numerically indicate the values of the current channel's segmented range. The spinners will increment or decrement the range value by one, while pressed. Alternately, the desired value can be typed directly into the appropriate text box.

**Invert:** This button reverses display of the current channel of the current class on the image. This button is disabled when there is more than one class on grayscale images.

**Automatic Threshold:** This button sets automatically determine values for segmenting the image. It will delete all classes and segment the image over a single range in a single class.

**Eyedropper Zoom Frame:** This frame displays the area under the eyedropper tool while it is active.

**Display Classes:** This drop-down list indicates and selects whether all classes are displayed on the image or only the current class.

**Display Method:** This drop-down list indicates and selects how classes are displayed (previewed) on the image. All combinations of class color, black, white, and transparent are allowed, enabling classes to represent either foreground objects (to keep) or background.

**Create Preview Image:** This button creates a new RGB class image from the image preview. The new image will be an RGB 24 class image when this process is applied to an original of any image class other than RGB 36 or RGB 48. In these cases, the resulting image class will be the same as the original image class.

You can use this button to create a mask of only one segmentation class, if you have created multiple segmentation classes. To do this, set your Preview Options to **Current Class** and **White on Black**. Then, click **Create Preview Image**. This will create a black and white mask of the currently selected segmentation class in an RGB image. If necessary, you can convert this mask to gray scale by choosing *Edit: Convert To: Gray Scale 8*.

**File:** The button brings up dialogs for saving and loading the current segmentation classes. When loading, the classes contained in the file may be merged with or replace the currently defined classes.

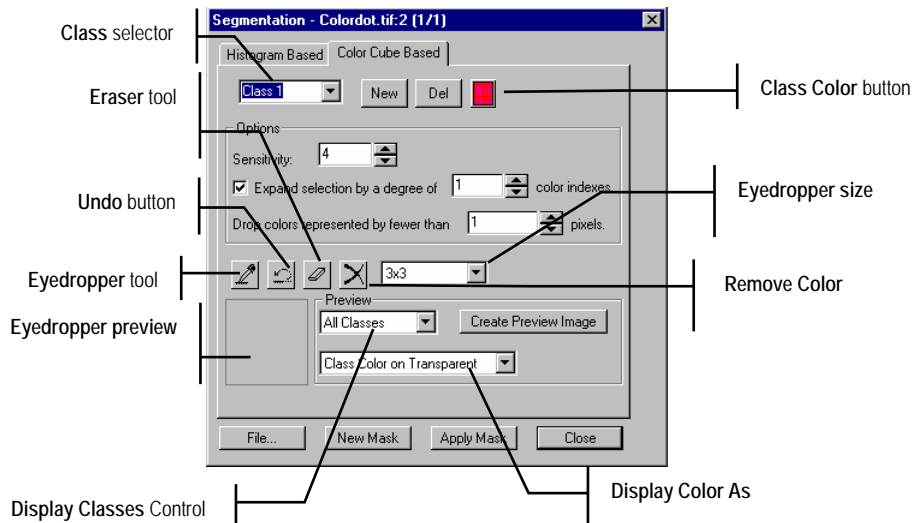
**New Mask:** This button creates a new image using the currently defined classes. All pixels contained in a class will be set to white, while those not in a class are set to black. The new image will be of the same image class as the original.

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**Apply Mask:** This button produces identical results as the **New Mask** button, except that the resulting image is written back to the original image.

## Color Cube-Based Model

This color selection model divides the color spectrum into a cube defined by intensity along red, green, and blue axes. Each element within the cube can be selected into a segmentation class independently, allowing vastly different colors to occupy the same class, which the histogram based model is incapable of doing. This definition granularity causes the eyedropper tool to be the only method of selecting and removing a class' colors. Although the **Color Cube-based Segmentation** property sheet shares many components with the **Histogram-based** property sheet, it also introduces several new controls as depicted below. This property sheet will not appear on grayscale class images.



**Class Selector:** This drop-down list identifies the current class (i.e. which class is represented in and will be changed via the **Range** controls). Changing the selected item in this drop-down list will change the current class.

**New:** This button will add a new class. On RGB images, the new class will encompass the entire range of all channels, but will not be displayed until the range value in one or more of its channels is changed. On grayscale images, the new class will encompass only the range defined from the lowest unsegmented value to the lowest

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segmented value. This difference is caused by the condition that classes may not overlap in grayscale images, although they may in RGB images. Pixels in an image that are encompassed by more than one class are represented visually by the class that is highest in the **Class Selector** (i.e. the class created first containing the pixel).

**Delete:** This button removes the current class.

**Class Color Selector:** This button opens a color selection dialog. The color chosen in this dialog will become the class color, which can represent pixels contained in the current class depending on the preview options.

**Sensitivity:** This text box and spinner combination controls the granularity of the color cube. As it is neither practical nor productive to define a color cube by every possible color value, each unit of the axis defining the color cube is composed of multiple color values that are very close in value. The value shown in the text box is a scale value defining how much granularity is used when defining the color cube (i.e. how “close” two colors must be before being considered the same color for purposes of selection). Higher values represent greater granularity. Note that this number is a scale value, and does NOT indicate the number of divisions on each axis of the color cube. As this value affects the way the entire image is represented, changing this value will reset segmentation (delete all classes and erase all selections).

**Expand Selection:** This check box, text box, and spinner combination controls how “big” an area of the color cube is selected into a class at the same time. When checked, the eyedropper and eraser tools will select not only the element of the color cube containing the area under the eyedropper, but those elements around the selected element on each axis. The higher the value in the text box, the further away the selection tools will reach when selecting and erasing colors in the class.

**Drop Colors:** This text box and spinner combination controls how much presence a particular color cube element must have before it is considered “noise”. When a color is selected the number of pixels in the image with that color cube element is considered. If there are fewer pixels with the selected color in the image than the value in the text box, that color cube element is not selected, although elements around it will still be considered, depending on the setting of the **Expand Selection** combination.

**Eyedropper:** This button will activate the eyedropper color selection tool and remain depressed while the tool is active. While the eyedropper is active, the eyedropper zoom frame will display an

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enlargement of the area under the eyedropper. Clicking the left mouse button will cause the color cube element(s) representing all colors under the eyedropper to be added to the current class. Press this button again to deactivate the eyedropper tool.

**Undo:** This button will reverse the last change to the current class' range values.

**Eraser:** This button will activate the eraser color removal tool and remain depressed while the tool is active. While the eraser is active, the eyedropper zoom frame will display an enlargement of the area under the eraser. Clicking the left mouse button will cause the color cube element(s) representing all colors under the eyedropper to be removed from the current class. Press this button again to deactivate the eraser tool.

**Remove Color:** This button will remove the current class' color from the segmentation preview.

**Eyedropper Size Control:** The drop-down list changes the size of the area covered by the eyedropper and eraser tools, measured in pixels. These tools can only be a square with an odd-numbered side length, from one to seven pixels.

**Eyedropper Zoom Frame:** This frame displays the area under the eyedropper and eraser tools when they are active.

**Display Classes:** This drop-down list indicates and selects whether all classes are displayed on the image or only the current class.

**Display Method:** This drop-down list indicates and selects how classes are displayed (previewed) on the image. All combinations of class color, black, white, and transparent are allowed, enabling classes to represent either foreground objects (to keep) or background.

**Create Image:** This button creates a new RGB class image from the image preview. The new image will be an RGB 24 class image when the original image class is RGB 24, Floating Point, or Palette. Otherwise, the new image will be of the same image class as the original.

**File...:** The button brings up dialogs for saving and loading the current segmentation classes. When loading, the classes contained in the file may be merged with or replace the currently defined classes.

**New Mask:** This button creates a new image using the currently defined classes. All pixels contained in a class will be set to white, while those not in a class are set to black. The new image will be of the same image class as the original.



*Process:Threshold/Segmentation*

**Apply Mask:** This button produces identical results as the New Mask button, except that the resulting image is written back to the original image.

## Operations

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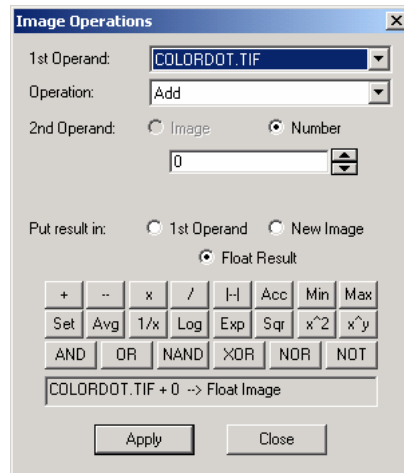
Use the *Operations* command to perform arithmetic and logic operations upon the active image, sequence, or AOI. Such operations can be performed between two images (such as subtracting a “Hi Pass” filtered image from an original image to emphasize high-contrast elements), or between an image and a constant value (such as “ANDing” an image with 240 to retain only the 4 most significant bits of information).

The *Operations* command can be used on both monochrome and color images. When applied to a *True Color* image, the operation will be applied to all three channels. For example, if you were subtracting a value of 10 from the image, 10 would be subtracted from the Red value, from the Green value and from the Blue value in each pixel.

Each image may have one or more AOIs. If neither image has an active AOI, the operation is performed from the upper left corner (position 0, 0) of each image. If only one image has an active AOI, the operation is performed between that AOI and the corresponding position in the other image. If both images have an active AOI, the operation is performed on the two AOIs, starting at the upper left corner of the bounding box of each.

If the active image has a multiple AOI, the modifications occur within each AOI. If the other image has an active multiple AOI, the bounding box for the AOI acts as the AOI for that image.

When you select the *Operations* command, the **Image Operations** dialog box will be presented. Within this box, select the kind of operation you want to perform, the image or value you want it to work with and a destination for the results.



**First Operand:** Select one of the files in this list box to tell *Image-Pro* what active image to use for the operation.

**Operation:** Select the kind of operation you want *Image-Pro* to perform from the drop-down list box, or click on the appropriate “calculator” button .

**AND:** Click this button to perform a logical “AND” between your active image and the specified operand. Only bit values that are “on” in both operands, will be “on” in the result.

**OR:** Click this button to perform a logical “OR” between your active image and the specified operand. Bit values that are “on” in either operand, will be “on” in the result.

**XOR:** Click this button to perform a logical “XOR” (exclusive OR) between your active image and the specified operand. Only when a bit value is “on” in one operand and “off” in the other will the bit be “on” in the result. If bit values are “on” in both operands, or if they are “off” in both operands, they will be “off” in the result.

**NAND:** Click this button to perform a logical “NAND” between your active image and the specified operand. “NAND” produces a result that is the complement of an “AND” operation. Bit values that are “off” in either, or both, operands, will be “on” in the result.

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**NOR:** Click this button to perform a logical “NOR” between your active image and the specified operand. ‘NOR” produces a result that is the complement of an “OR” operation. Bit values that are “off” on both images, will be “on” in the result.

**NOT:** Click this button to perform a logical “NOT” on the bit values of your active image. Every bit value that is “on” in the active image will be “off” in the result. Every bit value that is “off” in the active image will be “on” in the result. Use this command to invert the pixels in your image, producing a "negative" effect.

**Add:** Click this button to add the specified operand to the active image. If a pixel's result falls outside of the image's intensity scale, it will be clipped to the highest /lowest permitted value.

If you have set the **Offset** value, each result will be added to the **Offset** before it is written.

**Subtract:** Click this button to subtract the specified operand from the active image. If a pixel's result falls outside of the image's intensity scale, it will be clipped to the highest /lowest permitted value.

If you have set the **Offset** value, each result will be added to the **Offset** before it is written.

**Diff:** Click this button to obtain the absolute value between the specified operand and the active image. If a pixel's result falls outside of the image's intensity scale, it will be clipped to the highest /lowest permitted value.

If you have set the **Offset** value, each result will be added to the **Offset** before it is written.

**Multipl y:** Click this button to multiply the active image by the specified operand.

If you have set the **Scale** value, each result will be multiplied by the **Scale** before it is written.

**Divide:** Click this button to divide the active image by the specified operand.

If you have set the **Scale** value, each result will be multiplied by the **Scale** before it is written.

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**Average:** Click this button to replace each pixel with the mean value of the two operands.

**Log:** Click this button to replace each pixel with its logarithm. Pixel values less than or equal to 0 are invalid.

**Square Root:** click this button to replace each pixel with its square root. Pixel values less than 0 are invalid.

**Square :** Click this button to replace each pixel with its second power.

**In verse:** Click this button to replace each pixel with its inverse (1/pixel). Argument: scaling factor: new pixel = scaling factor / original pixel. Pixel values equal to 0 are invalid.

**X to the power of Y:** Click this button to replace each pixel with its Yth power. Y is limited to positive values. Pixel values less than 0 are invalid if Y is less than 1.

**Set:** Click this button to replace each pixel with the same given number or with the corresponding pixel in the second image operand.

**Exp:** Click this button to replace each pixel with its exponent.

**Maximum:** Click this button to replace each pixel with the largest value of the two operands.

**Minimum:** Click this button to replace each pixel with the smallest value of the two operands.

**Accumulate:** Click this button to replace each pixel with the sum of pixels of the two images. The operand image value will not be scaled to the destination image value before adding them.

If you have set the **Offset** value, each result will be added to the **Offset** before it is written.

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**Note:** **Accumulate** will not be available if you have selected **Number** as the operand.

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**Second Operand:** Click the appropriate radio button to tell *Image-Pro* what operand (besides the active image) to use for the specified operation.

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**Image:** Set this option if you want to use another image for the specified operation. You must also select, in the adjacent list box, the image you want to use. This image must be one that is currently open within *Image-Pro*; it cannot be an image on disk. The two images need not be of the same image class.

**Number:** Set this option if you want to use a constant value for the specified operation. If a logical operation has been selected, numbers from 0 - 255 can be entered. If an arithmetic operation has been selected, any value can be specified, including negative and fractional values. Scientific notation may be used.

If you are “ANDing” an 8-bit image to reduce its active bit planes, the following values should be used:

IF YOU WANT TO RETAIN	SET THE "NUMBER" TO
1 significant bit	128
2 significant bits	192
3 significant bits	224
4 significant bits	240
5 significant bits	248
6 significant bits	252
7 significant bits	254

**As Is:** This checkbox is only visible when operations between two integer images of different bit depths are being performed, such as GRAY16 and GRAY12, or RGB and RGB36. If checked, the IMC\_C\_DIRECT scaling method is used for image conversion prior to image operations. If unchecked, the IMC\_C\_SCALED method is used, so that an 8-bit image scaled up to a 16-bit image is effectively multiplied by 257, not 256, and the lower least significant bits have meaningful values.

**Place Result Into:** Select one of the options in this box to tell *Image-Pro* where to write the results of the specified operation.

**First Operand:** Click this button if you want to write the results back to the active image or AOI. Results will be placed into the same pixel positions from which they originated.

**New Image:** Click this button if you want *Image-Pro* to write the results into a new window. The new window will be of the same size and class as the original image or AOI.

**Float Result:** Click this button if you want *Image-Pro* to save the results into a floating point image.

**Image Equation:** *Image-Pro* summarizes your command here.

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**Note:** Log, Square root, Square, X to Y, and Exp will increase or decrease pixel values dramatically and may only be useful when used on floating point images. To apply these operations to 8 bit images, convert the image into a floating point image first.

Using Log, Square root, Inverse, and X to Y on images containing pixels whose values are invalid for the given operation, will result in the following approximations: Negative infinite results will be set to the minimum value supported by the image type (i.e. 0 for 8, 12, 16, 24, 36, 48 bit images). Positive infinite results will be set to the maximum value supported by the image type (i.e. 255 for 8 bit, 4096 for 12...). Indeterminate results (e.g. 0/0) will be arbitrarily set to the minimum value supported by the image type.

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***Some notes about operations on sequences:***

If the first operand image is a sequence (sequence 1) and has **Apply to Sequence** selected, then the active portion of the sequence will determine the first frame and number of frames operated on.

If the second operand is a single-frame image or does **not** have **Apply to Sequence** selected, then the active frame of the *second* operand will be applied to each frame operated on.

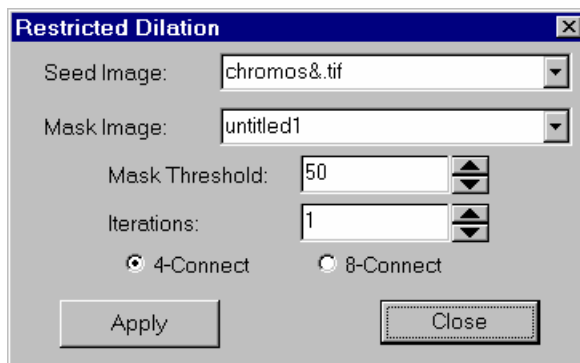
If the second operand image is a sequence (sequence 2) and has **Apply to Sequence** selected, then the active portion of that sequence will be used by the operation. If this portion has fewer frames than the operation requires (less than the active portion of sequence 1), then sequence 2 will wrap around, reusing frames as necessary to complete the operation.

## Restricted Dilation

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Use the *Restricted Dilation* command to grow objects in one image (the seed) up to the boundaries of a second image (the mask). The primary purpose of this function is to combine the results of two segmentation algorithms – the mask being generated by an algorithm that is less selective than the algorithm used by the seed image.

When you select *Restricted Dilation*, you will see the following dialog box:



**Seed Image:** Use this combo box to select an image where the non-zero areas will be dilated.

**Mask Image:** Use this combo box to select an image containing areas marking the limits of dilation. The seed image will not be dilated past the thresholded areas in the Mask.

**Mask Threshold:** The percentage intensity where the mask image will be thresholded.

**Iterations:** The number of dilations that are to be performed.

**4-Connect / 8-Connect:** Whether a 4 connected (horizontal and vertical) or 8-connected (all neighboring pixels) dilation will be performed.

**Apply:** Perform the Restricted Dilation.

**Close:** Close the dialog box.

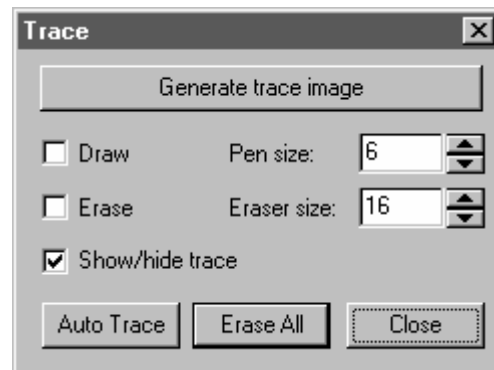


## Trace Objects

---

The *Trace Objects* command provides you with a tool to highlight specific features of a image manually. *Trace* works only when you have an image loaded into the *Image-Pro Plus* workspace.

When you click on this option, the **Trace** dialog appears:



*Trace Objects* is used to highlight dark features on a primarily light background, such as fingerprint ridges. These features can be drawn manually or automatically traced. The automatic tracing tool works by tracing the skeleton of dark features.

The following controls apply to the **Trace** command:

**Generate trace image:** Clicking this button after the trace operation is finished places the traced outlines in a separate image:

**Draw:** Check this box to begin drawing a traced line on the image.

**Erase:** Check this box to remove traced lines.

**Show/hide trace:** Check this box to display or hide traced lines in the image.

**Pen size:** Use the spin buttons to select the size of the drawing tool in pixels. The minimum pen size is 5 pixels; the maximum is 30.

**Eraser size:** Use the spin buttons to select the size of the erasing tool in pixels. The minimum eraser size is 5 pixels; the maximum is 100.

**Auto-Trace:** Click this button to automatically trace all the objects in an image.

**Erase All:** Click this button to automatically remove all the traced lines from an image.

**Close:** Click this button to exit the trace tool.

## Color Channel

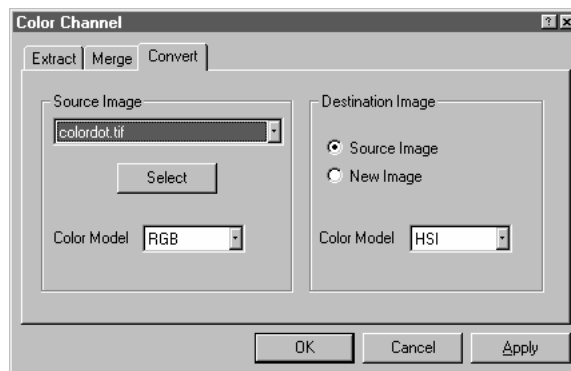
---

Use the *Color Channel* command to transform an image to another color model, to extract a specific color channel from a color image, or to merge an active, 8-bit gray scale image into a *True Color* image.

### Convert

Use the *Convert* selections to transform an image to another color model. You might do this to perform editing or analysis upon the image in another model. You might also do this to save the image data in a model other than RGB, for use with an external program.

The *Convert* command can only be used on *True Color* class images. If your image is of another class, you may convert it to *True Color* using the *Convert To* command on the *Edit* menu. Clicking on this tab displays the following dialog:



**Source Image:** From this drop-down box, select the Color Model through which you want the source *True Color* image interpreted.

**Select:** The source image can be selected either from the drop-down list box or by clicking the **Select** button. When you click this button, a small box appears (similar to the screen capture selection tool). Drag this box onto the image that you want to use as your source image.

**Destination Image:** Select the Color Model in which you want the results written.

**Source Image:** Click this button if you want the converted data written to the source image window.

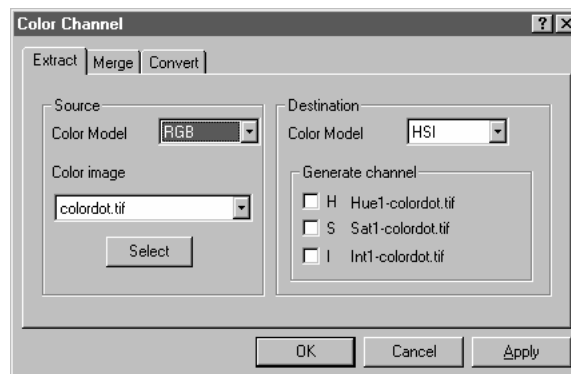
**New Image:** Click this button if you want the converted data written to a new image window.

## Extract

Use the *Extract Channel* selections to extract a specific color channel from a color image, and view it as an 8-bit *Gray Scale* image. You might do this to reduce the saturation values in a HSI image, or boost just the Blue values in an RGB image.

Once a color channel has been extracted, it can be edited and analyzed as you'd like (treat it like any ordinary *Gray Scale* image). After editing, it can be merged back into the original image or another image, using the *Merge Channel* command.

The *Extract Channel* command can only be used on *True Color* class images. If your image is of another class, you may convert it to *True Color* using the *Convert To* command from the *Edit* menu.



**Source:** Select the Color Model through which you want the active *True Color* image interpreted.

**Color Model:** Select the color model that describes the values contained in your source image. If you are extracting a channel that is from a model other than that of your source image, *Image-Pro* will automatically convert the current data to the appropriate model to extract the channel you've selected in the **Extract** option list.

**Color Image:** Indicate the name of the source image containing the channel that you wish to extract

### *Process:Color Channel*

**Select:** Select the color channel for which you want data extracted. When you click this button, a small box appears (similar to the screen capture selection tool). Drag this box onto the image that you want to use as your source image. You may extract a channel for a color model other than the one in which your image is currently rendered. For example, you can extract the **Hue** channel from an RGB image as long as your source is defined as **RGB** in the **From** option list.

---

**Note:** When a channel is extracted, its values are copied into the new image window; they are not removed from the original image.

---

**Destination Image:** Select the Color Model in which you want the results written.

**Generate Channel:** Select the color channel for which you want data stored after extraction. You may specify Hue, Saturation, or Color.

**Apply:** Click this button to extract the color channel of your active image.

## **Merge**

Use the *Merge Channel* command to merge an active, *Gray Scale* image into a *True Color* image. This is usually done to return a channel, extracted with the *Extract Channel* command, to an image after the channel data have been manipulated. However, you might use it to merge any *Gray Scale* image to a color channel in a *True Color* image.

When a *Merge Channel* function is performed, the values contained in the *Gray Scale* image replace the values in the specified color channel of the selected color image. If an AOI is active in the *Gray Scale* image when the merge is performed, only values from within the AOI rectangle will be merged into the color image (if the AOI is nonrectangular, the pixels from its rectangular bounding box will be used).

If a new color image is created, it will match the image class of the source image with the highest bit depth; Grayscale 8 will become RGB, Grayscale 12 will become RGB36, and Grayscale 16 will become RGB48.

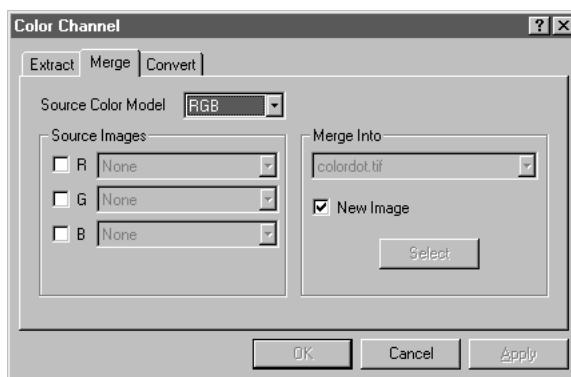
New grayscale images will be scaled to match the bit depth of an existing color image.

If an AOI is active in the receiving *True Color* image when the merge is performed, the *Gray Scale* values will be merged with those in the

*True Color* AOI. If the AOI is nonrectangular, only those pixels within the AOI will be affected; however the nonrectangular AOI's rectangular bounding box will be used for positioning purposes (i.e., the upper-left corner of the Gray Scale image/AOI will be matched to the upper-left corner of the bounding-box in the receiving AOI).

The *Merge Channel* command will work only if the source image is *Gray Scale* and the target image is *True Color* (both images must be open). The *Gray Scale* image (the source image) must be the active image when you select the *Merge Channel* command. If the active image is not *Gray Scale*, the command will be dimmed.

When you select the *Merge Channel* command, the following dialog box is presented.



**Source Color Model:** Select the color model that describes the values contained in your source image.

**Source Image:** Select the color channel image from which you want the active *Gray Scale* image or AOI written

**Merge Into:** From this list box, select the *True Color* image into which you want the active *Gray Scale* image or AOI merged. All open, *True Color* images will be listed here.

**New Image:** Check this box if you want to save the results of the color channel merges into a new image.

**Select:** You can select the destination image either with the Merge Into list box, or with the **Select** button. When you click this button, a small box appears (similar to the screen capture selection tool). Drag this box onto the image that you want to use as your source image. You may extract a channel for a color model other than the one in which your image is currently rendered. For example, you can

*Process:Color Channel*

extract the **Hue** channel from an RGB image as long as your source is defined as **RGB** in the **From** option list.

**Apply:** Click this button to merge the color channels.

---

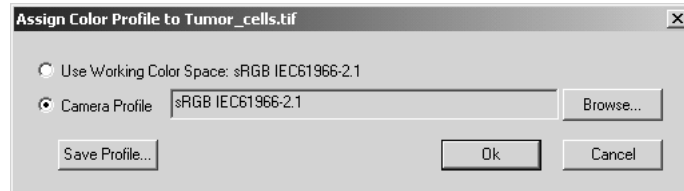
**Note:** If your incoming image is not RGB, but you want to merge the channels without any conversion, you should set the source color model to RGB.

---

## Assign Color Profile

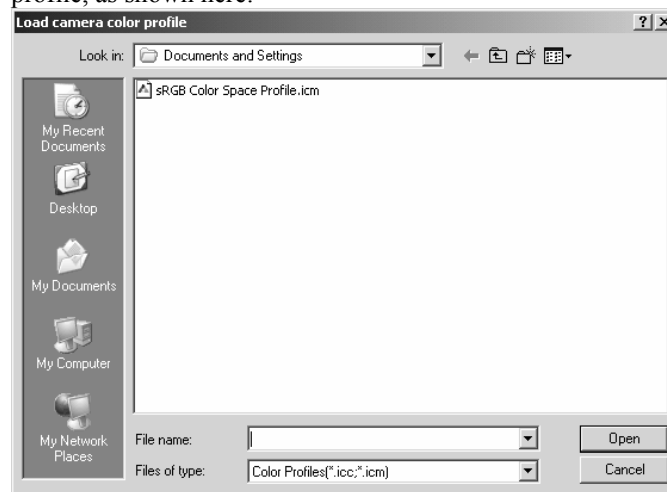
---

*Assign Color Profile* assigns color profiles to the image currently open in the *Image-Pro* workspace. Choosing this menu item displays the following dialog:



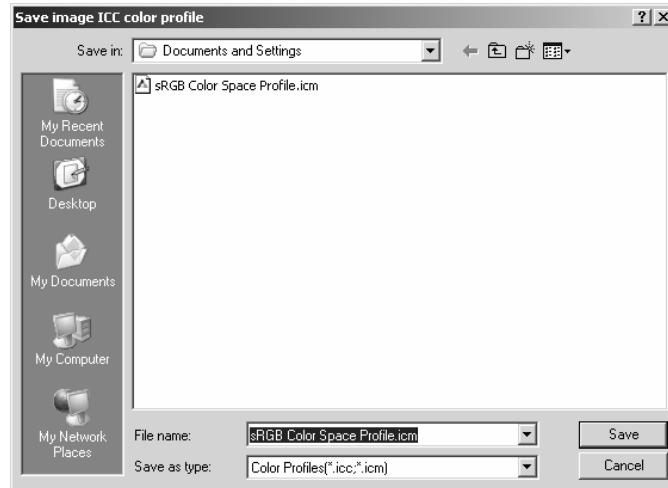
When the dialog is opened, it displays the active color profile associated with the image.

- **Working Color Space:** Click this button to use the working color space profile for the current image.
- **Camera Profile:** Click this button to assign a camera profile to the current image. Click the **Browse** button to load a different camera profile, as shown here:



- **Save Profile:** Click this button to save the color profile. You will see the **Save image ICC color profile** dialog:

*Process:Assign Color Profile*



- Select the correct file name and type, and click the **Save** button.
- Click **Cancel** to return to the **Assign Color Profile** dialog without saving a profile.

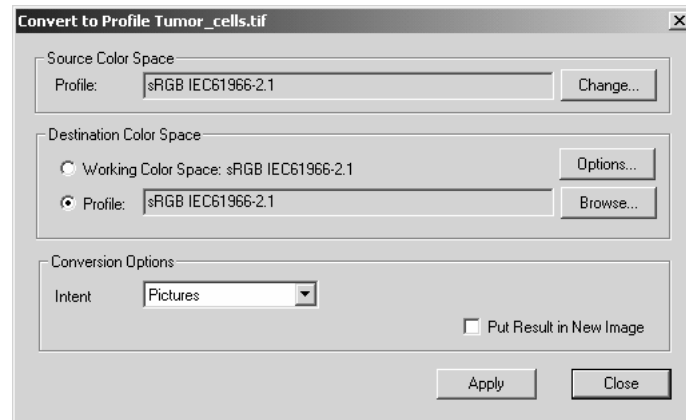
Once you have made your **Color Profile** selections, click **OK**. Click **Cancel** to return to the *Image-Pro* workspace, without making any changes.



## Convert to Profile

---

When you select *Convert to Profile*, you will see the following dialog:

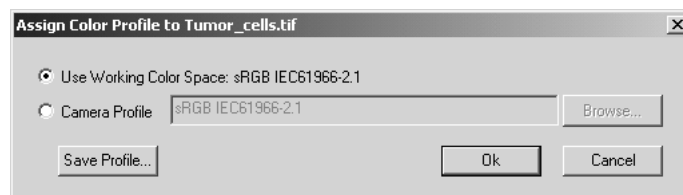


Using this dialog, the active image can be converted from one profile to another.

**Source Color Space:** The **Source Color Space** shows the current color profile assigned to the image.

- **Profile:** The **Profile** list box displays the color profile currently in use.
- **Change:** Click the **Change ...** button to assign a new color profile to the image. You will see the **Assign Color Profile** dialog:

■



**Destination color space:** The **Destination color space** can be either **Working Color Space** or **Profile**.

- **Working Color Space:** Click the **Working Color Space** button to convert the image to the default space. Click the **Options** button to change the working color space.
- **Profile.** Click this button to convert the image to the given profile.

*Process:Convert to Profile*

- **Conversion Options:** The conversion options use these **Rendering Intents**. Choose one of the following:

<b>Intent</b>	<b>ICC Name</b>	<b>Description</b>
Picture Perceptual	al	For images and photographs; attempts to maintain the relationship between colors.
Graphic Saturation	aturation	For charts and business graphics; attempts to maintain a color's saturation, rather than its hue or lightness.
Proof Relative	Colorimetric	Does not preserve the white point; if the output gamut does not support a given color, the nearest supported color is used.
Match Absolute	Colorimetric	Preserves the white point; if the output gamut does not support a given color, the nearest supported color is used.

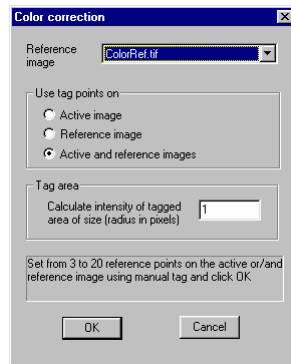
- **Put Result in New Image:** Check this box to create a new image with the profile results.
- **Apply:** Click the **Apply** button to convert the image to the chosen profile.
- **Close:** Click the **Close** button to return to the *Image-Pro* workspace without making any changes.

## Color Correction

---

The *Color Correction* feature in *Image-Pro Plus 7.0* is closely related to the *CIE L\*A\*B Color* feature. It is very difficult to make accurate color measurements without color correction. Color correction of images is usually done using a reference image containing known colors.

To use the *Color Correction* feature, you must have a color reference image open in the *Image-Pro* workspace. When you select *Color Correction* from the *Process* menu, you will see the following dialog:



To perform color correction an RGB color calibration must be created using a certain number of calibration points. You should select the number of calibration points assuming that:

- **3-point** calibration corrects changes in color gain
- **4-point** calibration corrects color shift and gain
- **7-point** calibration corrects the linear color mix
- **10-point** calibration corrects the color mix using polynomial approximation of power 2
- **20-point** calibration corrects the color mix using polynomial approximation of power 3 .

**Reference Image:** Choose the reference image from the drop-down list.

*Process:Color Correction*

**Use Tag points on:** Use *Image-Pro's Manual Tagging* feature (from the **Measure** menu) to select the calibration points on the following images:

- Active Image
- Reference Image
- Active and Reference Image.

**Tag Area:** Indicate the size in pixels of the area to be tagged.

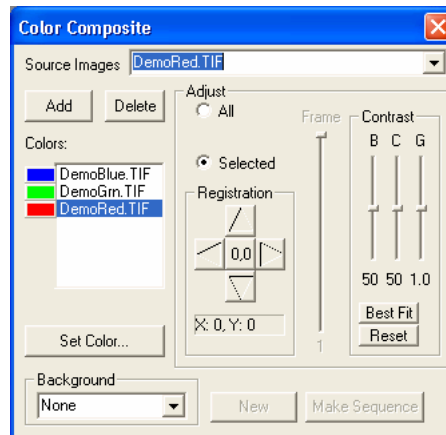
Select from 3 to 20 reference points on the image. Then click **OK**.

The program now takes to the *CIE L\*A\*B Color* feature where you can finish creating the color calibration.

## Color Composite

---

Use the **Color Composite** dialog box to create and configure color composites using black and white source images. You can access the **Color Composite** dialog box through the **Color Composite** menu item from the *Process* menu.



You can combine grayscale images into a color composite. Any group of grayscale images that are of the same size can be mixed in a color composite. Images of 8-, 12-, 16-bit integer or floating point format are combined into a 24-bit color composite. Each input channel will have individual LUT adjustments, as well as a registration offset to line it up with the rest of the images. You can also combine individual channels from a single image. Each channel will be listed separately.

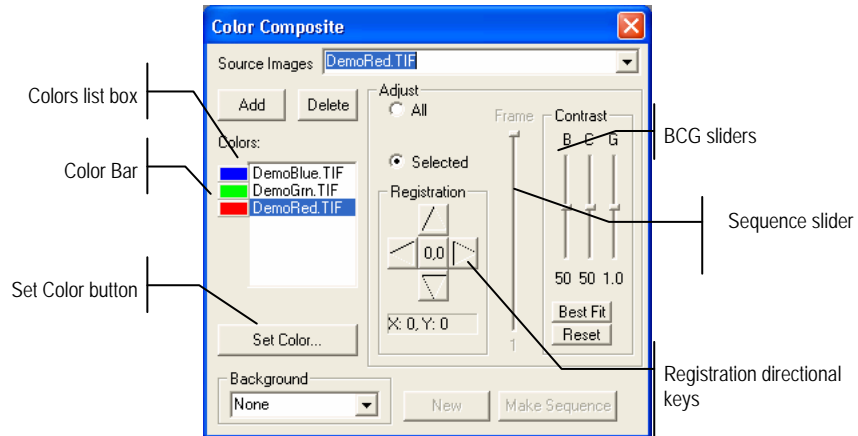
**Source Images:** The **Source Images** combo box displays the images available for color mixing. The Source Image list is initially filled with a list of the gray scale images that are currently open. The size of the color composite is determined by the first input image selected. Once this is entered, the list is filtered to contain only images of the same size as the initial selection.

**Add:** Click the **Add** button to add an image to the mix. You will be prompted for the color to be assigned to that image from the **Set Color** dialog.

**Delete:** Use this button to delete the selected image from the list.

*Process:Color Composite*

**Colors:** The **Colors** list box displays the images selected for color mixing.

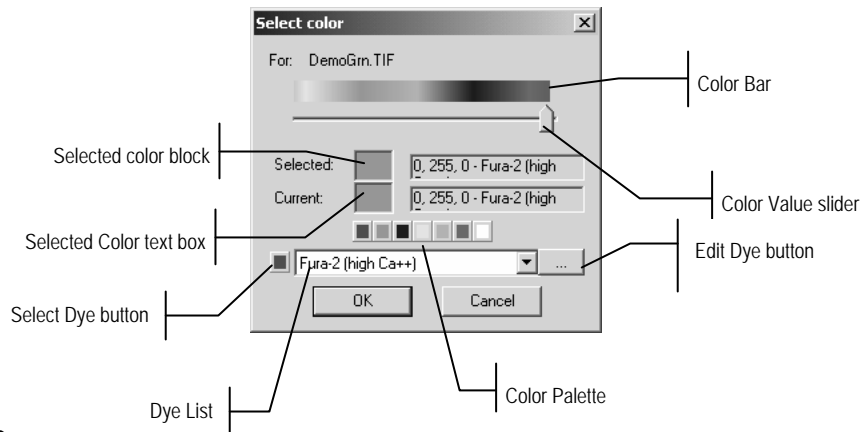


To change the color within the list box, double-click to select the image name (the **Select Color** dialog box appears) or highlight the name and click the **Set Color** button.

To delete colors/images from the **Colors** list box, first click on the color/image to highlight it then press the **Delete** button within the **Color Composite** dialog box.

**Color Bar:** The picture item box on the left side of the **Colors** list indicates the color associated with each image in the composite. The **Color Bar** will scroll with the contents of the **Colors** list box.

**Set Color:** This option determines the Color value used for the selected channel in the final image display. Clicking on the **Set Color** button brings up the **Select Color** dialog box.



Directly select a color by:

- 1) Dragging the **Color Value Slider** to the desired color on the **Color Bar** (range of 0 to 359°): or
- 2) Clicking the appropriate Color button on the **Color Palette**. The **Color Bar** represents Color degrees in the standard color wheel representation, and the **Color Palette** contains a subset of possible Colors (pure red, green, blue, cyan, magenta, and white): or
- 3) Select a dye in the dye list, and click the **Select Dye** button.

---

**Note:** You can navigate through the **Select Color** dialog box by using the <Tab> and <Shift + Tab> keys to cycle through the items in the dialog box.

---

**Intensity** is determined by the image the Color is applied to, and **saturation** will always be 100%.

The only exception to this is the “white” Color, which has by definition a saturation of 0%. Selecting a value greater than or equal to 360 (dragging the **Color Value** slider over to the far right) is a choice of white, which results in a non-tinted (gray) image.

The elements of the **Select Color** dialog box are:

**Color Bar:** The **Color Bar** shows a rainbow displaying the Colors underneath the appropriate point of the **Color Value** slider.

---

**Note:** If **Color Value Slider** is the selected (highlighted) selected control, then you can also control the slider with keyboard strokes:

- Use the left/right arrow << >> keys to move the slider incrementally.
- Press the <Page Up> and <Page Down> to move the slider in “chunk” sections along the **Color Bar**.
- Use the <Home> and <End> keys to move the slider to the beginning and end of the **Color Bar**.

---

**Selected:** This color block displays the new Color selected by the **Color Value** slider bar or the **Color Palette**.

**Selected Color:** The **Selected Color** text field displays the corresponding numeric Color value of the **Selected** color block described above. The **Selected Color** value is expressed as an RGB

## Process:Color Composite

color, with the red value first, followed by the green and blue values. If the color corresponds to a dye color, the dye name will be displayed. If the dye color corresponds to a color palette color, the name of the color will also be displayed.

**Current:** The **Current** color block displays the starting Color of the channel.

**Color Palette:** The **Color Palette** provides an alternate method of selecting a Color. Clicking on an item in the **Color Palette** will set the **Color Value** slider to the correct value and update the displays in both the **Selected** color block and **Selected Color** text field.

For example, clicking on the white button on the **Color Palette** will set the **Color Value** slider to the right, change the **Selected** color block to white, and cause the **Selected Color** text box to display "White".

**Adjust:** The controls in this section are used to adjust the color composite contribution from each image, or for the color composite preview itself. You can adjust the input image that is currently selected in the **Colors** list by clicking the selected button. When you want to adjust the color composite preview, or you want no image is selected in the **Colors** list, use the **All** button.

**Registration: Registration** allows you to correct for mis-registrations of images caused by filter-induced optical shifts. Use the directional keys to shift the selected image in the *x*- and/or *y*- direction, with respect to the rest of the input images. One click moves that channel one pixel in the direction indicated. Blank areas are filled with black. The key labeled "0,0" will re-center the selected image.

The registration shift for the selected channel or the overall image is displayed in the static text box below the directional keys.

**Contrast:** Use the **BCG sliders** along with the **Best Fit** and **Reset** buttons to adjust the contrast of the selected image or the composite.

**BCG sliders:** The Brightness, Contrast, and Gamma sliders are the similar to the Contrast Enhancement feature in *Image-Pro Plus*. You can also adjust each channel individually or collectively by using the **Selected** or **All** radio buttons (to the left of the **BCG** sliders in the **Adjust** group box).

**Best Fit:** Click this button to perform a Best-Fit contrast stretch on the selected input, automatically setting the Brightness and Contrast settings accordingly (Gamma will be reset to 1.0.). This method is identical to the **Best Fit** contrast adjustment under the standard



Contrast Enhancement feature of *Image-Pro Plus*. **Best Fit** is useful for first pass adjustments.

**Reset:** Pressing the **Reset** button automatically resets the BCG settings. If the **Adjust** radio button is set on **All**, the BCG settings for the overall color image will be reset.

**Frame:** Use the frame slider to adjust the frame of the selected image that is used for the preview, or the active frame of the composite. (Please see the discussion of **Color Composite** and **Image Sequences** that follows this section.)

**Background:** Select one of the available images to be the background color in this combo box. The rest of the image planes are subtracted from the background image — effectively punching “holes” into it and allowing the foreground colors to show without mixing with the background. This is most effective where the background covers large areas and the other images have smaller objects within the background objects.

**Back ground** allows the other inputs to be displayed “in front of” the background inputs, minimizing color mixing. For example, *Image-Pro* can display a red dot on a blue background without turning the dot to magenta.

Selecting **None** causes all images to be equally mixed.

**New:** Click on the **New** button to discard the current color composite image, and start over.

The **Source Images** combo box will reset to include all gray scale images (including floating point images). Selecting an image from the list will create a new composite the same size as the initial selection. As mentioned previously, the source images list will be filtered to contain only images of the same size as the initial selection.

## Color Composite and Image Sequences

When working with single-frame images, the **Frame** slider and the **Make Sequence** button will both be disabled and have no effect. When working with image sequences, the **Frame** slider affects both the single-frame *preview* and the way that the sequence will be built when **Make Sequence** is invoked.

**Frame:** The single-frame color composite workspace that is created once you've added at least one image, is built from the frame of each image that is indicated by the **Frame** slider. Note that when the image is added, the frame setting for that image defaults to the currently active frame of the sequence. Changing the **Frame** slider for an image does NOT change the image's active frame.

As you activate different images (or click them in the **Colors** list), the **Adjust** radio button switches to **Selected** to indicate the settings for that image's contribution to the color composite. The **Frame** slider will update to indicate the currently selected frame that is contributing to the color composite preview, and you can adjust the slider to select a different frame to contribute.

If you change the **Adjust** radio button to **All**, then you can use the **Frame** slider to change the frames of ALL images. This has the same effect as the sequence toolbar for sequences but the color composite preview is still a single-frame workspace. The color composite preview will also track the active frame when you navigate through the contributing sequences using the **Sequence Toolbar**.

The frame settings for each image determine how those sequences will be aligned when **Make Sequence** is used to create a color composite sequence. To illustrate, consider two workspaces of 5 and 7 frames, where the frame setting is frame 4 for the first sequence and frame 3 for the second (numbering from 1, with the selected frames being represented by Frame # (F1, F2, etc.) in the following diagram):

	S1	S2	Result
F1			
F2		F1	F2/F1
F3		F2	F3/F2
	F4	F3	F4/F5
F5		F4	F4/F5
		F5	
		F6	
		F7	

Note that the first frame of sequence S1 is ignored because of the way the two sequences are aligned. Note also that the “All” frame setting for this color composite will start at 3 - this is the point of alignment in the resulting sequence. If the sequences have been captured using the same Zstack settings, you will seldom if ever want to mis-align the sequences this way, but it also makes for some interesting effects. Note also that the length of the sequence built by **Make Sequences** is the amount of overlap between the sequences with the specified alignment, or 4 frames in this case.

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**Note:** *Image-Pro Plus 7.0* allows you to turn the display of color channels on or off by clicking on the color bar. The color bar will display the color of the selected channel only when that channel is displayed.

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## Pseudo-Color

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Use the *Pseudo-Color* command to “colorize” the active monochrome (*Gray Scale 8*, *Gray Scale 12*, *Gray Scale 16*, or *Floating Point*) image. You might do this to highlight certain features in a gray scale image. For example, you might want to display all densities above a certain point in red, or, if your imaging device recorded thermal information, all temperatures below a certain point in blue.

You might also use it to visually amplify specific intensities that are very difficult to distinguish from their surroundings. For example, features produced by pixels with values of 122 in a field of pixels with similar values would be impossible to see in a gray tone image, but would jump out if that value were to be rendered in color.

When you pseudo-color a monochrome image, you build a special palette through which your monochrome image is displayed. Pseudo-coloring an image does not modify the pixel values in your image bitmap in any way (it does not convert your image to *True Color* or *Palette*, for example). It simply associates a pseudo-color palette with the image, that *interprets* the gray-level values in the image as color.

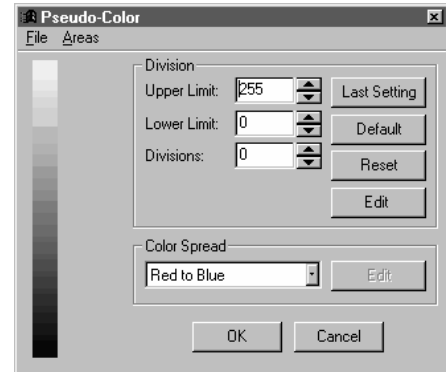
Pseudo-colored images are very similar in structure to *Palette* class images, but they differ in a couple of important ways. First, the pixel values in a pseudo-colored image actually represent continuous-tone intensity information, whereas a *Palette* image's pixels carries no intensity significance. Secondly, a *Palette* image includes a palette table that is actually part of the image file.

The pseudo-color palette associated with a pseudo-colored image is not a permanent part of the image. It is a palette that you assign to the image while you work with it in *Image-Pro*. Pseudo-color palettes can be saved to a disk file, and later loaded for subsequent use with any monochrome (*Gray Scale 8*, *Gray Scale 12*, *Gray Scale 16*, or *Floating Point*) image.

## Process:Pseudo-Color

When you select the *Pseudo-Color* command, the **Pseudo-Color** dialog box is displayed. It contains the tools you need to build, save and load pseudo-color palettes for monochrome images.

*Note - Because the **Pseudo-Color** command is only relevant to monochrome images, it will be dimmed if the active image is True Color. If you want to pseudo-color a True Color image, you must first convert it to one of the monochrome classes.*



The basic steps involved in pseudo-coloring the active, monochrome image are to:

- 1) Specify the range of intensities** you want to pseudo-color. The intensity range is specified using the **Upper Limit** and **Lower Limit** spin buttons.
- 2) Specify the number of distinct colors** you want to associate with the selected intensity range. The number of colors is selected using the **Divisions** spin buttons. When you set **Divisions** to a positive number, a second color bar appears to the right of the first one, and both color bars and your image assume the default pseudo-color settings. The color bar on the left represents the entire intensity range and illustrates the portion that is being colorized. The color bar on the right represents just the colorized portion of the intensity range.
- 3) Change the color assignments** and refine the intensity division widths. This is done using the color bar or the **Edit** button.
- 4) Save the settings for future use** (with the current image, or with another). This step is optional. You do not need to save the pseudo-color assignments in order for them to be applied to the active image. When you click the **OK** button, *Image-Pro* saves the current setting. If, however, you want to save it for continuing use or to exchange with a colleague, you need to save it.

When you have finished specifying/saving the pseudo-color palette, click **OK** to assign it to your image. Your image will be displayed through this palette for as long as it (your image) remains open. If you

## *Process:Pseudo-Color*

want to remove the effect of the palette upon the active image, select the *Pseudo-Color* command and click **Reset**. Your image will be returned to its normal monochrome state. Pseudo-colored images can be converted to *True Color* or *Palette* images using the *Convert To* command.

Each of the buttons and fields in the **Pseudo-Color** dialog box are described in more detail below.

**Division:** Within this group box, enter the intensity range and the number of distinct colors you want *Image-Pro* to use to pseudo-color. *Image-Pro* will divide the specified intensity range into the number of intervals you specify here, and assign a default color to each interval. The color intervals are displayed in the pseudo-color scale located along the left edge of the dialog box. Changing the number of divisions will divide the range into an equal number of intensities.

Using the **Upper** and **Lower Limit** controls, set the intensity scale to define the intensity range you want to colorize. If you want to colorize just a segment of low intensities, for example, set the scale to specify just that segment. If you want to pseudo-color a single intensity, set both limits to that intensity.

---

**Note:** When you set the range, the endpoint values are included in the range.

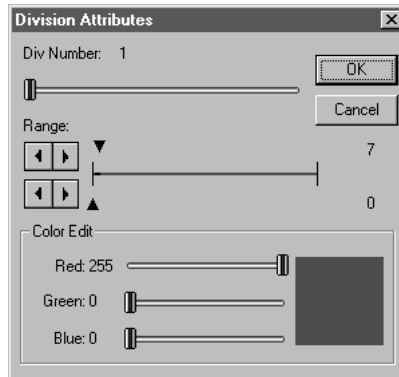
---

**Upper Limit:** Use the spin buttons or select and overwrite the current intensity value that is the highest one you want to colorize. If you have selected the number of divisions, the left color bars will reflect the change.

**Lower Limit:** Use the spin buttons or select and overwrite the current intensity value that is the lowest one you want to colorize. If you have selected the number of divisions, your image and the color bars at the left will reflect the change.

**Divisions:** Use the spin buttons or select and overwrite the current number of distinct colors to be pseudo-color. Your image and the color bars at the left respond to each change. You can specify up to 256 divisions.

**Edit:** Click this button to change a division's color or range. When you click **Edit**, the **Division Attributes** dialog box appears.



Within this box, select the number of the division you want to modify (in the **Div Number** field), then modify the interval width (using **Range** fields) and/or change the color assignment using the RGB sliders.

**Div Number:** This field indicates the number of the interval (on the pseudo-color scale) that you want to modify. You may move the slider or click on the bar itself at the relative point you want to activate. The color currently assigned to the division you select here, will be displayed in the swatch at the lower-right corner of the dialog box.

---

**TIP** You can access the **Division Attributes** options simply by double-clicking on the color bar in the **Pseudo-Color** window. This allows you to go directly to that color's division number.

---

**Range:** Use these fields to change the starting and ending values of the division associated with this color. Set the smaller value to specify the beginning of the range, and the larger value to the last value in the range (values are inclusive). The ranges of the divisions that adjoin the one you are editing will automatically be adjusted to compensate for your changes (e.g., if you changed Division #4's **Start** value from 64 to 62, Divisions #3's **End** value would be adjusted down to 61). If you extend a range by a large amount, the neighboring range(s) can be reduced to a single intensity value.

*Process:Pseudo-Color*

**Color Edit:** Use the **Red/Green/Blue** sliders in this group box to specify the color you want assigned to the specified division. A color is defined by entering the amount of each color component (RGB) it contains. The color you specify here will be displayed in the swatch at the lower-right corner of the dialog box, as you create it.

**Color Spread:** Within this group box, you may change the pseudo-color scale's predefined or custom color assignments.

**Blue to Red:** When *Image-Pro* sets the initial color values for your image, they are roughly in the order the colors appear in the spectrum. Click this button to array these colors with blue assigned to a lower value than red.

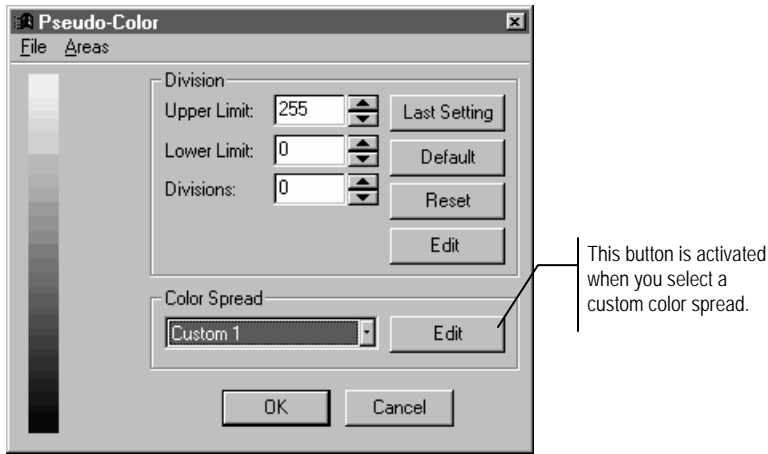
**Red to Blue:** When *Image-Pro* sets the initial color values for your image, they are roughly in the order the hues appear in the spectrum. Click this button to array these colors with red assigned to a lower value than blue.

**Red, Green, Blue, Cyan, Magenta, Yellow:** Selecting one of these colors will display the color values in your images as graduated shades of that particular color.

**Custom 1 - Custom 4:** You may define one to four custom color palettes for pseudo-color. To define a custom color, highlight the custom selection in the list box, and click on the **Edit** button.

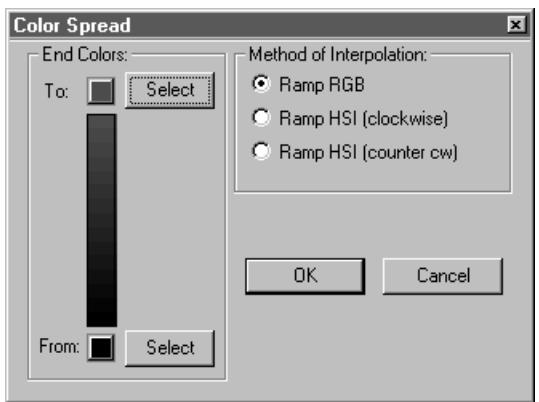
**Note:** When creating a custom color, you must use the Edit button. Double-clicking on the Color bar (above) will not save the adjusted color in your custom settings.



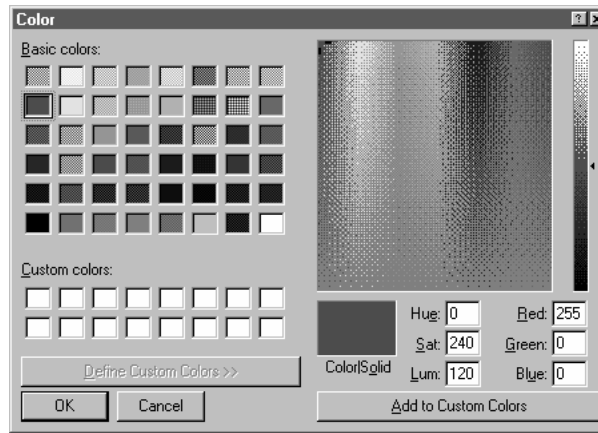


This button is activated when you select a custom color spread.

Clicking the **Color Spread Edit** button displays the **Color Spread** dialog box. This group box is enabled only when one of the four color spreads is selected:



**End Colors:** In this group box, select the colors for the top and bottom ends of your custom color range. Clicking on either **Select** button displays the **Color** dialog box:



**Basic colors:** Click on the appropriate color square to choose one of the basic colors for the end of your color palette.

**Custom colors:** Click on the **Define Custom Colors >>** button to create a new color. You can create a custom color by adjusting the Hue, Saturation, and Luminance values, and/or the Red, Green, and Blue color values. The 4-way cursor will appear on the color selection area. You can move this cursor around the color selection area, and the HSL and RGB values will change with it.

**Method of Interpolation:** In this group box select the method for displaying your custom colors:

**Ramp RGB:** Use this method to go directly across the Hue color wheel *from color to color* with no intermediate colors. For example, in gradations from blue to red.

**Ramp HSI (clockwise):** Use this method go around the Hue color wheel clockwise. For example, from blue to red going through shades magenta.

**Ramp HSI (counter-clockwise):** Use this method go around the Hue color wheel counter-clockwise. For example, from blue to red going through shades of yellow and green.

The **Color/Solid** block will show your custom color as the values change. When you are satisfied with your new color, click on **the Add to Custom Colors** button, and the new color will appear in the Custom Colors sampler.

When you have finished your color selections, click **OK**

**Reset:** Click this button to reset the image to its prior (monochrome) representation.

**Default:** Click this button to select the pseudo-color default settings. There will be 32 divisions. The pseudo-color default in *Image-Pro* is the **Bue to Red** color assignment.

**Last Setting:** Click this button to recall the pseudo-color settings as of the last time you saved them. When you click the **OK** button on the **Pseudo-Color** dialog box, you save the settings.

---

*Note:* Any changes you have made to your settings will be lost when you recall the **Last Setting**. If you want to keep them, use the **Save** command on the **Pseudo-Color** dialog box's **File** menu.

---

**OK:** Click the **OK** button if you want to close the **Pseudo-Color** dialog box and assign the pseudo-color palette to the active image.

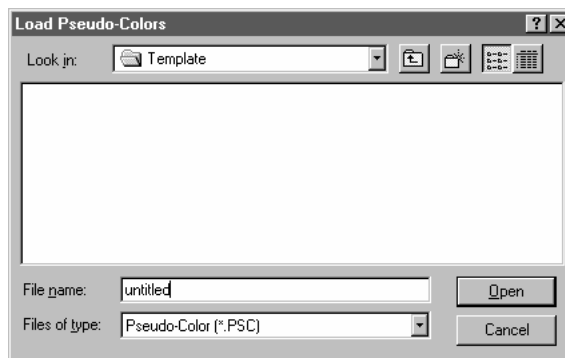
**Cancel:** Click the **Cancel** button if you want to close the **Pseudo-Color** dialog box without making changes to the colorization of your image.

## Pseudo-Color File Menu

Use the commands on the *Pseudo-Color : File* menu to load or save pseudo-color settings.

**Load Pseudo-Color:** Select this command to load a previously saved pseudo-color palette file (.PSC), and apply it to the active image. Pseudo-color palette files are created using the **Pseudo-Color Save** button (see below).

When you select the **Load** command, the **Load Pseudo-Colors** dialog box is issued:



Specify the name of the file that contains the pseudo-color palette you want to load, then click **OK**. The palette will be loaded into the **Pseudo-Color** dialog box. You may edit the palette using any of the **Pseudo-Color** tools described above. When you click **OK**, the palette will be assigned to the active image.

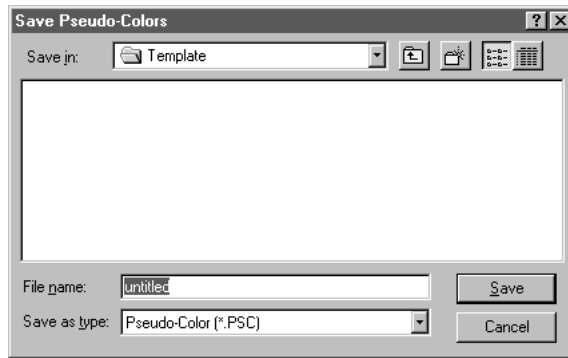
**Save Pseudo-Color:** Select this command to save the current pseudo-color palette to an *Image-Pro* pseudo-color file (a .PSC file). It is important to save a pseudo-color palette file if you want to use it with the active image at some future point. This is because only the most recent pseudo-color palette is stored automatically. If you do not store the pseudo-color palette, you may need to manually recreate it next time you work with the image. If the palette has been saved, it can simply be reloaded using the *Pseudo-Color Load* command (see above).

---

**Note:** The **Last Setting** palette is the one last used for any image. If you want to keep settings specific to the active image, use the Save command.

---

When you select the **Save** command, the **Save Pseudo-Colors** dialog box is issued:



Specify the name of the file to which you want the pseudo-color palette saved, then click **Save**.

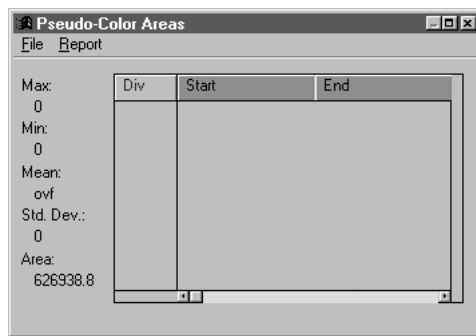
Process:Pseudo-Color

## Pseudo-Color Areas Menu

Use the command on the *Pseudo-Color : Report* menu to show the counts associated with pseudo-color settings.

**Areas:** Select this command to display a report of the number of pixels in each color representation (or the calibrated value if a spatial calibration is attached to the image) and to enable copying the report.

When you select the *Areas* command, the **Pseudo-Color Areas** dialog box is issued:



Use the *File* menu to save, copy, or link the report to other applications or to a file.

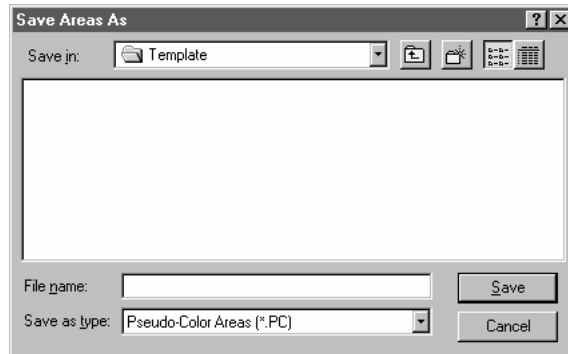
Use the *Report* menu to modify the amount of information given in the report, and you can select tabular or graphic representation.

## Pseudo-Color : Pseudo-Color Areas File Menu

The commands on the *Pseudo-Color :Areas:* window's *File* menu are used to perform operations that copy and save your pseudo-color percentage areas to other applications or to disk files.

**Save Areas As :** Select the *Save Areas As* command to store the current pseudo-color areas to disk. Pseudo-color areas are saved in an ASCII file containing the starting and ending values within each division, the area in pixels (or calibrated area if the image is attached with a spatial calibration), and the percentage of total area covered by the division.

When the *Save Areas As* command is selected, the **Save Areas As** dialog box is displayed.

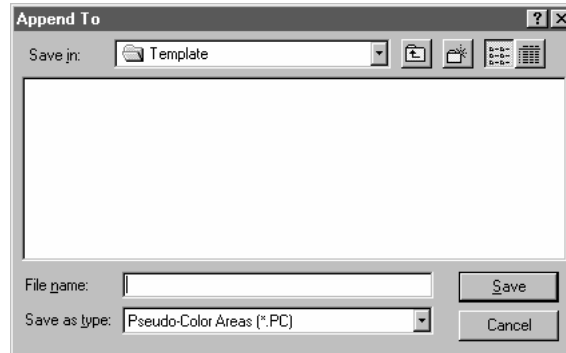


Enter the name of the file to which you want the pseudo-color areas stored, then click **OK**. Your pseudo-color areas will be written to the file.

**Append To :** Select the *Append To* command to store the current pseudo-color areas at the end of an existing pseudo-color file.

When the *Append To* command is selected, the **Append To** dialog box is displayed:

## Process:Pseudo-Color



Select the file to which you want the current areas appended, then click **Save**. The current pseudo-color areas will be written to the end of the specified file.

*Note -If you enter the name of a file that does not exist, Image-Pro will create a new file with that name.*

**Copy To Clipboard:** Select the *Copy To Clipboard* command to copy the current pseudo-color areas to the Windows Clipboard. From there, the areas can be pasted into any application that accepts text from the Clipboard.

**Save Options:** Select the *Save Options* command to change the style in which pseudo-color areas files are written.

Click once on your selection to choose (if checked) or omit (unchecked) it.

- ✓ **H**header: Select *Header* to save the heading information at the top of the pseudo-color areas file.
- ✓ **D**iv Number: Select *Div Number* to save the division number to the left of the pseudo-color areas.
- ✓ **S**tatistics: If *Statistics* is selected, the statistical information will be appended to the end of the areas file.

**Data Export:** Select the *Data Export* command to transfer pseudo-color areas to Microsoft's Excel spreadsheet. For additional information about DDE, see the *Dynamic Data Exchange* discussion.



## Pseudo-Color : Pseudo-Color Areas Report Menu

The commands on the *Pseudo-Color : Areas* window's *Report* menu are used to determine the report type and style.

**Table:** Select the *Table* command to display the current pseudo-color settings in tabular form. The information that is displayed is determined by the settings in *Table Style* . If *Table* is selected, *Graph* is deselected.

**Graph:** Select the *Graph* command to display the current pseudo-color settings in graphic form. Choose the form of the graph in *Graph Style* . If *Graph* is selected, *Table* is deselected.

**Table Style :** Select the *Table Style* command to select the properties to include in the table. When *Graph* is selected, this option has no effect.

When you select the *Table Style* command, you will see a menu from which you may choose the settings you want listed in the table.

You may choose any or all of these options. A check mark to the left indicates that the option is selected. Each option is described below.

- ◆ **Start of Div:** Select this option to display the starting pixel value in the division.
- ◆ **End of Div:** Select this option to display the ending pixel value in the division.
- ◆ **Absolute Area:** Select this option to display the total count of pixels whose value falls within the division (or the calibrated value if the image is spatially calibrated).
- ◆ **Relative Area(%):** Select this option to display as a percentage the number of pixels whose value falls within the division, divided by the number of pixels in the image or AOI.

**Graph Style :** Select the *Graph Style* command to display the graph as a line or bar graph. When *Table* is selected, this option has no effect.

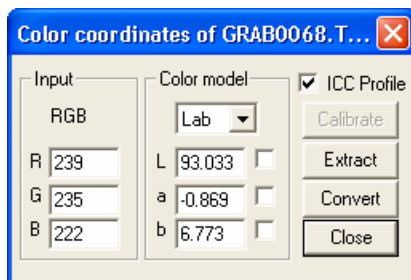
Process:CIE L\*A\*B Color

## CIE L\*A\*B Color

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The *CIE L\*A\*B* color module is designed for absolute color measurements in *CIE L\*A\*B* color coordinates. This module provides a simple and reliable color correction tool that can be used to correct image colors.

When you select *CIE L\*a\*b color* from the *Process* menu, you will see the following dialog:

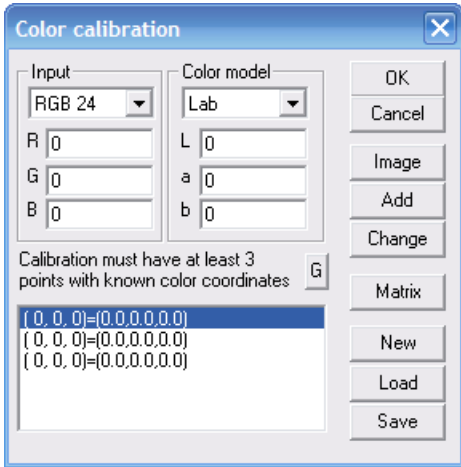


**ICC Profile** : The **ICC profile** checkbox appears in the upper right corner of the dialog. When you check this box, the **XYZ** and **L\*a\*b color** coordinates for the color space associated with the image will be reported. The **Calibrate** button will be disabled. When the **ICC Profile** is box is *not* checked, the active color conversion matrix, as defined in the **Calibrate** dialog, is used to calculate **XYZ** and **L\*a\*b** values.

**Color model**: Choose a color model from the drop-down list box. This feature supports XYZ, LAB, RGB, CMY, and YIQ. Note that you can measure LAB and XYZ color coordinates only when you XYZ or LAB calibration. If your calibration is in RGB, LAB and XYZ are not available.

**Input**: The information in this group box reflects information from the active image in the *Image-Pro* workspace.

**Calibrate**: Clicking the **Calibrate** button displays the following dialog:

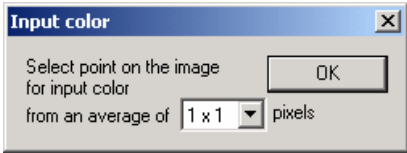


The current calibrations appear in the window at the bottom of this dialog.

**OK:** Click **OK** to accept the current calibrations and return to the previous dialog.

**Cancel:** Click **Cancel** to return to the previous dialog without making any calibration changes.

**Image:** Click the image button to place a new calibration marker on the current image. You will see the following dialog:



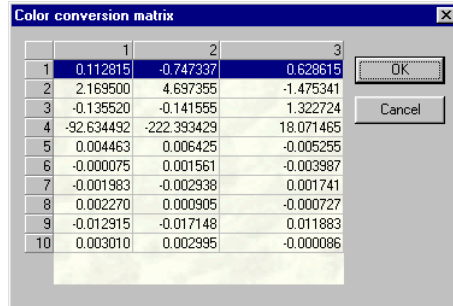
Use the eyedropper tool to select a point on the image for an input color calibration, then click **OK**.

**Add:** Clicking the **Add** button after selecting a new input calibration adds that calibration to the current list.

**Change:** Use your cursor to highlight one of the numbers in the calibration list. Click **Change** to substitute a new calibration for the selected one.

**Matrix:** Clicking the **Matrix** button displays the XYZ color conversion matrix, as shown here:

Process: CIE L\*A\*B Color



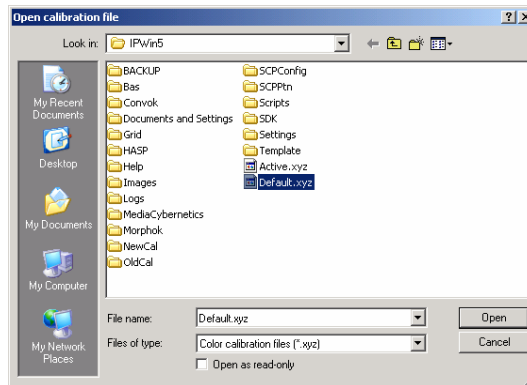
The number of rows in the matrix corresponds to the number of reference points used to create it. Each column represents a color channel. You can edit the cells in the matrix by clicking on the selected cell and entering a new number.

**OK:** Click **OK** to accept your changes and close the **Matrix** dialog.

**Cancel:** Click **Cancel** to close the **Matrix** dialog without making any changes.

**New:** Clicking the **New** button begins the process of creating a new color calibration by clearing any calibration points from the window on the **Calibration** dialog.

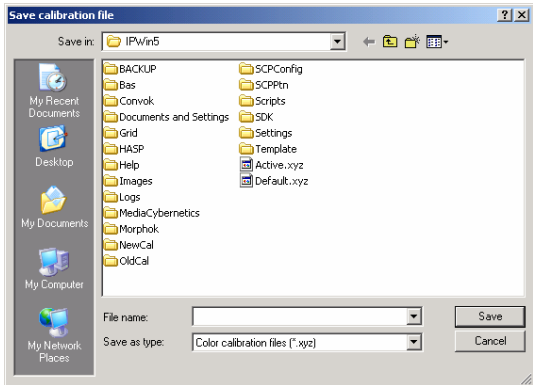
**Load:** Click this button to load existing color calibration settings from a file, as shown here:



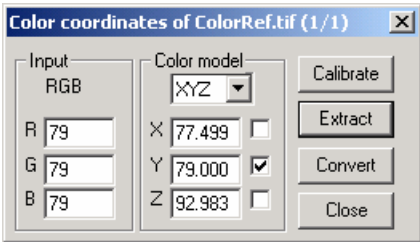
Color calibration settings files have the extension \* .xyz .

**Save:** Click this button to save your current calibration settings to a file with the extension \* .xyz , as shown here:

Process:CIE L\*A\*B Color



**Extract:** Click this button to extract a color channel from the active image. Check the box next to the channel you want to extract, as shown here:



An image with the extracted color channel will be placed in the *Image-Pro* workspace.

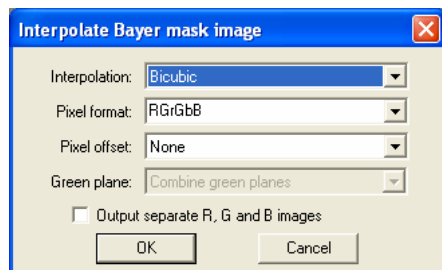
**Convert:** Click this button to convert the active image from the input color model to the one you've selected. The converted image will be placed in the *Image-Pro* workspace.

**Close:** Click this button to close the **Color coordinates** dialog.

## Bayer Interpolation

This feature, new in *Image-Pro Plus v. 7.0*, allows you to process grayscale *Bayer Mask Images* into color workspaces.

When you select *Bayer Interpolation* from the *Process* menu, you will see the following dialog:



**Interpolation Options:** Please choose one of the following from the drop-down list:

- None
- Bilinear
- Bicubic

**Pixel Format Options:** Select one of the following from the drop-down list. The **Pixel Format** options can be better visualized as follows:

R	Gr
Gb	B

RGrGbB

Gr	R	First row
B	Gb	Second row

GrRBGb

Gb	B
R	Gr

GbBRGr

B	Gb	First row
Gr	R	Second row

BGbGrR

*Process: Bayer Interpolation*

Since the interpretation of the pixels is location-dependent, when an image is acquired with a region of interest, the algorithm needs to know the offset of the top-left pixel from the normal pixel layout.

**Pixel Offset:** Choose one of the options from the drop-down list.

The **Pixel Offset** options include none, one horizontal, one vertical or one in each direction.

**Green Plane:** Since there are two green pixels, the **Green Plane** options are to combine the green planes or just use the Gr or Gb pixel planes. This **Green Plane** option is for use in Bayer mask camera QA/QC/R&D, so the option will only be supported when the interpolation type is set to pixel-replication.

**Output separate R, G and B images :** This option allows you to output 3 separate grayscale images. Most of the time, the user will want an RGB true-color image with the same per-sample bit depth as the original image (e.g. for a 12-bit Bayer mask image, the result will be 36-bit color). However, for some QA/QC purposes, it may be valuable to return the separate planes rather than combining them into a true-color image.

---

**Important note:** The available algorithm is designed to work with pixel data as it is being acquired. It therefore applies to the entire image and has no concept of Area Of Interest. Consequently, the algorithm will ignore any AOI on the image, but will copy the AOI to the new image(s) created.

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## Registration

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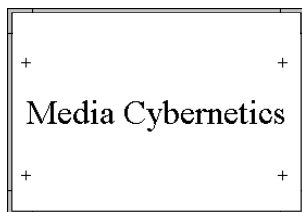
Use the *Registration* command to align one image with another by defining a set of “anchor points” common to both images. This command is often used to prepare images, acquired from different angles or distances, for comparative analysis. For example, if you needed to measure and compare vegetation growth of an area depicted in two aerial photographs taken several months apart from substantially different angles and / or altitudes, you would want to register one photograph to the other to ensure comparable measurements.

The *Registration* command requires two input images: a “reference” image and an “object” image. The reference image defines the standard perspective of the subject; the object image is the one that is to be spatially transformed to the reference's standard (i.e., the object image is warped to match the reference's anchor point coordinates). The result of the transformation is written to a new image window.

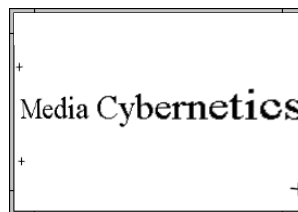
If you attempt to use the *Registration* command when you have only one image open, you will receive an error message:



The examples below show how a distorted image was corrected by registering it to a reference image.



*the "reference" image*



*the "object" image*





*the corrected result*

---

**Note:** The registration process merely warps the object image to the specified anchor points; there is no blending of the two images, nor is there any attempt to match similar features. The image data in the reference image are entirely ignored; only its anchor point coordinate information is used by the transformation process.

---

To register one image to another, follow the steps below:

- 1. Open the reference image and the object image** (the one to be warped).
- 2. Select the *Registration* command from the *Tools* menu.** The **Registration** dialog box will be presented, and a set of “anchor point” markers will be placed in each of the images.
- 3. Select the object image's name in the *Transform* list box.**
- 4. Select the reference image's name in the *Into* list box.**
- 5. Use your mouse to position the anchor point markers in the registration and object images.** An anchor point identifies a point of correspondence between the two images. In the example above, an anchor point was established over each of the registration cross hairs in the reference and object images. In an aerial photograph you might tie the images together with landmarks (e.g. buildings or geologic formations) or intersecting features (e.g., rivers, roads, fences). When positioning your anchor points, keep in mind the following:
  - ◆ Position the anchor points as far apart as possible. If anchor points are too close together, your registration request will be ignored.
  - ◆ Position the anchor points as accurately as possible. Use the **Zoom** tool to magnify your images so that the markers can be positioned *exactly* over the designated point (you can use the **Pan To Point** button to move quickly from one point to

## Process:Registration

another when you are zoomed-in on the image. See *Pan To Point* below).

- ◆ Be sure the anchor point numbers correspond between the two images. If, for example, anchor point #1 marks the corner of Building A in the reference image, anchor point #1 in the object image must also mark the corner of Building A.

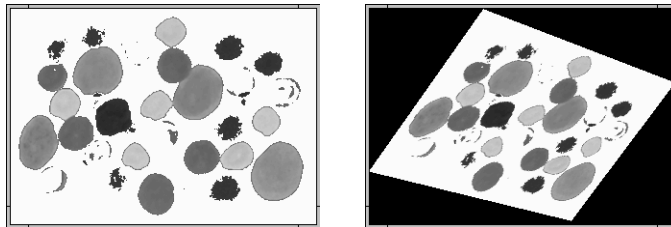
---

**Note:** The order of your anchor points is not significant (i.e., anchor point #1 does not have to be near #2, nor does it have to mark the upper-left corner of a quadrilateral). Anchor point markers can appear in any order you'd like; just be sure that the #'s correspond between the two images.

---

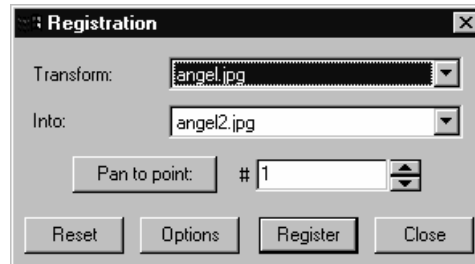
- ◆ You must define at least 4 anchor points (you may include more; see **Options**, below). When more than four are defined, *Image-Pro* reduces your set to 4 points using a least-squares method before performing the transformation.
- 6. Click the Register button.** The object image is spatially transformed by matching its anchor points to the anchor points in the reference image. The result is placed in a new image window.

If the image has been transformed to a smaller or nonrectangular shape, the unused image space will be padded with black pixels, as shown in the example below.



The object image on the left was skewed using the registration command (the reference image is not shown here). Note that in the result (on the right) the unused pixels are set to black.

When you select the *Registration* command, the **Registration** dialog box will be presented:



**Transform:** In this list box, select the name of the object image (this image must already be open). The object image is the one that will be spatially transformed.

**Into:** In this list box, select the name of the reference image (this image must already be open). The reference image is the one that defines the perspective to which the object image will be transformed.

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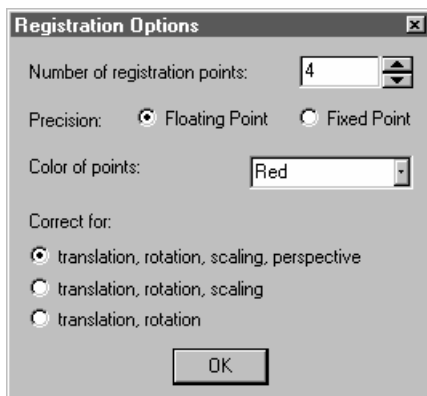
*Note:* Generally the *Registration* command is used to align two images of the same subject; however, it can also be used to deliberately distort an image for aesthetic purposes (to change perspective, or to create an unusual effect, as was done in the previous example). When used for this purpose, the reference image can be any image at all (it need not be a different "view" of the subject). You merely need a workspace large enough to position the anchor points required to produce the effect you want. In the example above, the reference image (not shown) was simply an empty (all white) image window of the same size as the object.

---

**Pan to point:** Click this button to display the specified anchor point marker. This feature is useful for quickly moving to a specific anchor point when you are zoomed-in on your image (it is a quick alternative to scrolling or zooming-out and zooming back in again). To move to an anchor point, first select the anchor point number in the “#” list box, then click **Pan to point**.

**Reset:** Clicking the **Reset** button will reset all the registration points to their default locations on the two registration images.

**Options:** Click this button to set the options associated with the *Register* command.



**Number of points:** Select the number of anchor points that you want to define in each image. You must define at least 4, and no more than 10, points. If you use more than four anchor points, *Image-Pro* will fit your set to just 4 points (using a least-squares method) before it performs the transformation.

**Precision:** Select the precision, **Floating** or **Fixed**, in which you want the registration process performed. This setting determines the level of accuracy that will be maintained by the transformation, as described below.

**Floating Point:** Select this option if your application demands a very high level of data accuracy (if you are registering an image for measurement purposes, you should use **Floating Point** mode). Floating-point transformations are time consuming when performed on a system without a math co-processor — if your work involves frequent transforms of this type, you will want to have one installed.

**Fixed Point:** Select this option if your application does not demand a high level of data accuracy (if you are registering an image for aesthetic purposes, **Fixed Point** mode is appropriate). A fixed-point transform will be significantly faster than a floating-point transform if your system is not equipped with a math co-processor.

**Color of Points:** Select the color in which you want the anchor point markers displayed.

*Process:Registration*

**Correct for:** Select the combination of transformations that you want performed. The placement of the anchor points is a critical factor in determining the outcome. For this reason, a small displacement of an anchor point may introduce a skew or a scale factor that you don't want. In that instance you may choose not to perform those transformations.

Select from the following combinations:

**translation, rotation, scaling, perspective:** Select this option to perform all transformations.

**translation, rotation, scaling:** Select this option if you know there is no skewing to correct for.

**translation, rotation:** Select this option if you know there is no scaling or skewing to correct for.

## FFT

---

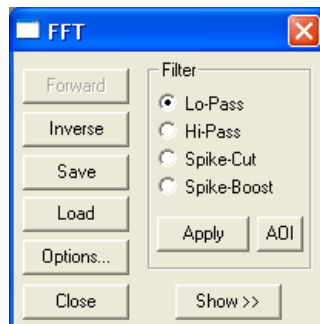
Use the *FFT* command to edit a grayscale image in its spectral form. FFT editing is useful for removing periodic noise from an image. This type of noise can be very difficult to remove using spatial filters, but tends to generate isolated energy spikes that are easily de-emphasized or removed in the FFT spectrum.

The following general steps are required to perform an FFT transformation:

- 1. Selecting the area of the active image that will be transformed.** Define an AOI if you want *Image-Pro* to transform just a certain part of your image. If a nonrectangular AOI is defined, the pixels within its bounding-rectangle will be recognized as the AOI. If no AOI is active, the entire image will be transformed.
- 2. Performing an *FFT : Forward* transformation** using the **Forward** button to display the image as an energy spectrum.
- 3. Editing the spectrum** to enhance, attenuate or eliminate certain characteristics. This is done by drawing an AOI around a particular group of frequencies to isolate them from the rest of the spectrum, then filtering the spectrum using the **Filter** button.
- 4. Performing an *FFT : Inverse* transformation** to write the edited data back to its original window, or to a new window.

Additionally, the *FFT* command lets you save FFT results to a file. You might want to do this to use the data as input to an external program. You can also reload the saved data back into *Image-Pro*.

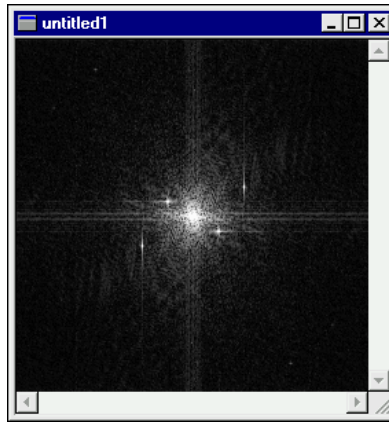
When the *FFT* command is selected, the **FFT** dialog box is presented.



The options available to you in the **FFT** dialog box are determined by whether the active window contains an image, or an FFT spectrum.

This dependency is discussed in each of the FFT dialog-box components below.

**Forward:** Click this button to generate a transformation of the active AOI or image. The **Forward** button will produce a new window containing a representation of the FFT data for the image. The FFT data will be represented in the form that you have specified in the FFT **Options** dialog box (see **Options** below).

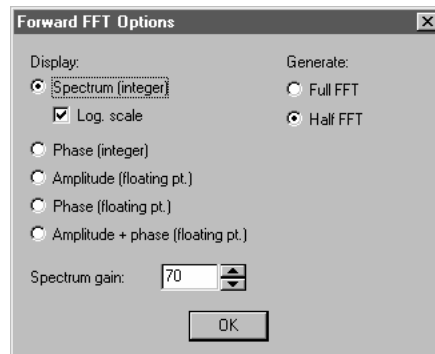


If an AOI is active when the **Forward** button is clicked, just the pixels within the AOI will be transformed.

Before clicking the **Forward** button, you should click the **Options** button to make sure your transformation options are set as you would like. See **Options** below.

The **Forward** button will only be enabled when an image is in the active window. If the FFT window is active, this button will be dimmed.

**Forward FFT Options:** Click this button to select the options for the **Forward** transformation. The **Forward FFT Options** dialog box will be displayed if the **Forward** button is active:



---

**Note:** If the **Inverse/Filter** dialog box is displayed when you press the **Options** dialog box, it means that the active window contains FFT data, not an image. To get the **Forward FFT Options** dialog box, simply close the **Inverse/Filter** dialog box, and click on your image window. Then select **Options** again.

---

The **Forward FFT Options** dialog box allows you to specify the way in which you want the data represented, and how much of it you want to generate.

**Spectrum:** Select this **Display** option if you want the FFT data represented as the traditional “cloud of points” spectrum.

**Log Scale:** Check this box if you want to display the FFT spectrum as a logarithmic scale.

**Phase:** Select this **Display** option if you want to display the phase of the FFT. You may display it as an integer scale or as floating point (see note ).

**Amplitude:** Select this display option if you want the FFT data displayed in varying degrees of brightness (amplitude).

---

**Note:** The difference between floating point and integer display options is that in the case of floating point options, the FFT data is directly available as images (spectrum and phase images), whereas in the integer case the FFT data is kept hidden and separate from the displayed spectrum and phase. The only way to process the



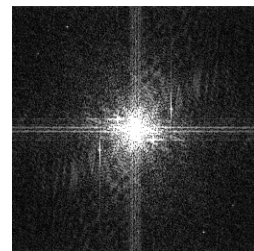
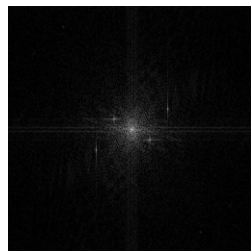
FFT data in the integer case is via the built-in lopes, hi-pass, spike cut and boost options. In the floating point case, since the FFT data is the image itself, you can apply any type of processing using any module in Image-Pro. In the floating point case we need to display the raw FFT data and therefore we cannot display the FFT using a log scale.

---

**Half FFT:** Select this **Generate** option if you want to create just half of the FFT data. Because an FFT data set is symmetrical around its “zero frequency factor,” it is not always necessary to create the full set of data, since the missing half may easily be calculated from the half that is generated. If you do not plan to export your FFT data, use this option instead of **Full FFT** as **Full FFT** generations take longer to produce.

**Full FFT:** Select this **Generate** option if you want to create the full set of FFT data. You might select this option if you want to export the FFT data to an external program that is not capable of calculating the missing half of an FFT data set generated with the **Half FFT** option.

**Spectrum Gain:** You can select a number between 1 and 200 in order to provide the best visual representation of the spectrum for your processing. The image on the left below was processed with a **Spectrum Gain** of 30. The one on the right, a **Spectrum Gain** of 150.



**Filter:** Use the buttons in this box to edit the data in the FFT window. The FFT spectrum is edited by:

1. **Selecting an FFT *Filtering* option** in the **Filter** group box (see filtering descriptions below).
2. **Defining an AOI that isolates the frequencies** you want to have addressed by the selected filter. Only an elliptical or rectangular AOI can be used here - irregular AOIs will not work.



**3. Clicking *Filter* to initiate the filtering operation.** Before clicking the **Filter** button to perform the specified filtering operations, you should click the **Options** button to make sure your filtering options are set as you would like. See **Options** below.

**Low Pass:** Select this filtering option if you want to eliminate or attenuate the frequencies above a specified range. When you select the **Low Pass** option, you must define an AOI around the center of the spectrum. Adjust the AOI so that it encompasses only the frequencies you want to remain in the image. Frequencies outside of the AOI will be eliminated if the **Rectangle** filtering option is selected, or attenuated if the **Hanning** filtering option is selected.

Low Pass frequency editing has the same effect as applying a Low Pass spatial filter in that it softens the image. The advantage of using the **Low Pass** frequency filter over the **Low Pass** spatial filter is its flexibility and ease of use. Experimenting with the frequency spectrum is usually easier to do than developing the exact spatial kernel configuration to produce the desired effect.

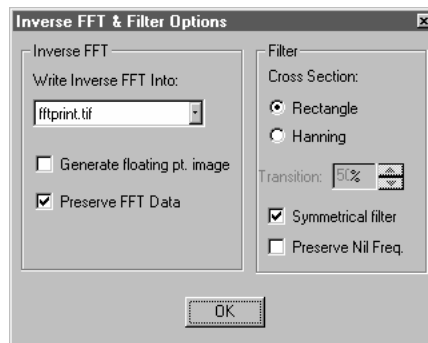
**Hi Pass:** Select this filtering option if you want to eliminate or attenuate the frequencies below a specified range. When you select the **Hi Pass** option, you must define an AOI around the center of the spectrum. Adjust the AOI so that it encompasses the frequencies you want to remove from the image. Frequencies inside of the AOI will be eliminated if the **Rectangle** filtering option is selected, or attenuated if the **Hanning** filtering option is selected.

Hi Pass frequency editing has the same effect as applying a Hi Pass spatial filter in that it produces an image with only high contrast elements. The advantage of using the **Hi Pass** frequency filter over the **Hi Pass** spatial filter is its flexibility and ease-of-use. Experimenting with the frequency spectrum is usually easier to do than finding the exact spatial kernel configuration to produce the desired effect.

**Spike Cut:** Select this option if you want to eliminate or attenuate just a certain frequency, or group of frequencies, in an image. When you select the **Spike Cut** option, you must define an AOI around the group of frequencies you want to cut. Adjust the AOI so that it encompasses just the frequencies you want to remove from the image. Frequencies inside of the AOI will be eliminated if the **Rectangle** filtering option is selected, or attenuated if the **Hanning** filtering option is selected.

**Spike Boost:** Select this option if you want to accentuate a certain frequency, or group of frequencies, in an image. When you select the **Spike Boost** option, you must define an AOI around the group of frequencies you want to enhance. Adjust the AOI so it surrounds just those frequencies that you want to boost. The energy of the frequencies inside the AOI will be increased up to twice their value.

**Inverse FFT Options :** Click this button to select the options for the **Inverse FFT** and **Filter** operations. The following **Inverse/Filtering** dialog box will be displayed.



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**Note:** If the **Forward FFT Options** dialog box is displayed when you press the **Options** dialog box, it means that the active window contains an image, not FFT data. To get the **Inverse/Filter Options**

dialog box, simply close the **Forward FFT Options** dialog box, and click on your FFT window. Then select **Options** again.

---

**Filter:** Within this group box, select the type of filter you want to use, the transitions, whether or not you want spikes to be cut or boosted symmetrically, and whether or not you want to preserve the average intensity of the image.

**Rectangle:** Select this option if you want to set the specified frequencies to null. If the **Hi Pass** or **Spike Cut** frequency filter has been selected, the **Rectangle** option will set all frequencies *within* the masking-AOI to null. If the **Low-Pass** filter has been selected, the **Rectangle** option will set all frequencies *outside* the masking-AOI to null.

---

**Note:** Avoid using the **Rectangle** option with AOI masks that are tightly defined around the center of the spectrum, as this will introduce an artifact known as ringing.

---

**Hanning:** Select this option if you want to attenuate the specified frequencies, rather than setting them to null. Attenuation is performed at the rate specified in the **Transition** field. If the **HiPass** or **Spike Cut** frequency filter has been selected, the **Hanning** option will attenuate all frequencies *within* the masking-AOI. If the **Low-Pass** filter has been selected, the **Hanning** option will attenuate all frequencies *outside* the masking-AOI.

**Transition:**  % Set this value to specify the rate at which the specified frequencies will be attenuated. The closer the **Transition** value is to 0, the more the result will resemble the **Rectangle** option (setting the specified frequencies to null).

**Symmetrical:** Check the **Symmetrical** check box if you want the **Spike Cut** and **Spike Boost** filters to affect the specified frequencies in both halves of the FFT data set. When the **Symmetrical** option is set, you will see that the corresponding group of frequencies in the opposite half of the spectrum are also cut/boosted when you perform a **Spike** filtering operation. If you do not want this to occur, disable the **Symmetrical** option.

---

**Note:** Disabling the **Symmetrical** option affects only full FFT data sets. When only half of an FFT data set has been generated, spike filtering is always performed symmetrically. This happens

because the missing half is automatically derived from the half that was initially generated. The two halves, by definition, will mirror each other.

---

**Preserve Nil Freq:** Check the **Preserve Nil Freq** check box if you want to preserve the average intensity of the image when using a **HiPass** frequency filter. This check box has no effect with **LoPass**, **Spike-Cut**, or **Spike-Boost** filters.

**Inverse FFT:** Within this group box, select the image into which you want the inverted image to be placed and whether or not the FFT data should be kept after inversion is complete.

**Write Inverse FFT into:** In this list box, select the window you want the inverse FFT data written to when the **Inverse** button is clicked. FFT data can be inversely transformed into any open image window, or to a new window.

This button has no effect on the filtering process; it is used by the **Inverse** operation only.

**Preserve Data:** Check this box if you want to maintain the FFT data after an **Inverse** operation is performed. If this option is disabled, the FFT data will be cleared from the FFT window when the **Inverse** operation is complete. Please note that this will not close the FFT window; the spectrum will still exist as an image; however, the data from which it was generated will no longer exist.

**OK:** Click **OK** when the options for inverting the image are set to your satisfaction.

**Inverse:** Click this button to generate an inverse transformation of the active FFT window. The **Inverse** button will write the FFT data back to its spatial form. Data will be written to the window specified in the **Write Inverse FFT into** list box in the **Options** dialog box. An FFT can be inversely transformed into any open image window, or into a new window.

The inverted data will be written back to the AOI if the original window is specified as the destination. If a different window has been specified, the data will be written to the equivalent position within the image (it will match the position of the upper-left corner), or into an AOI, if one is active.

## Process:FFT

The FFT window will remain open after an **Inverse** operation. If you have checked **Preserve Data** in the **Options** dialog box, the FFT data will remain, too. This lets you continue to edit the frequency spectrum until you are satisfied with the results.

The **Inverse** button will only be active when an FFT is in the active window. If an image window is active, this button will be dim.

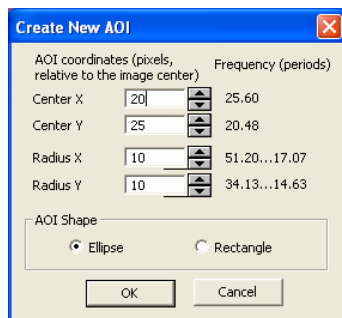
**Load:** Click this button to load FFT data from a previously saved FFT data file (FFT data files are created using the *FFT* command's **Save** button -- see below). All other functions perform as described in *Open and Load Dialog Boxes*.

**Save:** Click this button to save FFT data from the active FFT window to an FFT data file. FFT data are saved to a binary file containing the coefficients of the FFT array. The saved data can be used as input to an external program, or can be reloaded into *Image-Pro* at a later time using the *FFT* command's **Load** button.

You may supply an extension or let *Image-Pro* automatically append an extension of .FFT to the file name you provide. If you assign your own extension, you must give the full name with that extension to *Image-Pro's* *Load* routine and use the *FFT* file type. Using an .FFT extension assures that *Image-Pro* will identify and list your file when you are ready to *Load* it. All other functions perform as described in *Save Dialog Boxes*.

**Apply:** Click this button to apply the FFT to the active image.

**AOI:** Clicking this button opens the AOI dialog, which allows you to set a center, radius, and type of AOI that will be used for the FFT process.



*Process:FFT*

**Center X, Center Y, Radius X, Radius Y:** These coordinates are in pixels, and relative to the center of the FFT image.

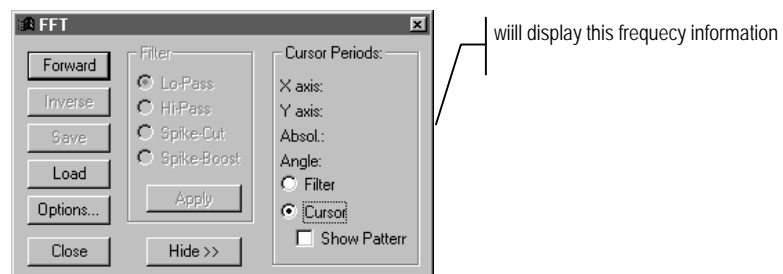
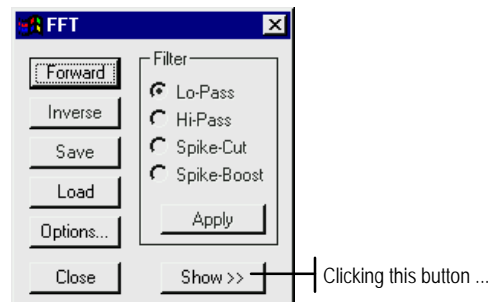
**AOI Shape:** Indicate which shape your AOI should take by clicking the radio button next to **Ellipse** or **Rectangle**.

**OK:** Clicking **OK** sets the AOI on the FFT image.

**Cancel:** Click **Cancel** to return to the main FFT dialog.

**Show >>:** Click this button to show frequency period, distance and angle values for the current cursor position or for a group of selected frequencies.

When you click the **Show** button, the **FFT** dialog box is expanded to display these values:



**Cursor Periods:** After you have performed the **Forward FFT** on your image, these options become available. When you have the **Cursor** option selected, the x-axis, y-axis, absolute and angle values are displayed in response to the placement of your cursor on the frequency image. Moving the cursor around changes the values that are displayed.

Process:FFT

**Show Pattern:** Check the **Show Pattern** check box if you want *Image-Pro* to display the frequency pattern generated by your filter on the source image. In order to see this effect, be sure your source image is visible on your desktop, then in the inverted image create an AOI that surrounds the area that you want to alter. The source image reflects where the changes will occur. **Show Pattern** works only when the **Cursor** option is selected.

**Filter:** Click the **Filter** button at the bottom of the period group box when you want to display information relating to the currently selected FFT filter.

**Cursor:** Click the **Cursor** button when you want frequency information associated with the current cursor position.

**Hide >>:** Click the **Hide** button to suppress the display of frequency information.

### **Working with FFT Data Files**

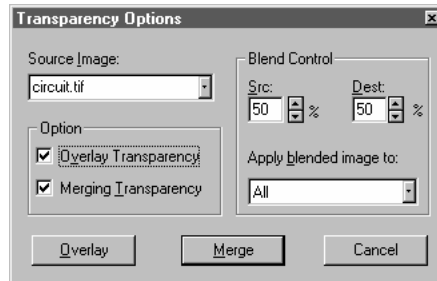
If you plan to develop programs that read *Image-Pro* FFT files, you may want to consult the source in *Appendix C: Source Code*.



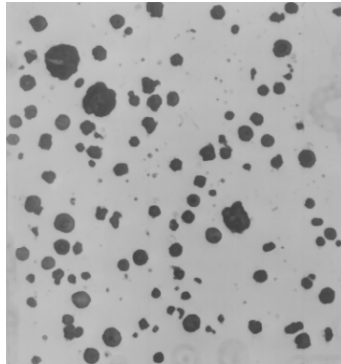
## Image Overlay

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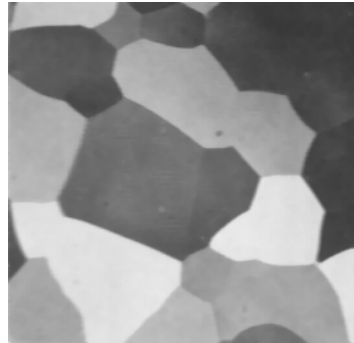
Use the *Image Overlay* command to combine two images with varying degrees of transparency. It is similar to the *Paste Options* command. Selecting *Image Overlay* displays the **Transparency Options** dialog:



**Source Image:** The source image is the image with the characteristics you want to copy. The target or destination image is the active image.



*Source Image*

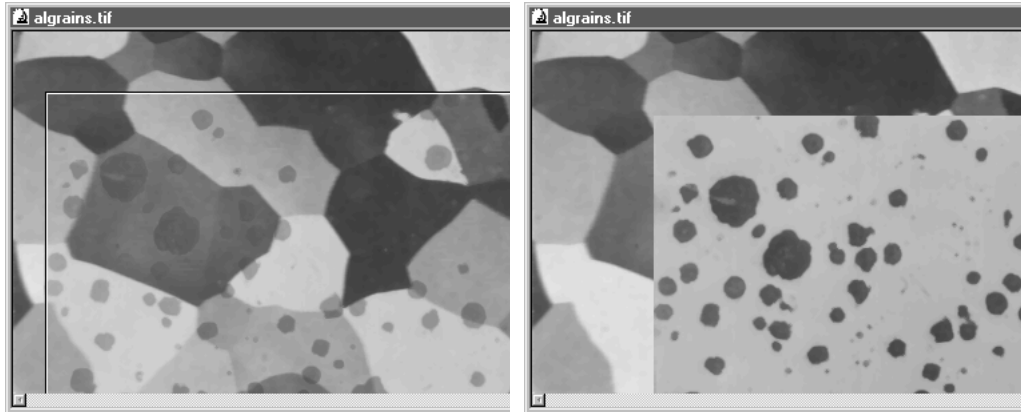


*Destination Image*

*Process:Image Overlay*

**Options:** The check boxes in this group indicate how the two images will be combined.

**Overlay Transparency** determines how much of the destination image will be visible through the overlaid image. Turning this check box off means that that the overlaid portion will be opaque when placed onto the target image.

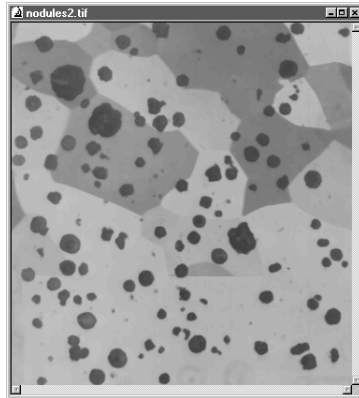


*Image with Overlay Transparency On*

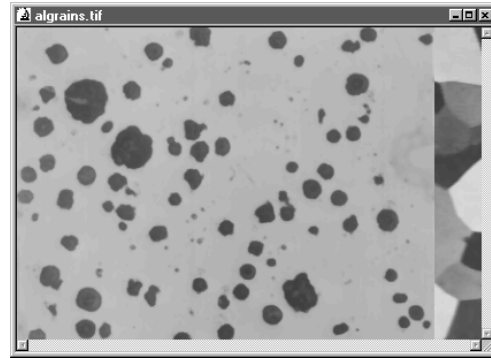
*Image with Overlay Transparency Off*

**Overlay:** Click this button to paste one image or AOI onto another temporarily. You can move the overlaid area around using the cross-shaped cursor. When you have the overlaid area positioned where you want it, click the right mouse button to merge the two images.

**Merging Transparency** determines how much of the destination image will be visible through the overlaid image when the two images are merged.



*Image with Merge Transparency On*



*Image with Merge Transparency Off*

**Merge:** Click this button to combine the two images (or AOI). This is identical to clicking the right mouse button after you have overlaid two images.

**Blend Control:** Use the options in this group box to control the ratio at which Clipboard data is blended with the destination image. These values are only utilized when the **Overlay Transparency** or **Merging Transparency** options are set (see above).

**Src:** Select the percentage at which merged data will be applied to the image. The larger the number, the heavier the merged data is applied (e.g., if 100% is set, only the pasted merged data will be visible in the result). This value is linked to the **Dest** value, such that when **Src** is changed the necessary change is automatically made to **Dest** to maintain a sum of 100%.

**Dest:** Select the percentage of the destination image allowed to “show through” the applied merged data. The smaller the number, the less the original image will remain visible (e.g., if 0% is set, only the Merged data will be visible in the result). This value is linked to the **Src** value, such that when **Dest** is changed the necessary change is automatically made to **Src** to maintain a sum of 100%.

*Process:Image Overlay*

**Apply Blended Image To:** Specify the intensity values that are to be affected by the blend. Select from the following:

- ◆ **All:** The merged data will be applied, in the specified blend ratio, to all pixels in the paste area, regardless of their intensity.
- ◆ **On Lighter Only:** The merged pixels will be pasted (in the specified blend ratio) only in places where the destination pixel is lighter than the corresponding merged pixel.
- ◆ **On Darker Only:** The merged pixels will be pasted (in the specified blend ratio) only in places where the destination pixel is darker than the corresponding merged pixel.

## Grid Mask

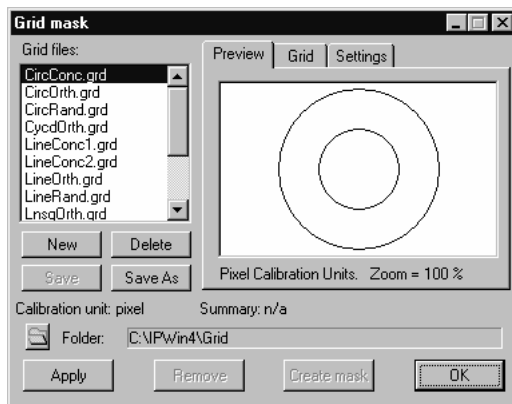
---

The *Grid Mask* tool allows you to define and create precise grids for intercept counting in either a manual (*Manual Tag*) or semi-automated (*Image Operations*) fashion.

This tool allows you to create and save a grid of your own design, including such information as length of the grid lines or number of points in the grid. You may also define the following type of grids: point, lines (horizontal or vertical or both), random lines, line segments (horizontal or vertical or both), circles, and cycloids. In addition, you may set the X and Y intervals for the line grids, spacing for point grids and combine multiple point grids in one grid overlay.

The *Grid Mask* tool will generate an overlay on the image for viewing purposes, although you also have the option to create a new image mask of the grid. The grid is not attached to the image, but stored in a separate file (\*.grd).

When you select the *Grid Mask* tool, you will see the following tabbed dialog:



The following selections are common to all the *Grid Mask* pages:

**Grid Files:** The **Grid Files** list box lists the saved grid files (\*.grd) in the default folder that are available to be reloaded and used.

**New:** Click the **New** button to create a new “untitled” grid file.

**Delete:** Click the **Delete** button to remove the selected grid file.

**Save:** Click the **Save** button to save or update the selected grid file.

## Process:Grid Mask

**Save As:** Click the **Save As** button to save the current grid file under another name.

The **Calibration/Summary** area provides a brief description of your calibration units and grid specifications.

**Default Folder:** This area displays the path and name of the folder where your grid files are stored. Click the folder icon to select a different folder.

**Apply:** Click this button to display the grid overlaid on your image.

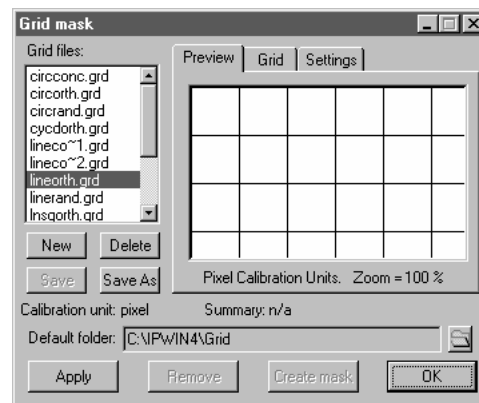
**Remove:** Click this button to erase the grid overlay from the image.

**Create Mask:** Click this button to create a separate black and white image containing the grid mask overlay.

**OK:** Click this button when you are finished with your grid. All the grid mask features on the image will be removed.

## Preview

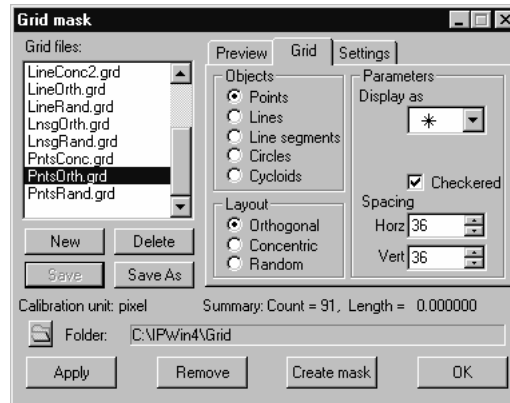
The **Preview** area lets you see what your grid looks like before applying it to the image.



Use the selections on the **Grid** page to change the format and display of your grid.

## Grid

Grid settings define the appearance of the overlay on the image.



**Objects:** Click one of the radio buttons to indicate what type of grid symbols you want to use. Your choices are:

- ◆ Points
- ◆ Lines
- ◆ Line Segments
- ◆ Circles
- ◆ Cycloids.

**Layout:** Click one of the radio buttons to indicate the placement of the objects on the image to create the grid. You have the following choices:

**Orthogonal:** The objects on the grid are displayed in a regular pattern of horizontal rows and vertical columns.

**Concentric:** The objects on the grid are displayed in a regular pattern of concentric circles.

**Random:** The objects on the grid are displayed in a random arrangement. Selecting **Random** allows you to choose a **fixed seed** parameter, so that the same random pattern can be repeated.

**Parameters:** The information and selections in this group box will change, depending on the type of objects you have selected. Your choices are:

**Display as:** When you have selected *points* as the object, this list box offers a selection of symbols to mark the objects on the grid.

**Checked:** Click this box to create a “checkerboard” pattern with the objects. You can apply this to points, line segments, circles, and cycloids.

**Spacing:** Use the spin buttons to indicate the horizontal and vertical spacing of the objects on the grid, using the measurement units selected on the **Settings** page. This selection appears when you have chosen the **Orthogonal** layout. **Points, lines, line segments,** and **circles** allow you to select both horizontal and vertical spacing. **Cycloids** let you select vertical spacing only.

**Length: Line Segments** lets you indicate the length of a line segment in percent of measurement units.

**Radius: Circles** and **Cycloids** lets you indicate the radius of the objects.

**Radial Spacing:** This parameter lets you indicate the distance between objects when you have selected **Concentric Circles** or **Concentric Points**.

**Segments:** This parameter lets you indicate the number of objects when you have selected **Concentric Points**.

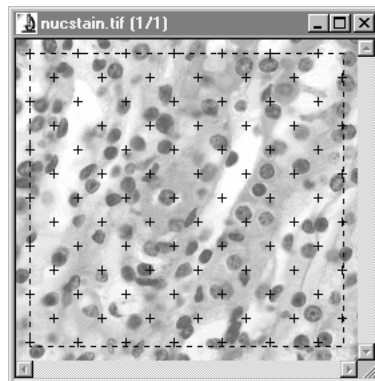
**Count:** This parameter lets you indicate the number of objects when you have selected **Random** objects.

---

*Note:* The parameter and spacing selections may change depending on the type of object you have selected.

---

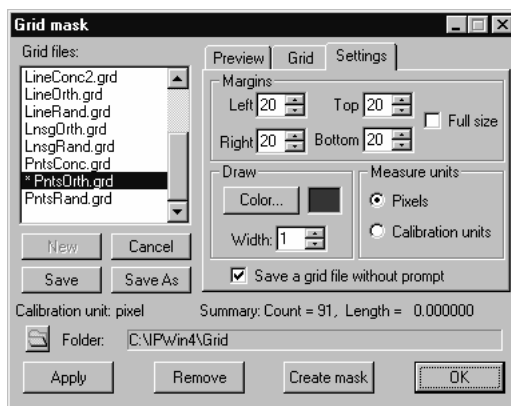
The example grid below displays the points marked as +’s in a checkerboard pattern spaced 20 pixels apart.





## Settings

Options on the **Settings** page help you to define the size of your overlaid grid.



**Margins:** The **Margins** group box lets you define the grid by creating margins within the image. Enter a number in each box, or check the **Full Size** box to cover the entire image with the grid.

**Draw:** Click the **Color** button in this group box to assign a color to the grid.

**Width:** These spin buttons let you determine the thickness of the lines used to draw the grid.

**Measure Units:** Click the appropriate radio button to use either **Pixels** or the **Calibration units** taken from the image (microns, for example) to define the grid spacing and margins in the appropriate units.

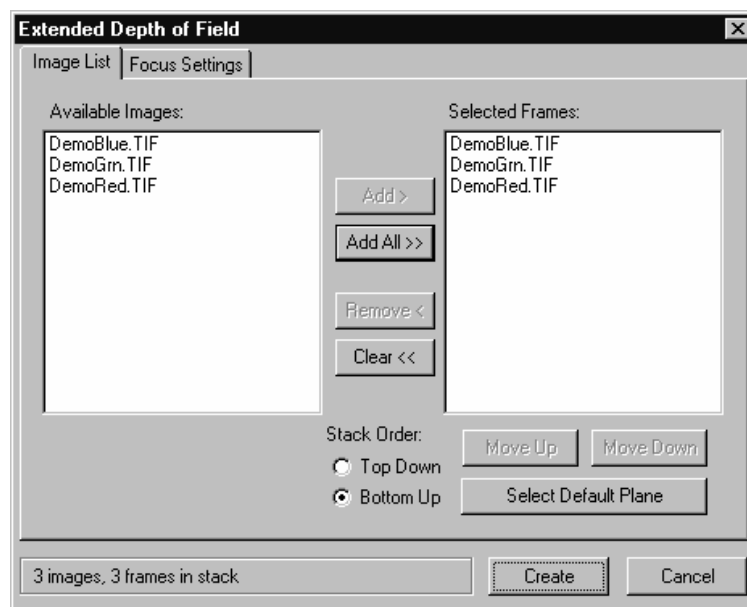
**Save Grid File without Prompt:** Check this box to automatically save your grid settings file without *Image-Pro* prompting you to do so.

## Extended Depth of Field

---

The *Extended Depth of Field* feature comprises the multi-plane focus feature, along with a new contrast detection algorithm and an option to return a topographic map of the image. This feature allows you to combine individual images or sequences into a single, in-focus, composite image.

When you choose this option from the *Process* menu, you will see the dialog below:



### Image List Tab

The **Available Images** contains a list of all the images or sequences that could be used to create a best-focus image. Any number of single-frame images or image sequences can be moved from the **Available Images** list to the **Selected Images** list. Use your cursor to highlight the name of the image or sequence that you want to move into the **Selected Frames** area.

Available images that are sequences will indicate how many of the frames are selected for the operation. If **Apply to Sequence** (on the

**Sequence** toolbar) is not selected, only the active frame will be used. This indicates that **Apply to Sequence** is not active. When **Apply to Sequence** is active, the length of the active portion of the sequence will be indicated.

After the first image is selected, the **Available Images** list will be filtered to list only images that are the same type and size as the selected image (no conversion or resizing is supported).

**Add:** Click this button to move the highlighted image or sequence from **Available Images** into **Selected Frames**. The **Selected Frames** are used to create the image stack. **Add** will add frames from any images selected in the **Available Images** list to the **Selected Frames** list, and is enabled whenever there are one or more images selected. When an image with multiple frames is added, the **Selected Frames** list will indicate the range of frames that will be used, e.g. **Heart.seq (5-25)** .

**Add All:** Click this button to move all the images or sequences from **Available Images** into **Selected Frames**. **Add All** is always enabled, and will add all images from the **Available Images** list to the **Selected Frames** list. This feature does not check to see if there are duplicate images, so if there are already images in the **Selected Frames** list, the whole list from **Available Images** will be added to them.

**Remove:** Click this button to move the highlighted image or sequence from **Selected Frames** back into the **Available Images** list. **Remove** will remove any images selected in the **Selected Frames** list, and is enabled whenever there are one or more images selected.

**Clear:** Click this button to erase the images or sequences from the **Selected Frames** list. **Clear** is always enabled and will remove all images from the **Selected Frames** list.

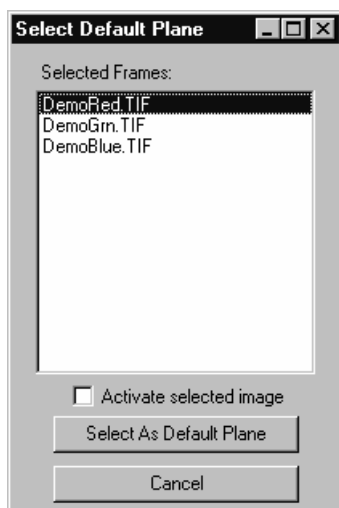
**Stack Order:** The order of the images in the **Selected Images**, in combination with the **Stack Order** controls, list will determine the order of the stack.

**Top Down:** If you click this button, the first image at the top of the list will be the first image in the stack and the bottom image the last.

**Bottom Up:** If you click this button, the order of the images is reversed. The image at the top of the list will be the last image in the stack, and the image at the bottom of the list will be the first image in the stack. Note that the perceived “position” of the images will affect the **Topographic Map**, and may affect which pixel is selected for some of the focus analysis operations.

**Move Up/Move Down:** The **Move Up** and **Move Down** buttons are enabled when only one image is selected in the **Selected Images** list, and *only* if the selected image does not have Z position information. These buttons will move the selected item up or down in the list.

**Select Default Plane:** This feature is enabled whenever there are images in the **Selected Frames** list. Clicking this button displays the following dialog:



**Select as Default Plane:** Click this button to set the selected image frame as the default plane to use when the multi-plane focus algorithm does not find “in-focus” material on any of the supplied planes. Since one or more of the source images may be multi-frame image sequences, any sequence images are expanded to list each included frame, with the frame number indicated in parenthesis.

**Cancel:** Click this button to cancel the default plane select and return to the **Extended Depth of Field** dialog.

At the bottom of the **Extended Depth of Field** dialog box are 3 controls that are shared by all tab pages, as shown here:



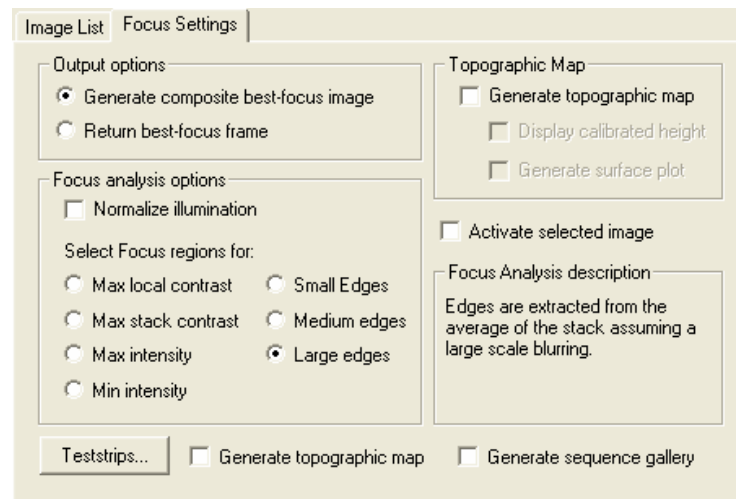
The message box at the far left contains a text field indicating the current number of images and frames in the stack. This field is updated whenever any of the pertinent settings change. The first portion of the field indicates the number of images; the second portion of the field indicates the number of frames currently selected for inclusion in the stack.

**Create:** Click this button to start the process of creating the image stack. This button will only be enabled when there are at least two selected image/frames. The dialog will not close when the stack is created.

**Cancel:** Click this button to close the dialog. If images have been added but an image stack has not been created, you will see a message asking you if you want to continue.

## Focus Settings Tab

The **Focus Settings** tab page allows you to select the various focus analysis and output options.



The **Output options** provide the same functionality as the *multi-plane focus* and *software auto-focus* options on the **Acquire** feature of *Scope-Pro*.

**Generate composite best-focus image:** Clicking this button creates a single image composed of the best pixels from the set of input images, as determined by the **Focus Analysis Options**.

**Return best-focus frame:** Clicking this button creates a new workspace containing a copy of the selected frame with the most in-focus material.

**Normalize Illumination:** Use this option when the illumination or intensity of emitted light will change in the time that it takes to acquire all of the planes. Basically, this option corrects for uneven illumination as the Z plane changes.

### *Process:Extended Depth of Field*

An example would be a sample that uses a fluorescent dye that degrades with exposure to light. The first captured plane will have a higher intensity than the last plane captured. In this case all captured planes will be normalized before being processed into the final image.

**Select Focus Regions for:** There are four options to select what area is “in-focus”:

**Maximum Local Contrast:** This method considers areas with high/maximum contrast to be in focus. Pixels are analyzed in the area around the current pixel location. The pixel from the plane with the largest variance or local contrast is selected.

**Maximum through-depth contrast:** The mean value of the pixels is calculated. The pixel from the plane with the largest variance from the mean is selected.

**Max. Intensity:** This method is used for samples that are more intense than the background such as light emitting fluorescent dyes. The pixel from the plane having the highest intensity at the current pixel location is selected.

**Min. Intensity:** This method is used for regions that are darker than the surrounding area to be in focus. The pixel from the plane having the lowest intensity at the current pixel location is selected.

**Small Edges:** Edges are extracted from the average of the stack assuming a moderate blurring.

**Medium Edges:** Edges are extracted from the average of the stack assuming a mid-scale blurring.

**Large Edges:** Edges are extracted from the average of the stack assuming a large-scale blurring.

The **Topographic Map** options allow you to select whether or not to generate a topographic map from the focus, and if so, whether to add an intensity calibration.

**Generate Topographic Map:** Clicking this button creates a topographic map from the focused image.

**Display calibrated height:** Clicking this button adds an intensity calibration that translates the plane number values in the image to the height. This option will be disabled if **Generate Topographic map** is not selected, and also if the Z position of the image frames is not known.

**Activate Selected Image:** When this box is checked, selecting an image in the in the selected frames list will also cause the associated workspace to be activated.

*Process:Extended Depth of Field*

**TestStrips:** Clicking the **Test strips** button will generate a sequence containing one frame for each combination of the Focus Analysis options (Normalize on and off and the 4 “Select regions” options) and order options (top-to-bottom and bottom-to-top).

**Generate Topographic Map:** Checking this box will cause the test to use the topographic map output when selected; otherwise the composite output is used.

**Generate Sequence Gallery:** Checking this box will cause the resulting sequence to be made into a sequence gallery.

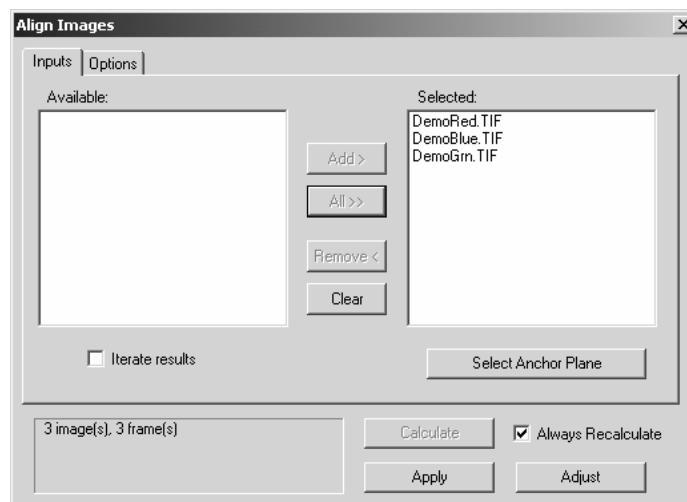
## Align Images

---

The *Alignment Correction* feature allows you to align individual images, or images in a sequence, for translation, rotation, or scaling. This feature is useful if you are working with images taken over time, images with some camera movement, or if you are aligning channels in multiple images from a beam splitter, or correcting for offset angles through a stereo microscope Z-stack.

### Input Tab

When you select *Align Images* from the *Process* menu, you will see the following dialog:



The **Input** tab allows you to select two or more images to align. This tab displays the list of available images on the left and the selected images or sequences on the right.

**Add:** Click this button to move the highlighted image or sequence from **Available Images** into **Selected Images**. The **Selected Images** are used to create the aligned image stack. **Add** will add frames from any images selected in the **Available Images** list to the **Selected Images** list, and is enabled whenever there are one or more images selected. When a sequence containing multiple frames is added, the **Selected Images** list will indicate the range of frames that will be used, e.g. **Heart.seq (5-25)**.



**Add All:** Click this button to move all the images or sequences from **Available Images** into **Selected Images**. **Add All** is always enabled, and will add all images from the **Available Images** list to the **Selected Frames** list. This feature does not check to see if there are duplicate images, so if there are already images in the **Selected Frames** list, the whole list from **Available Images** will be added to them.

**Remove:** Click this button to move the highlighted image or sequence from **Selected Images** back into the **Available Images** list. Remove will remove any images selected in the **Selected Images** list, and is enabled whenever there are one or more images selected.

**Clear:** Click this button to erase the images or sequences from the **Selected Images** list. **Clear** is always enabled and will remove all images from the **Selected Images** list.

**Iterate results:** Checking this box allows you to select the output sequence as the next input, once the initial alignment has been performed. Some data sets may have incomplete registration, due to data being distributed away from the center of the image, and a second or third pass may improve the results.

**Select Anchor Plane:** This feature is enabled whenever there are images in the **Selected Images** list. Clicking this button displays the following dialog:



## Process:Align Images

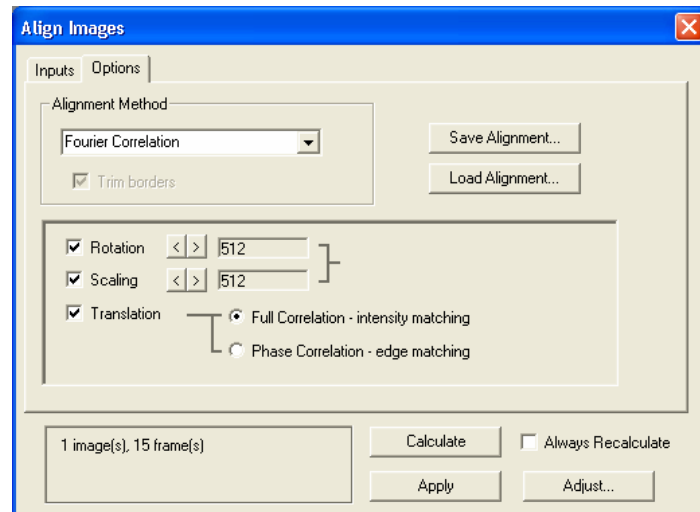
**Select as anchor frame:** By default the reference image (which will not be moved) will be the first item on the list or the first image in a sequence. You can change the default by highlighting an image with the cursor and clicking the **Select as anchor frame** button. All other images will be moved to align relative to that image.

Images are presented as individual workspaces, as sequences with active ranges, or as portions of *Set Manager Z* stacks. If any subset of a sequence is chosen, all other subsets from that workspace will be removed. Unselected Z stacks will remain in the input list. The input list is filtered to include only images of the same size as those selected.

Selected images will be shown as workspace and frame number. Note that set information is currently not preserved in the selection list box.

## Options Tab

The **Options** tab allows you to select the appropriate alignment method; choose rotation, scaling, or translation, and set limits on how far the alignment should go. The **Options** tab is shown here:



**Alignment Method:** Currently, you can choose between **Fourier Correlation** and a user-specified model.

**Save Alignment/Load Alignment:** Click one of these button to save or load a set of calculated alignments to/from the Documents and Settings file. Alignment files have the extension \*.ipa .

**Trim Borders:** If the alignment is purely translation, checking this box allows you to select trimming the output to include only those pixels valid in each image. Otherwise the output image is the same size as the input images, and empty pixels are filled with black.

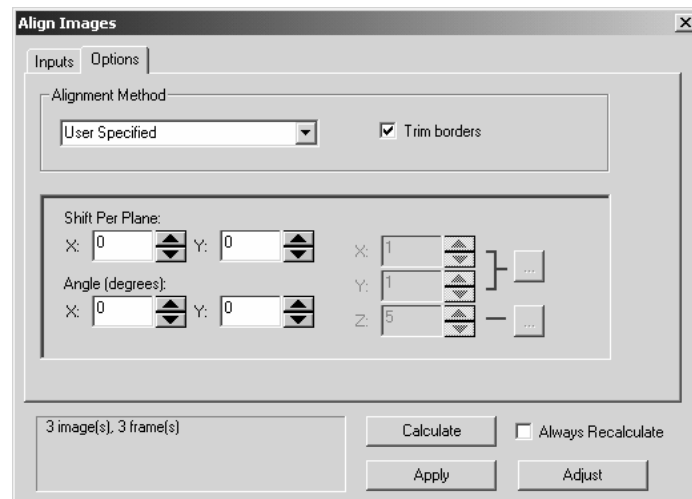
If you select **Fourier Correlation**, you will see the following options:

**Rotation/Scaling/Translation:** Click the radio button to choose which alignments to perform.

**Rotation/Scaling angles and scales:** These options let you choose how many angles and scales to test. Rotation can be  $\pm 90$  degrees, while scaling is possible over a range of about 0.7 to 1.4. These ranges are divided by the number of angles or scales to test. Higher numbers will require more computation. Note that these must be power of 2 factors for the FFT algorithms.

**Full/Phase correlation:** Select one of these radio buttons to perform the FFT translation correlation as a *full correlation* or as a *phase-only correlation*. Full correlation works better for most images. Phase-only correlation ignores image intensity, and may be preferable if the overall image intensities vary while the patterns remain similar.

If you have **User-Specified alignment** selected, you will see the following options:



**Shift Per Plane:** Indicate the number of pixels to shift the images on a per plane basis. The XY calibration may be attached to the frames using the **...Edit** button on this options page

**Angle in degrees:** Indicate the angle to shift the images. This requires calibrated XY images and a Z distance between images. The

## Process:Align Images

Z distance may be attached to the frames if they were captured using *Stage-Pro*, or applied using the **...Edit** button on this options page .

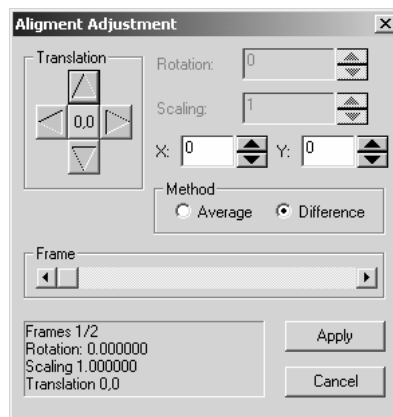
**Calibrated Distances...** Click this button to set the calibration for XY distance (using the Spatial Calibration dialog) or the Z spacing for stacks lacking inherent Z information. This affects the relationship between the user specified shift per plane and the angle

**Calculate:** Click this button to run the alignment calculations on the selected set of images.

**Always Recalculate:** Check this box to recalculate the alignment whenever you click the **Apply** button.

**Apply:** Click this button to apply your alignment to the selected images. This will create a new sequence of aligned images.

**Adjust:** Click this button to manually adjust the alignment. You will see the following dialog:



**Translation:** Use the arrow buttons to adjust the translation .

**Rotation:** Use the spin buttons to adjust the rotation. Rotation can be +/- 90 degrees.

**Scaling:** Use the spin buttons to adjust the scaling. scaling is possible over a range of about 0 . 7 to 1 . 4 .

**X:** Use the spin buttons to adjust the X position of the image for alignment.

**Y:** Use the spin buttons to adjust the Y position of the image for alignment.

**Method of comparing images:** Click the appropriate radio button to choose the average or difference method.

*Process:Align Images*

**Frame:** The slider bar indicates which frame in the sequence is being processed.

**Apply:** Click this button to apply your alignment to the selected images. This will create a new sequence of aligned images.

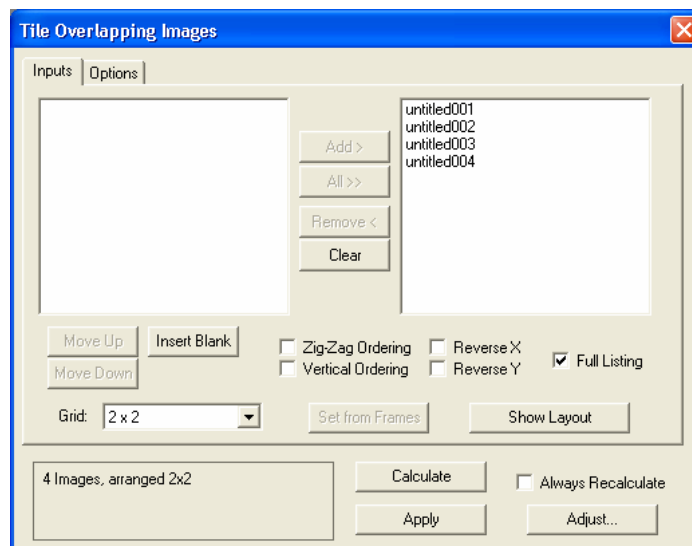
**Cancel:** Click this button to cancel the alignment changes.

## Tile Images

---

The *Tiling* feature allows you to align and stitch images together. This might be useful when constructing panoramic views, or for linking together multiple images taken with a motorized stage

When you select the *Tile Images* feature from the *Process* menu, you will see the following dialog:



### Inputs Tab

The **Input** tab allows you to select two or more images to align. This tab displays the list of available images on the left and the selected images or sequences on the right.

**Add:** Click this button to move the highlighted image or sequence from **Available Images** into **Selected Images**. The **Selected Images** are used to create the tiled image stack. **Add** will add frames from any images selected in the **Available Images** list to the **Selected Images** list, and is enabled whenever there are one or more images selected. When a sequence containing multiple frames is added, the **Selected Images** list will indicate the range of frames that will be used, e.g. **Heart.seq (5-25)**.

**Add All:** Click this button to move all the images or sequences from **Available Images** into **Selected Images**. **Add All** is always enabled, and will add all images from the **Available Images** list to the **Selected Frames** list. This feature does not check to see if there are duplicate images, so if there are already images in the **Selected Frames** list, the whole list from **Available Images** will be added to them.

**Remove:** Click this button to move the highlighted image or sequence from **Selected Images** back into the **Available Images** list. Remove will remove any images selected in the **Selected Images** list, and is enabled whenever there are one or more images selected.

**Clear:** Click this button to erase the images or sequences from the **Selected Images** list. **Clear** is always enabled and will remove all images from the **Selected Images** list.

**Move Up/Move Down:** The **Move Up** and **Move Down** buttons are enabled when only one image is selected in the **Selected Images** list, and *only* if the selected image does not have Z position information. These buttons will move the selected item up or down in the list. These buttons are enabled only if **Full Listing** is on.

**Insert Blank:** Clicking this button will insert an empty frame into the input list of available images.

**Grid:** Use this box to select the tiling arrangement. Note that only rectangular arrangements are allowed, such as 3x4, 1x9, 5x2, etc. The available arrangements are listed in the **Grid** combo box. Inserting blanks may allow tiling if not all images are available for a rectangular layout

**Zig-Zag Ordering:** Checking this option arranges the images in a zig-zag pattern, like this:

1 2	
4 3	

**Vertical Ordering:** Checking this option arranges the images in sequential order, as shown here:

1 3	
2 4	

**Reverse X:** Checking this option arranges the images in a reversed X-order, as shown here:

*Process:Tile Images*

2 1	
4 3	

**Reverse Y:** Checking this option arranges the images in a reversed Y-order, as shown here:

3 4	
1 2	

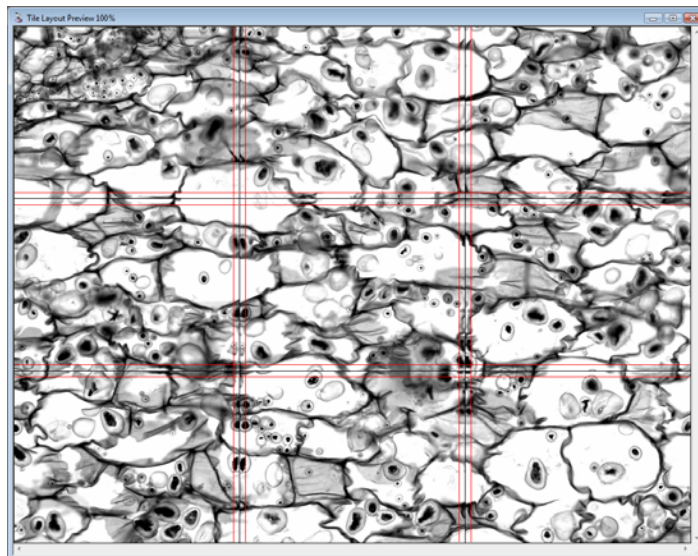
**Full Listing:** Clicking this button displays a list of all the images in the sequence in the selected images list, in a regular order, as shown here:

1 2	
3 4	

Otherwise, sequential frames in sequences are grouped together.

**Set From Frames:** Click this button to use the current position information to create the tile.

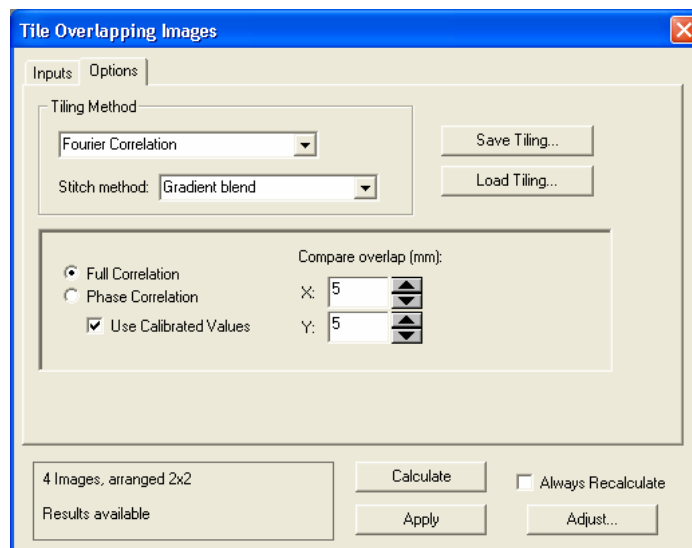
**Show Layout:** Clicking this button displays a thumbnail of the tiled image layout, as shown here:





## Options Tab

The **Options** tab allows you to choose the mosaic dimension, alignment method, specify how much overlap is to be expected between images, and limit how far the alignment may go. You will also be able to choose whether edges of the images will be clipped midway between images or blended in a graduated fashion. The **Options** tab is shown here:



**Tiling Method:** Choose either **User Specified** or **Fourier Correlation** from the drop-down list.

**Stitch Method:** Use the drop-down list to choose one of the stitching methods: **None**, **gradient blend**, or **trim to fit**.

**Save Tiling/Load Tiling:** Click one of these buttons to save or load a set of calculated alignments to/from the Documents and Settings file. Tiling files have the extension `*.ipt`.

**Full Correlation: Full:** Click this radio button to perform the FFT translation correlation as a *full correlation*. Full correlation works better for most images.

**Phase Correlation:** Click this button to perform the FFT correlation as a phase-only correlation. Phase-only correlation ignores image intensity, and may be preferable if the overall image intensities vary while the patterns remain similar.

## Process: Tile Images

**Use Calibrated Values:** If spatial calibrations are available and consistent for all input frames, it is possible to specify the overlap region in calibrated units.

**Compare Overlap:** Specifies how much of the image should be considered when calculating the tiling offsets. This is shown on the **Layout Preview** with a red border between images. Note that the shift between images is limited to these distances in X and Y.

**Center of edges only:** Check this box to perform the tiling using the center of the edges of the image only.

**Horizontal / Vertical:** Specify the X-Y offset for horizontal (row) pairings and for vertical (column) pairings, as described here:

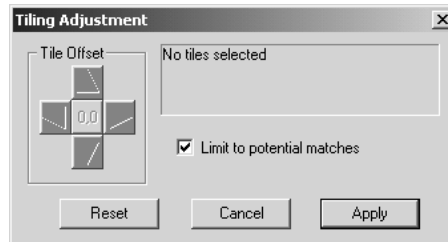
**Max offset (X and Y):** Indicate the maximum offset in pixels for the image tiling overlap.

**Min offset (X and Y):** Indicate the minimum offset in pixels for the image tiling overlap.

**Show Layout:** Clicking the **Show Layout** button displays a new dialog and a new workspace, showing all of the images overlaid with a 50-50 blending or clipping at the edges. Each tile is indicated with a crosshair in the center. Clicking on a tile recreates the data with the selected tile shown as a **Paste** preview on the image, which allows you to move it about with the mouse. This tile will be highlighted with an additional graphic showing the active tile. Here is a preview of the tiled images.



**Adjust:** Clicking this button allows you to adjust the image tiling manually. You will see the following dialog:



Use the spin buttons to adjust the tile offsets manually.

**Limit to potential matches:** Checking this box limits the tiling to adjacent images meeting a certain number of matching criteria.

**Reset:** Click this button to discard any changes and return to the original image layout.

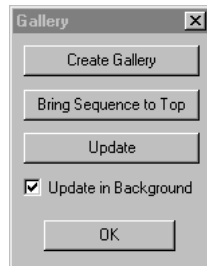
**Cancel:** Click this button to discard any changes and close the **Adjustment** dialog.

**Apply:** Click this button to apply the tiling and stitching methods selected to the images.

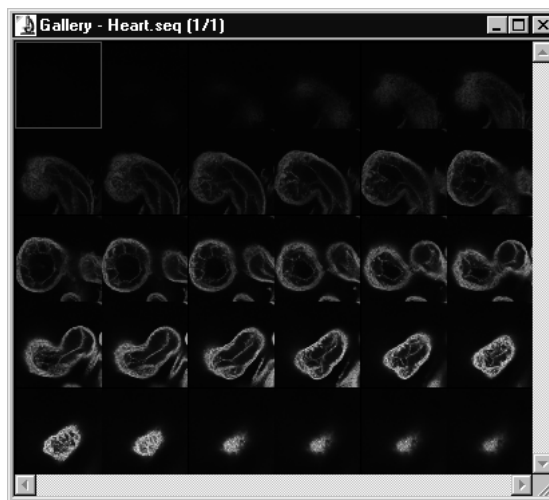
## Sequence Gallery

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The **Sequence Gallery** feature creates a workspace from an active image sequence. The individual frames in the sequence are displayed in a gallery format; hence it creates a “sequence gallery.” Clicking the **Sequence Gallery** menu item displays the following dialog:



**Create Gallery:** Clicking this button creates a sequence gallery workspace from the active workspace. This feature is disabled if there are no active images, or if the active image is a single-frame workspace. Here is a sample sequence gallery:



**Bring Sequence to Top:** Clicking this button activates the sequence workspace on which the gallery is based, and is only enabled when the active workspace is a sequence gallery.

**Update:** Clicking this button will update the sequence gallery with any changes to the image sequence in the active workspace.

*Process:Sequence Gallery*

**Update** will be disabled until a sequence gallery is the active workspace, and a change is made to the sequence that the gallery cannot track, or when other changes are made and **Update in Background** is not selected.

**Update in Background:** Checking this box provides a live link between the sequence workspace and its gallery so that any changes made to the sequence are updated in the gallery as they are made.

**OK:** This button closes the **Sequence Gallery** dialog.

The red square in the **Sequence Gallery** workspace indicates the active frame in the larger *Image-Pro* workspace. Use your cursor to click on different frames in the **Sequence Gallery**, and the red square will move to the selected frame, and the active image in the *Image-Pro* workspace will change to the selected frame as well.

---

*Note:* When a change is made to the length of the original sequence, e.g. frames are added or removed, the sequence gallery workspace will no longer be associated with the sequence, and the **Update** and **Update in Background** links will no longer be active. The only valid option will be **Create Gallery**.

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*Process:Sequence Gallery*

## **The Measure Menu**

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Use the *Measure* menu commands to perform calibration, counting, and measurement operations. If you have not done so already, please review the *Measuring and Counting* discussion in *Section 1* of your *Start-Up Guide* to provide you with an overview of how the different tools work together.

## Calibration

---

Use the *Calibration* command to work with intensity and spatial calibration scales. It is used to modify the scales, store modified scales to disk, and re-load them when needed. If you are not familiar with the calibration process, you may want to review the *Intensity Analysis* and *Measuring and Counting* discussions in *Section 1* before proceeding.

When you select the *Calibration* command, you will receive a menu from which you must choose the calibration operation you want to perform.

- ◆ Calibration Wizard
- ◆ Select Spatial
- ◆ Spatial
- ◆ Set System
- ◆ Intensity
- ◆ Step Tablet
- ◆ Open
- ◆ Save Active
- ◆ Save All
- ◆ Linearize Image
- ◆ Linearize New

Each of these operations is described in the following pages.



## Reference Calibrations

*Image-Pro Plus* uses *reference calibrations*. A *reference calibration* is generally a spatial calibration for a particular configuration of your microscope optics (e.g. an objective) but any calibration that you use frequently may be used as a reference calibration.

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**Note:** Previous versions of *IPP* saved every calibration used from session to session, and after frequent use, the list of saved calibrations could get cluttered. *IPP 7.0* saves *only* reference calibrations. If you want to import your calibrations from previous versions of *Image-Pro* into version 7.0, copy the *IPCAL .CAL* file from old version's folder into the *IPREF .CAL* folder in version 7.0.

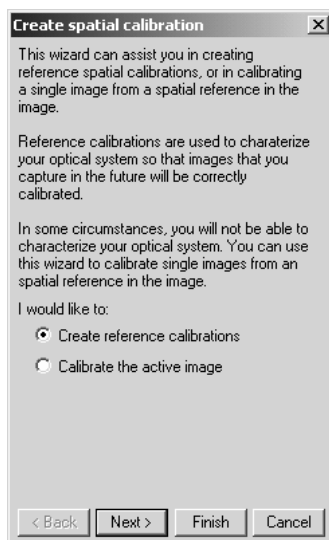
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You will generally want to create a reference calibration for each value or total magnification of your optical system. Some of the elements that affect total magnification are the microscope objectives, any magnification-changing optics in the light path, and the camera used for acquiring images. The recommended method for creating reference calibrations is the *Calibration Wizard*, discussed in the next section, and in your *Image-Pro Plus Start-Up Guide*.

The *system calibration* is a selected reference calibration. It is applied automatically to each captured image. If you change the optics in the light path, by changing the objectives or the camera, you should select the corresponding calibration as the system calibration. The easiest way to do this is to use the **System Calibration toolbar**, also discussed in the following section.

## The Spatial Calibration Wizard

The *Calibration Wizard* is designed to make it easier for you to calibrate single images or to define a set of reference calibrations. Reference calibrations are used to calibrate each microscope objective, used by *Stage-Pro* and *Scope-Pro*, and the system calibration will be drawn from the list of reference calibrations. When you select the *Spatial Calibration Wizard* from the *Measure: Calibration* menu, you will see a **Welcome** screen.



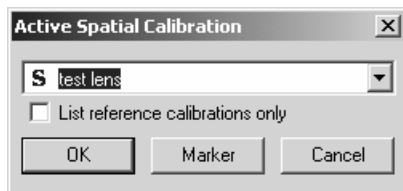
The *Spatial Calibration Wizard* is designed to walk you through the process of creating both active and reference calibrations quickly and easily. You can follow the directions on each screen, and click **Next** to continue.

The following table indicates the steps required by both actions:

<b>Step</b>	<b>Calibrate the active image</b>	<b>Create reference calibrations</b>
<b>1</b>	<b>Welcome page</b>	<b>Welcome page</b>
<b>2</b>	<b>Name and Units page</b>	<b>Reference Process page</b>
<b>3</b>	<b>Image Calibration page</b>	<b>Capture Setup page</b>
<b>4</b>	<b>Finish Image page</b>	<b>Magnifier page</b>
<b>5</b>		<b>Objective page</b>
<b>6</b>		<b>Capture Preview page</b>
<b>7</b>		<b>Reference Calibration page</b>
		<b>Finish Reference page</b>

Additional instructions and advice for using the *Spatial Calibration Wizard* can be found in Chapter 10 of your *Image-Pro Plus 7.0 Start-Up Guide*.

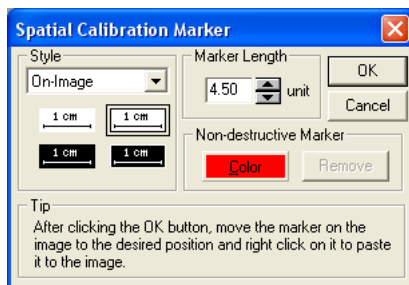
- ◆ Use the **Select Spatial** option from the pop-out menu to calibrate the current image. Clicking on this menu item displays the following dialog box:



Choose the required spatial calibration from the drop-down list box, and click **OK**. The correct calibration will be applied to the current image.

**List reference calibrations only:** Check this box to see only the list of saved IPP reference calibrations.

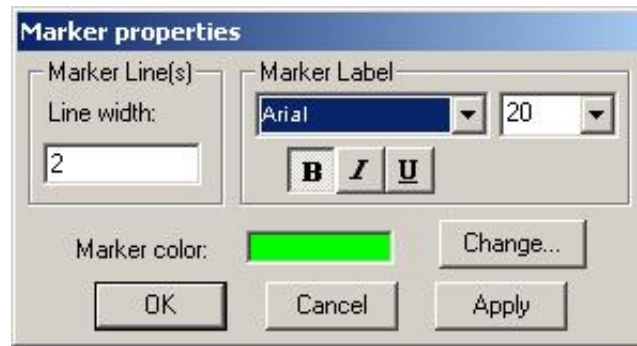
To place a marker on the image, click the **Marker** button. You will see the following dialog:



The **Style** drop-down list allows you to choose either the **On-Image** or **Non-Destructive Markers**. **On-Image** is the method that has been used in previous versions of *IPP*, and is shown in the example above. **Non-destructive** uses the same graphics overlays used in the *Annotate* and *Image Overlay* features.

The **Non-Destructive Marker** options include a **Color** button which sets the on-image color in the same way described for the other **On-Image** color buttons, and **Remove**, which is enabled if there is a non-destructive marker on the active image and will remove the marker if selected.

As the **Tip** indicates, once a non-destructive marker is placed on the image (select one and click **OK**), the style of the marker can be edited by double-clicking any of the marker elements (the text or either line):

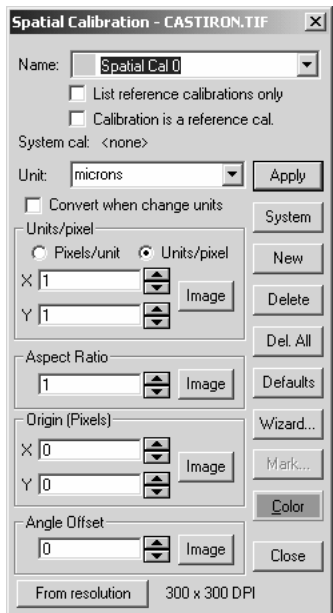


The **Marker Properties** dialog allows you to change the marker color (which will affect all on-image **Calibration** objects, in the same way as all the previous on-image color buttons), and the settings for the marker label, in a similar manner to the **Annotation** properties dialog. In *IPP version 7.0*, you can now adjust the line width as well as the color.

- 
- ◆ **Select *Spatial* from the pop-out menu** if you want to calibrate the spatial scale. By default, *Image-Pro* expresses spatial measurements in terms of pixels. The *Spatial* command is used to change the terms in which *Image-Pro* reports such measurements. You would use this command if you wanted to measure objects in terms of microns, or miles.

The *Spatial* command is also used to establish **Aspect Ratio**, **Image Offset**, and **Angle Offset** values.

When you select the *Calibration : Spatial* command sequence, the **Spatial Calibration** command window is displayed. This window is linked to the active image, as is indicated by the image name in the **Spatial Calibration** title bar.



From within this command window, you can apply an open set of calibrations to the image by selecting the name of the set in the **Name** field, or you can create a new set of calibrations by clicking the **New** button and entering your calibration values in the appropriate group box. When creating a new set, or modifying an existing set, you can use the **Image** buttons to obtain the calibrations from within the image itself.

---

**Note:** Your first step in using this window must be to either select an existing set of calibrations in the **Name** field, or click **New** to create a new set. The fields and buttons in this window will remain dimmed until this is done. See **Name** and **New** discussions below.

---

**Name:** In this list box, select the set of calibration values you want to apply to the image. If you want to use the uncalibrated (pixel) values, select **(none)**. Any reference calibrations that were loaded (using the *Calibration : Open* command), and all calibrations used on open images created since the *Image-Pro* application window was last opened, will be listed in this list box.

You will not be able to enter calibration values until a name other than **(none)** has been selected in the **Name** list box. If **(none)** is

the only set listed, you can create a set by clicking the **New** button and specifying its calibration values.

By default, *Image-Pro* will assign the name “Spatial Cal 0” to a new calibration set; however, you may change this to a more descriptive name if you’d like. Up to 128 characters can be entered as a description. Spaces and punctuation are allowed.

---

**Note:** Naming a calibration set does not save the set to disk. When a calibration set is created, it is stored in memory. The name you assign in the Name field is used to reference the set while it exists in memory. If you want to permanently save a set of calibrations, you must use the **Save** command on the **Calibration** command’s pop-out menu.

---

**Apply:** Clicking this button applies the selected calibration to the active image. When **None** is selected, clicking this button removes any calibrations from the active image. **Apply** is disabled when there is no active image.

**List reference calibrations only:** This checkbox determines if only reference calibrations will be listed. When this box is checked, you will see a list of reference calibrations. This list does *not* necessarily include the calibration of the current active image. If this box is not selected, the calibration for the current active image will appear in the list of all available system calibrations.

**Calibration is a reference calibration:** This checkbox indicates the state of the current calibration. It can be used to make the calibration a reference calibration if it isn’t already, or make a reference calibration a normal non-reference calibration (if the calibration is also the system calibration, the system calibration will be set to **none**).

**System:** Clicking this button makes the current calibration the system calibration. It will be automatically applied to captured images since the system calibration must be a reference calibration, the current calibration is added to the list of reference calibrations if it is not already there.

**System Cal:** The **System Cal** text field indicates the current system calibration, which may or may not be in use.

**New:** Click this button to create a new set of calibration values. When this button is clicked, *Image-Pro* will place the “Spatial Cal 0” name in the **Name** list box (the 0 digit may be incremented to

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make the set name unique) and activate the calibration fields in the 4 group boxes. The values you enter in the group boxes will make up the new set. You can change the “Spatial Cal 0” name to something more descriptive by simply typing over this name in the **Name** field. You can store the new set of values to disk using the *Save* command on the *Calibration* command’s pop-out menu.

**Delete:** Click this button to delete the current calibration.

**Del All:** Click the button to clear the list of all the reference calibrations.

**Unit:** Type the name of your unit in this field (e.g., microns, inches, miles) or select it from the list in the combo box . This name will appear when *Image-Pro* reports spatial data in a report or graph.

**Convert when change units:** This option determines what action will be taken when you change the calibration units in the unit list. If this option is *not* selected, or if either of the calibration units are not absolute physical units (such as meters or miles) then no action will be taken. If this option is selected, and the units are absolute physical units, then the pixels/unit (or units/pixel) scaling value will be adjusted to converting the existing calibration to the new units.

For example, if you convert a one-pixel per millimeter calibration to use meters, the converted calibration will be set to 1000 pixels per meter.

**Pixels/Unit:** In this group box, specify the number of pixels that represent a single unit in both the horizontal (X) and vertical (Y) direction.

The **pixels/unit** and **units/pixel** radio buttons determine how the scaling values are used. The **Pixels/Unit** group box name is changed to indicate the scaling in use, and the current scaling is adjusted accordingly (e.g. 2 pixels/unit will convert to .5 units/pixel).

You may either enter the **Pixel/Unit** values directly into the “X” and “Y” fields (by typing the values or using the  $\sigma$  and  $\tau$  buttons), or you can click the **Image** button to specify the values from an object of known length in your image.

---

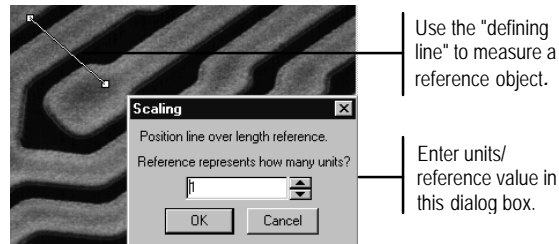
**Note:** If you enter “X” and “Y” values that are not the same, *Image-Pro* will automatically update the **Aspect Ratio** field to



reflect the relationship between the two values (i.e., X/Y). Similarly, if you change the **Aspect Ratio** value to anything but 1, *Image-Pro* will change the Y value to reflect the selected **Aspect Ratio**.

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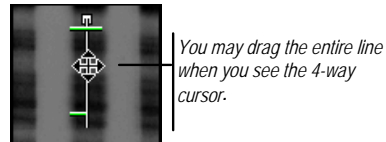
When you select the **Image** button, the **Scaling** dialog box is presented, and a “defining line” is placed in your image.



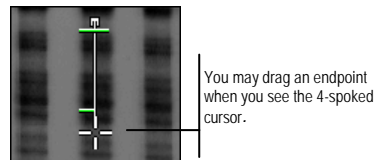
In the **Scaling** dialog box, specify the number of units your reference object represents. If you will be calibrating from an object that is exactly one unit in length, leave the reference value at “1.” If it measures anything but 1, you must specify its known length here. Then, you must place the defining line over the length of the object to specify its size in pixels.

Use your mouse to position the defining line over your object. When you move your cursor over the line, special cursor symbols appear, letting you know that the line or one of its endpoints can be moved.

To move the entire line, place your cursor over the middle of the line. When the 4-way arrow appears, drag the line to a new position.

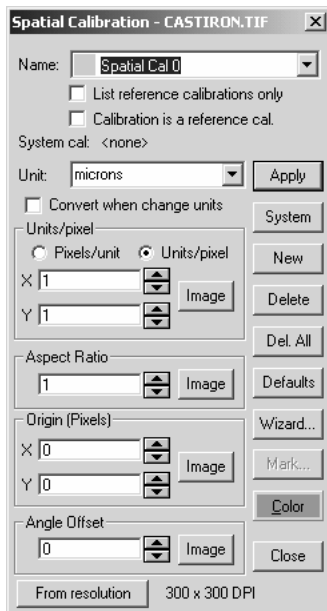


To move an endpoint, place your cursor over the end of a line. When the wheel-cursor appears, drag the endpoint to its new position.



Position the defining line such that it stretches the length of the reference object. Then click **OK** to return to the **Spatial Calibration** dialog box.

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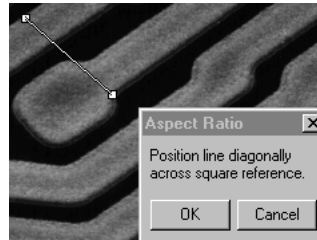


*Image-Pro* will calculate the calibration by dividing the number of pixels under the defining line by the unit number you specified in the **Scaling** box. The result will be placed in both the “X” and “Y” fields of the **Pixel’s/Unit** group box.

**Aspect Ratio:** Use this group box to specify the relationship between the horizontal and vertical axes in your image. The value in this field represents X/Y. It is used to compensate for an image acquired with a camera having an aspect ratio different from that of the displaying device.

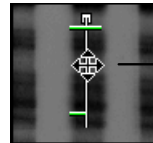
You may either enter the value directly into the **Aspect Ratio** field (by typing the value or selecting it with the  $\sigma$  and  $\tau$  buttons), or you can click the **Image** button to specify the value from an object that is known to be square in your image.

When you select the **Image** button, the **Aspect Ratio** message box is presented, and a “defining line” is placed in your image.



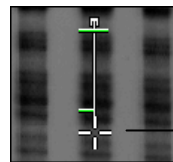
Use your mouse to position the defining line diagonally over your square object. When you move your cursor over the line, special cursor symbols appear letting you know that the line or one of its endpoints can be moved.

To move the entire line, place your cursor over the middle of the line. When the 4-way arrow appears, drag the line to a new position.



You may drag the entire line when you see the 4-way cursor.

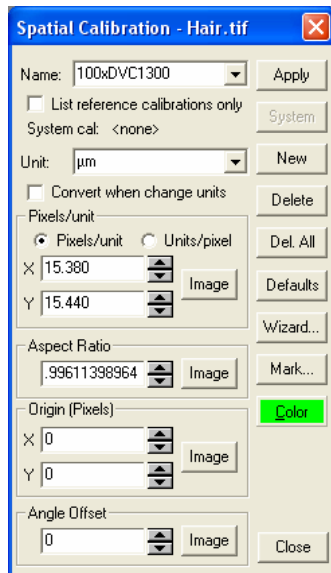
To move an endpoint, place your cursor over the end of a line. When the wheel-cursor appears, drag the endpoint to its new position.



You may drag an endpoint when you see the 4-spoked cursor.

Position the defining line such that it stretches the diagonal length of the square object, from corner-to-corner. Then click **OK** to return to the **Spatial Calibration** dialog box.

*Image-Pro* will calculate the **Aspect Ratio** and update the **Y** value in the **Pixels/Unit** group box if necessary.



You may also indirectly specify **Aspect Ratio** by setting the “Y” value in the **Pixels/Unit** group box to a value not equal to the “X” value. When “X” and “Y” values are not equal, *Image-Pro* will automatically calculate and update the **Aspect Ratio** value.

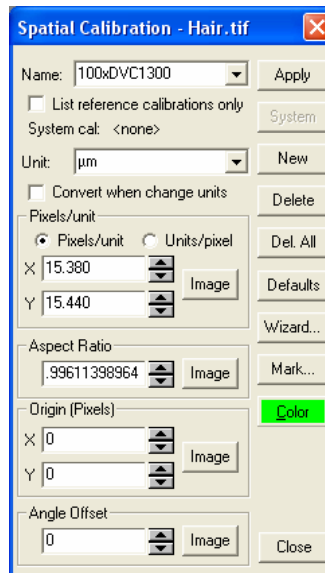
**Origin:** Use this group box to specify an offset to the horizontal and/or vertical origin of your image. These values establish what will be considered position 0,0 within your bitmap. For example, if you wanted pixel 10,10 to be considered the upper-left corner of your image, you would enter offset values of 10 in each **Origin** field. The **Origin** offset values affect the results produced by the **Centr-X** and **Centr-Y** measurements, which report the “X” and “Y” position of the center of a counted object..

You may either enter the **Origin** values directly into the “X” and “Y” fields (by typing the values or selecting them with the  $\sigma$  and  $\tau$  buttons), or you can click the **Image** button to specify the values from a point in your image.

When you select the **Image** button, the **Origin Offset** message box is presented, and a “cross hair” is placed in your image.



Use your mouse to position the cross hair over the pixel that represents the upper-left corner of the image. Then click **OK** to return to the **Spatial Calibration** dialog box. *Image-Pro* will calculate the “X” and “Y” origin offsets based upon the point you selected.



Both positive and negative values are allowed. Negative “X” values move the origin to the left of the left edge of the image, and Negative “Y” values move the origin up above the top line of the image.

**Angle Offset:** Use this group box to specify an offset, in degrees to the vertical axis. This value affects the **Angle** results reported by the *Count/Size* command, which measures the angle between the vertical axis and the major axis of the measured object. The vertical angle is equal to 0 unless an offset has been specified here. If an ellipse measured 90° from vertical, but an offset of 10 had been specified, *Image-Pro* would report the measurement as 80.

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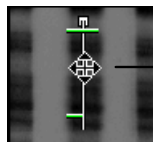
You may either enter the **Angle** value directly into the field (by typing it or using the scroll buttons), or you can click the **Image** button to specify the angle from the image.

When you select the **Image** button, the **Angle Offset** message box is presented, and a “defining line” is placed in your image.



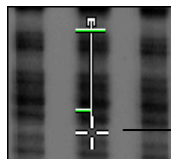
Use your mouse to position the defining line along the vertical axis of your image.

To move the entire line, place your cursor over the middle of the line. When the 4-way arrow appears, drag the line to a new position.



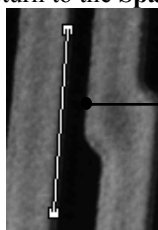
You may drag the entire line when you see the 4-way cursor.

To move an endpoint, place your cursor over the end of a line. When the wheel-cursor appears, drag the endpoint to its new position.



You may drag an endpoint when you see the 4-spoked cursor.

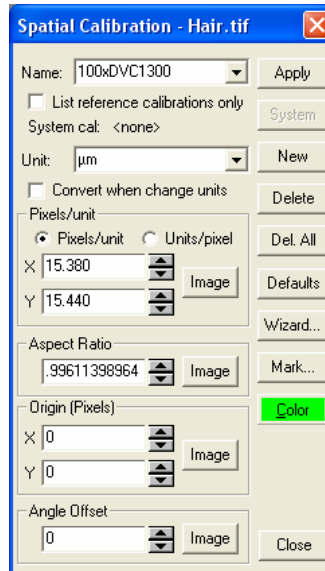
When the defining line is positioned along your vertical axis, click **OK** to return to the **Spatial Calibration** dialog box.



Stretch the defining line vertically along the reference

*Image-Pro* will calculate the vertical offset in degrees and place the result in the **Angle** field.

*Measure:Calibration*



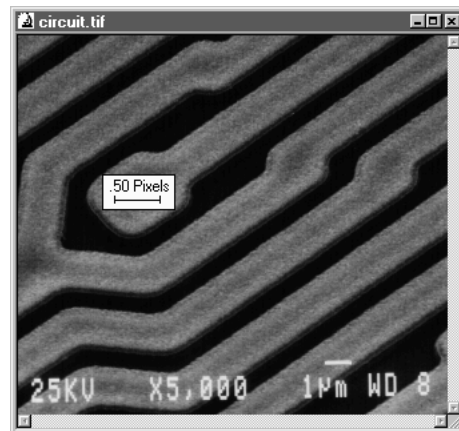
When you are satisfied with your **Spatial Calibration** settings, press the **Close** button. This holds them in memory. To save them, see the *Calibration : Save* command sequence below.

**Wizard:** Click this button to launch the **Calibration Wizard**, which is described earlier in this section.

**Mark:** Use this button to add a **Calibration Bar** to your image. The **Mark** button will be enabled, and you will see this message:



Use the spin buttons to set the marker size. To set the markers on the image, click **OK** and move the marker on the image to the desired position, and click the right mouse button. The marker remains on the image:



This marker now represents one of whatever unit of measure you have selected. To change the number of units represented, use the spin buttons under **Marker Length**.

---

**Note:** The image itself is modified by marking the calibration bar with on-image markers. If you want to be able to return to the original image, make a copy before marking it, or use the non-destructive markers.

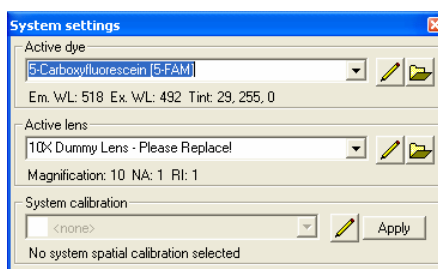
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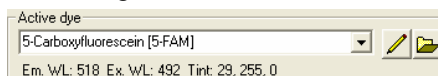
**Color:** Click this button to set the color of overlay objects and the non-destructive calibration markers used on the active image.

**From Resolution:** Click this button to create a new calibration from the image's resolution.

- ◆ **Select Set System from the Calibration pop-out menu if you want to set a system calibration.** The **System Settings** dialog appears, displaying the current active calibration:



**Active Dye:** The **active dye** displays the dye currently applied to the active image.

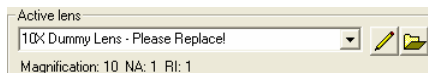



Click this button to open the **Edit Dye List** dialog box. You can select a different dye from the drop-down list. Please see the *Edit: Edit Dye List* pages preceding this section for more information.




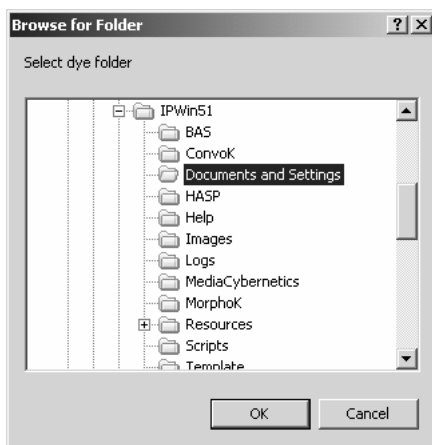
Clicking the folder browse button allows you to select a new dye from a different folder. This button displays a standard Windows folder selection dialog.

**Active Lens:** The **active lens** box displays the name of the lens currently in use.



 Click this button to open the **Edit Lens List** dialog box. You can select a different lens dye from the drop-down list. Please see the *Edit: Edit Lens List* pages following this section for more information.

 Clicking the folder browse button allows you to select a new lens from a different folder. This button displays a standard Windows folder selection dialog, as shown here:

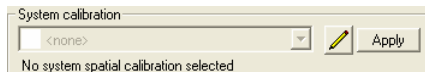


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*Note:* If your calibrations have been created by the Calibration Wizard, or have been manually named so that they contain an exact match for the name of one of your lenses, selecting a new system lens will also set the correct system calibration. This will not work if you have more than one calibration for each lens.

---

**System Calibration:** The **system calibration** is a selected reference calibration .

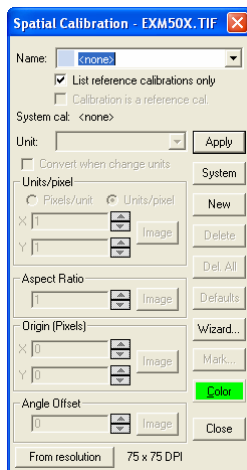


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It is applied automatically to each captured image. If you change the optics in the light path, by changing the objectives or the camera, you should select the corresponding calibration as the system calibration.



Clicking this button displays the full **Spatial Calibration** dialog, where you can modify the spatial calibration settings.



Please refer to the *Spatial Calibration* section under the *Measure* menu for more information.

**Apply:** Click the **Apply** button to apply the dye, lens, or calibration to the active image.

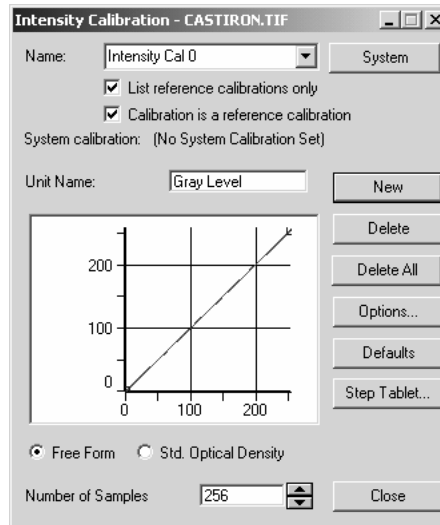
- 
- ◆ **Select *Intensity* from the Calibration pop-out menu** if you want to calibrate the intensity scale. By default, *Image-Pro* expresses intensity measurements in terms of the actual pixel value contained in the image bitmap. The *Intensity* command is used to express this value in other terms. You would use this command if you wanted to relate intensity to optical density, or relate intensity to temperature or relate intensity to protein content, for example.

The *Intensity* calibration command is also used to establish **Black** and **Incident** levels for optical density experiments.

When you select the *Intensity* command, the **Intensity Calibration** command window is displayed. This window is linked to the active

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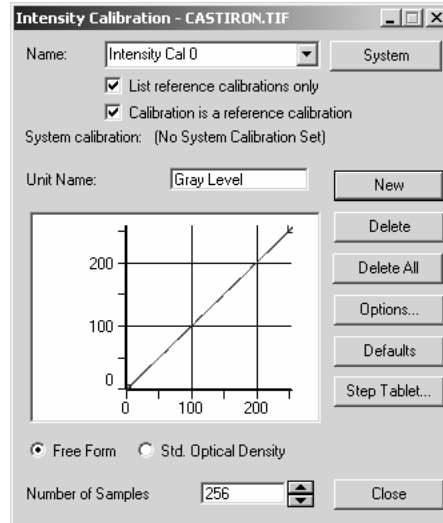
image, as is indicated by the image name in the **Intensity Calibration** title bar.



When the window is initially activated, the curve window is blank, and most buttons and fields are dimmed. This indicates that *Image-Pro's* default intensity calibration is in effect for the image.

To change the default calibration, you can apply an open set of calibrations to the image by selecting the name of the set in the **Name** field. Or, you can create a new set of calibrations by clicking the **New** button and specifying your curve type.

Once a calibration set has been selected, the curve window is filled with the specified calibration curve, and the buttons along the right are activated. Your window will look similar to the one below.



The curve describes how the intensity values within your image will be interpreted by *Image-Pro*. The horizontal axis depicts the incoming intensity value, and the vertical axis, the output value produced by calibration. Using the **Options** button, you can modify the shape of this curve.

Using the **Intensity Calibration** window to create a new intensity calibration involves the following basic steps:

- 1) **Clicking the New button to create a new set.** The default intensity curve will be displayed.
- 2) **Selecting the kind of curve you want to work with.** Highlight the **Standard Optical Density** option button if you want to apply the standard optical density curve to your image. Otherwise, leave the **Free Form** option button enabled.
- 3) **Modifying the curve with the Options button.** If the **Free Form** option button is active, the **Options** button lets you define the calibration curve. If the **Standard Optical Density** is active, the **Options** button lets you define incident-light and black levels.

The specific elements of the *Intensity Calibration* window are defined below.

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**Name:** In this list box, select the set of intensity calibration values you want to apply to the image. If you want to use the *Image-Pro* default values, select **(none)**. Any intensity calibration sets that were loaded (using the *Calibration : Open* command), or created since the *Image-Pro* application window was last opened, will be listed in this list box.

You will not be able to enter intensity calibration values until a name other than **(none)** has been selected in the **Name** list box. If **(none)** is the only set listed, you can create a set by clicking the **New** button and specifying its calibration values.

By default, *Image-Pro* will assign the name “Intensity Cal 0” to a new calibration set; however, you may change this to a more descriptive name if you’d like. Up to 20 characters can be entered as a description. Spaces and punctuation are allowed.

---

**Note:** Naming a calibration set does not save the set to disk. When a calibration set is created, it is stored in memory. The name you assign in the Name field is used to reference the set while it exists in memory. If you want to permanently save a set of calibrations, you must use the **Save** command on the **Calibration** command’s pop-out menu.

---

**List reference calibrations only:** This checkbox determines if only reference calibrations will be listed. When this box is checked, you will see a list of reference calibrations. This list does *not* necessarily include the calibration of the current active image. If this box is not selected, the calibration for the current active image will appear in the list of all available system calibrations.

**Calibration is a reference calibration:** This checkbox indicates the state of the current calibration. It can be used to make the calibration a reference calibration if it isn’t already, or make a reference calibration a normal non-reference calibration (if the calibration is also the system calibration, the system calibration will be set to **none**).

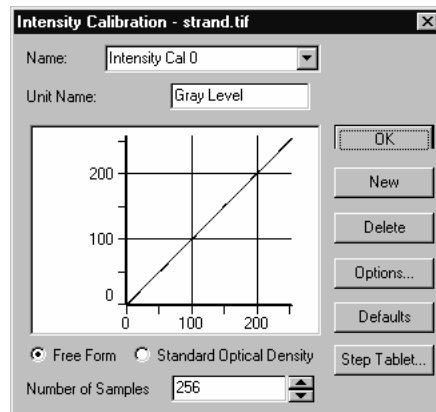
**New:** Click this button to create a new set of calibration values. When this button is selected, *Image-Pro* will place the “Intensity Cal 0” name in the **Name** list box (the 0 digit may be incremented to make the set name unique) and activates the curve window. The values you enter in the **Options** dialog box will make up the new set.

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You can change the “Intensity Cal 0” name to something more descriptive by simply typing over this name in the **Name** field. You can store the new set of values to disk using the *Save* command on the *Calibration* command’s pop-out menu.

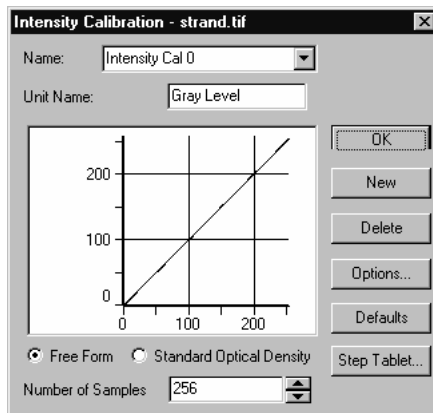
**Unit Name:** Type the name of your unit in this field (e.g., density, degrees, disbursement). This name will appear when *Image-Pro* reports intensity data in a report or graph.

**Free Form:** Select this option button if you need to define a calibration curve unique to your application.



When the **Free Form** button is enabled, the **Options** button is used to specify (X,Y) pairs that define the shape of the calibration curve. See **Options** on the following pages

**Standard Optical Density:** Select this option button if you want to interpret pixel values according to the standard optical density curve (see *Optical Density* discussion in *Section 1* for a definition of this curve). When **Standard Optical Density** is selected, the calibration curve appears as follows:



When **Standard Optical Density** is selected, the **Options** button is used to establish **Incident** and **Black** levels.

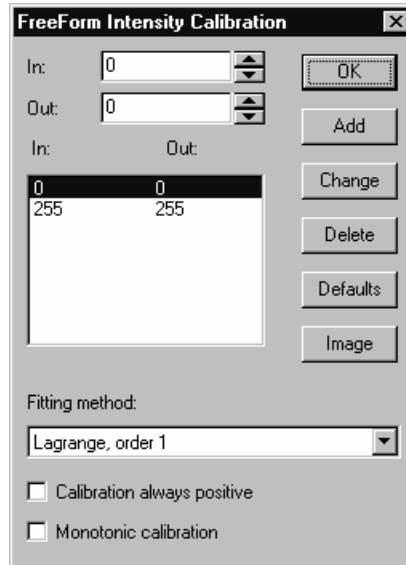
**Number of Samples:** Enter the number of samples to be used along the X-axis. By default this value is set to the maximum value for the intensity range. For *Floating Point* images, the sample value defaults to 1000, but can be re-defined by setting a new value here.

**Options:** Click this button to access the options dialog box for the selected curve type: *Free Form* or *Standard Optical Density*.

---

◆ **Free Form Optical Density Options:** If this button is checked the **FreeForm Intensity Calibration** dialog box is presented.





Within this box, specify the (X,Y) pairs that define your particular curve. If your curve is linear, only two pairs need to be entered. If your curve is nonlinear, enter as many pairs as you need to define the curve to your satisfaction (you may enter a pair for each point on the X-axis if you want - e.g., 256 pairs for a *Gray Scale* image). Once you have entered all your points, click **Close** to return to the **Intensity Calibration** window. The calibration curve will be updated to reflect the points that you specified.

The following fields and buttons are used to create a list of coordinates that define your curve:

**In/Out:** Enter the coordinate-pairs here. The **In** value specifies the X-axis value (the intensity value actually contained in your image), and the **Out** value specifies the Y-axis value (the interpreted value). For example, if you want the intensity value of 10 expressed as 50, type 10 as the **In** value, and 50 as the **Out** value.

The **In** value can also be obtained from within the image itself by using the **Image** button. See below.

---

**Note:** The suggested workflow for **Freeform Intensity Calibration** is to set the **In** and **Out** values first, then click **Add** to add a new point with those values. To edit an existing point, click the point in the list, change the values in the edit boxes, and then click **Change**.

---

**Add:** Click this button to add the **In** and **Out** values to the coordinate list. (You can also use this button to add a 0,0 pair to the coordinate list, then use the **Change** button to assign new **In** and **Out** values to each of these 0,0 pairs).

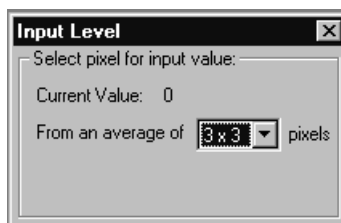
**Change:** Click this button to change the highlighted pair in the list to the new values specified by the **In** and **Out** fields.

**Delete:** Click this button to delete the highlighted pair in the list.

**Defaults:** Click this button to delete the entire list, and return to the *Image-Pro* default coordinates.

**Image:** Click this button to select the **In** value from within the image itself. This is generally used if you have a test image containing known calibration values. For example, you might have an image that provides intensity examples for a range of temperatures. The intensity value (**In**), would be selected from the image, and the temperature value it represents would be typed into the **Out** field (for optical density applications, such a test strip is known as a “density wedge”).

When the **Image** button is selected, the **Input Level** dialog box is presented. This box will report the actual intensity value of the pixel beneath the cursor.



To select an intensity value from your image, move the cursor to a representative pixel; the Current Value is updated as you move the cursor. To use a bigger pixel “neighborhood” instead of just one pixel, you may choose an average pixel value from the drop-down list. When satisfied, click with the left mouse button or press **Enter**. The value will be placed in the **In** field.

---

**Note:** Clicking **OK** places in the **In** field the value at the time you are clicking **OK**. This may not be what you want.

---

**Fitting Method:** These fitting options are available:

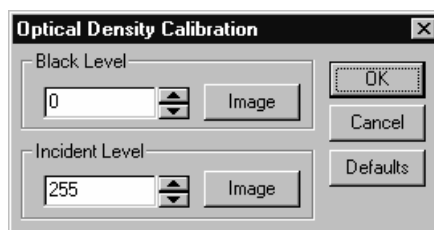
- ◆ **LaGrange order 1 to 3:** These are identical to fitting modes 1 to 3 in previous versions of *Image-Pro Plus*. This is also known as “piecewise polynomial” fitting, where groups of 2 to 4 adjacent sampling points are fitted with a polynomial function of order 1 to 3.
- ◆ **Polynomial order 1 to 3:** Polynomial fitting means that a single polynomial function is fitted to all the sampling points.
- ◆ **Calibration always positive:** This option ensures that the fitted calibration curve or function never displays a negative calibrated intensity.
- ◆ **Monotonic calibration:** This option ensures that the fitted calibration function always increases or always decreases ( i.e. no two gray levels can have the same calibrated intensity value unless the calibration function is flat between these two gray levels).

---

*Note:* The two check boxes are needed most when using a polynomial fitting, since Lagrange fitting ensures that the calibration function goes through the sampling points, and unwanted negative values or non-monotonic behavior is unlikely.

---

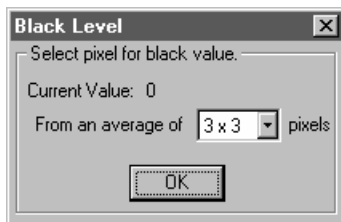
- ◆ **If the *Standard Optical Density* option button is checked,** the **Optical Density Calibration** dialog box is presented.



Use the options in this box to set you **Black** and **Incident** levels. You may enter these values directly into the edit boxes, or set them by selecting a representative pixel within an image (that you have acquired from your camera) using the **Image** button.

**Black/Incident Level:** In these two fields, specify the values that represent **Black** (no light being transmitted) and **Incident** (all possible light being transmitted) states in your image. You may type the values directly into these fields, or use the **Image** button to obtain them from the image.

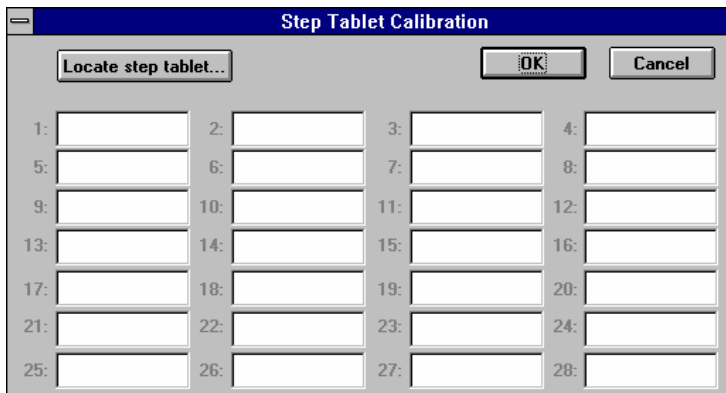
**Image:** Use this button to obtain the Black or Incident level by reading the value of a pixel in the image. When you select the **Image** button the **Black Level** or **Incident Level** message box is presented, as shown here:



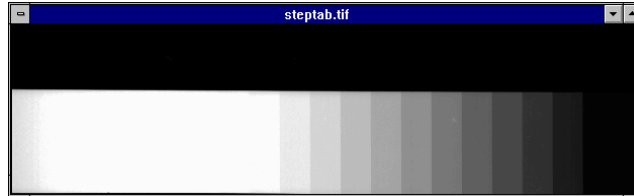
Move the cursor to a pixel that represents the level you want to set, and click your left mouse button. To use a bigger pixel “neighborhood” instead of just one pixel, you may choose an average pixel value from the drop-down list. The intensity value will be placed in the **Black Level / Incident Level** field.

- ◆ **Select Step Tablet from the Calibration pop-out menu** if you want to use the Step Tablet to create a new intensity or optical calibration curve by using a calibrated step tablet.

When you select the *Step Tablet* command, the **Step Tablet Calibration** dialog box appears as follows:



Open the image of an intensity or density calibration step tablet, similar to the one following:



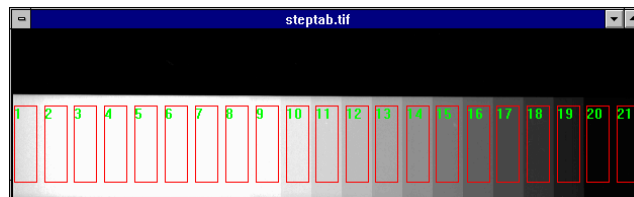
Click on the **Locate Step Tablet** button. Follow the instructions that appear in the additional dialog box below:



Use the left mouse button to click on the first step in the image and then click on the **OK** button.

Follow the next set of instructions by clicking on the last step.

*Image-Pro* displays the outline of the various steps and labels them with step numbers, as shown below.



Enter the optical density or intensity values in the appropriate cell corresponding to each of the steps of the **Step Tablet Calibration** dialog box. *Image-Pro* allows you to enter values for up to 28 steps for the calibration. When you are finished entering the step values in the **Step Tablet Calibration** dialog box, click on **OK**.

*Image-Pro* creates a new intensity or optical density calibration curve according to the step values just entered, and returns to the **Intensity Calibration** dialog box described in the previous section.

- 
- ◆ **Select *Open* from the Calibration pop-out menu** if you want to load a calibration file that had been created using the *Calibration* command's **Save** option. Once a calibration file is loaded, its values

are made available to any open image window. The calibration values are applied to an image by selecting them in the **Spatial Calibration** and **Intensity Calibration** dialog boxes.

When the *Open* command is selected, the **Open Calibration** dialog box is presented.

**File Name:** From this list box, select the name of the file you want to open. You can either type the name of the file (with its entire path, if it is not in the current directory), or use the **Look in**, and **File Type** selections to obtain a list of file names from which you can select.

Double-clicking on a file name in the **File Name** list box will automatically open it.

**File Type:** Only calibration files (\*.CAL) can be selected in this list box.

- 
- ◆ **Select *Save Active* from the Calibration pop-out menu** if you want to save the Intensity and/or Spatial calibration values currently applied to the active image. Saved calibration files can be re-loaded at a later time and attached to any open image. You might also distribute the calibration file to other *Image-Pro-for-Windows* users in your work group who need the same calibration settings for their analyses.

It is important to understand that it is the selections of the active image that are saved to a file. If the active image has both Intensity and Spatial calibration sets applied to it, *both sets* of data will be saved into the calibration file. If the active image has only one type of calibration values applied, and the other type is set to **(none)**, only the selected type will be saved to the file (for example, if **Spatial** values are set but **Intensity** values are not, only the **Spatial** values would be stored into the file).

Similarly, reference calibrations need to be applied to the active image to be saved with the image data. Otherwise, you need to use the **Save All** feature to save both system and reference calibrations.

When you select the *Save Active* option from the **Calibration** pop-out menu, the **Save Calibration** dialog box is presented.

**File Name:** Enter the name of the file you want the calibration values saved to. To specify the file's location, you can either enter its entire path (disk and directory), or specify its location using the **Save in** list box.

Do not type an extension. *Image-Pro* will automatically assign a “.CAL” extension to your file name.

Clicking once on a file displayed in the list of files under the **File Name** box will select that file name as the one to which you want the calibration values stored (i.e., you are requesting that the file be overwritten). Double-clicking on the file name will automatically save the values to that file. Any time you save calibration values to an existing file, *Image-Pro* will request confirmation.

**File Type:** Only \*.CAL can be selected here. Calibration values are stored in ASCII format.

- 
- ◆ **Select Save All from the Calibration pop-out menu** if you want to save the Intensity and/or Spatial calibration values currently selected for the active image. Saved calibration files can be re-loaded at a later time and attached to any open image. You might also distribute the calibration file to other *Image-Pro-for-Windows* users in your work group who need the same calibration settings for their analyses.

It is important to understand that it is the selections of all open images that are saved to a file. All Intensity and Spatial calibration sets assigned to any open image will be saved into the calibration file. If the open images have only one type of calibration values and the other type is set to **(none)**, only the selected type will be saved to the file (for example, if **Spatial** values are set but **Intensity** values are not, only the **Spatial** values would be stored into the file).

When you select the *Save All* option from the **Calibration** pop-out menu, the **Save All Calibrations** dialog box is presented.

**File Name:** Enter the name of the file you want the calibration values saved to. To specify the file’s location, you can either enter its entire path (disk and directory), or specify its location using the **Save in** list box.

Do not type an extension. *Image-Pro* will automatically assign a “.CAL” extension to your file name.

Clicking once on a file displayed in the list of files under the **File Name** box will select that file name as the one to which you want the calibration values stored (i.e., you are requesting that the file be overwritten). Double-clicking on the file name will automatically save the values to that file. Any time you save calibration values to an existing file, *Image-Pro* will request confirmation.

*Measure:Calibration*

**File Type:** Only \*.CAL can be selected here. Calibration values are stored in ASCII format.

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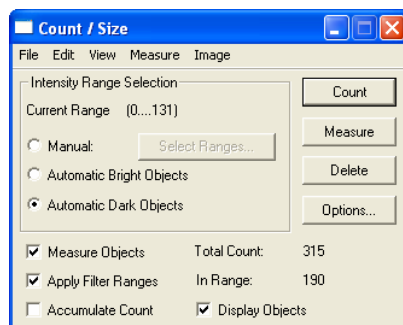
- ◆ **Select *Linearize* from the Calibration pop-out menu** if you want to display the current image with the calibrated pixel values that were applied as a result of the intensity calibration.
- ◆ **Select *Linearize New* from the Calibration pop-out menu** if you want to create a new image with linearized, calibrated pixel values.



## Count/Size

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Use the *Count/Size* command to perform counting and measurement operations. The **Count/Size** dialog contains many functions and options. Moreover, it contains a menu bar with an entire set of its own commands.



Counting/Sizing can be performed upon an entire image, or upon an active AOI.

**Important** - if you want to express your measurement results in terms other than the default intensity and pixel-width values, you must calibrate the intensity and spatial scales before you count and measure. See the **Calibration** command.

The basic steps involved in a counting task are to:

1. **Set the range of intensities** (monochrome images) or the colors (color images) that identify the object you want to count using the tools in the **Intensity Range Selection** group box.
2. **Select the kinds of measurement you want to record** in the *Count/Size* : *Measure* menu's *Select Measurements* command.
3. **Count and measure the objects** using the **Count** and/or **Measure** buttons.

Then, using the *Count/Size* menu commands, your measurements can be viewed, saved, and edited. You can also classify, sort and graph your data using the menu commands.

When you select the *Count/Size* command, the **Count/Size** window is displayed.

## Measure:Count/Size

Before performing your counting or measurement operation, be sure your intensity and spatial scales have been calibrated for your application. See the *Calibration* command.

---

**Note:** During a **Count/Size** operation, *Image-Pro* reports intensity and size data in terms of the currently specified intensity and spatial units. In the command and option descriptions below, the general terms “intensity” and “pixels” are used, but keep in mind that these properties will actually be reported in the terms that you have specified with the **Calibration** command.

---

**Intensity Range Selection:** Use the options in this box to specify the intensity values that define the objects you want counted and measured. You can manually select the range by highlighting a segment of the intensity or color scale, or you can request to have *Image-Pro* automatically determine the range.

**Current Range:** This informational field displays the boundaries of the currently selected intensity range. All pixels containing intensities within this range (endpoints included) are considered part of a countable object. These values are set using either the **Manual** or **Automatic** intensity selection options.

**Automatic Bright Objects:** Click this option button if you want *Image-Pro* to analyze your image and automatically set the intensity range when it performs the count. *Image-Pro* will interpret **Bright Objects** as foreground objects (on a dark background).

**Automatic Dark Objects:** Click this option button if you want *Image-Pro* to analyze your image and automatically set the intensity range when it performs the count. *Image-Pro* will interpret **Dark Objects** as foreground objects (on a bright background).

If *Image-Pro* does not successfully extract your objects from your image, you may need to use the *Flatten Background* command on the **Count/Size** window's *Image* menu to eliminate background intensities that compete with your objects.

**Manual:** Click this option button if you want to define your intensity range manually. After you have selected **Manual**, click the **Select Range** button (for monochrome) or **Select Color** button (for color), to specify the range of intensities/colors that define the objects in the active image.

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- ◆ **If you are working with a monochrome image**, click the **Select Ranges** button to set from **one to 16** intensity ranges that

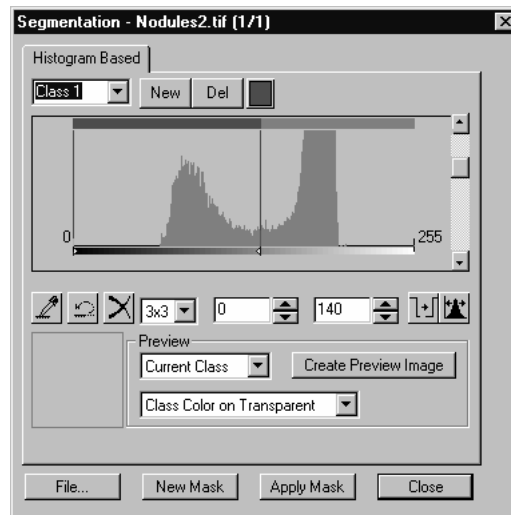
define the objects in the active monochrome image. You might want to do this to distinguish objects at a particular intensity range from their background or from other objects. An object is defined as that area whose pixels fall within the intensity values of its defined range.

---

**Note:** *Image-Pro* attempts to preview your segmentation on the image while you adjust the ranges. This is done using a process that divides the current display range into 256 bins and assigns the preview colors for each bin. If you are segmenting a high-precision image type (i.e. 16-bit grayscale), this process is inherently less accurate than the actual segmentation will be. You can make the preview more accurate by using *Enhance: Display Range* to narrow the range of intensities that are displayed during the preview process.

---

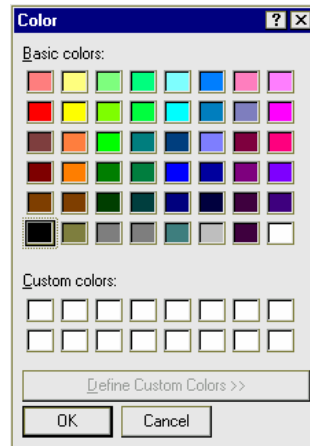
When you click *Select Ranges* , the **Segmentation** dialog box is presented.



Initially, one range will be selected. You will set it and additional ranges, one range at a time, until you have as many as you need. For a description of this dialog, please refer to the *Segmentation* section.

Click the **Color Button** to change the color representation of the current range. The **Colors** dialog box appears.

Measure:Count/Size

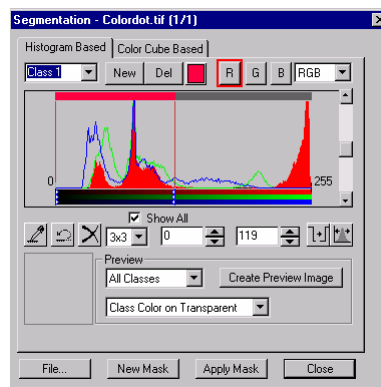


To choose a different **Basic Color**, click the desired color, then click **OK**. Custom colors are not enabled. For more information about the **Colors** dialog, see the *Process: Pseudo-Color* section earlier in this manual.

Click the **OK** button when you have completed your multiple threshold selections.

You may proceed with counting objects based upon the value ranges you have selected.

- ◆ **If you are working with a color image**, click the **Select Color** button to specify the range of colors that define the objects within your image. When you click *Select Color* the **Segmentation** dialog box is presented. For a description of this dialog, please refer to the *Segmentation* section



Use this dialog box to segment out the objects that are to be counted and/or measured. See the *Segmentation* command for procedures on segmenting color ranges from an image.

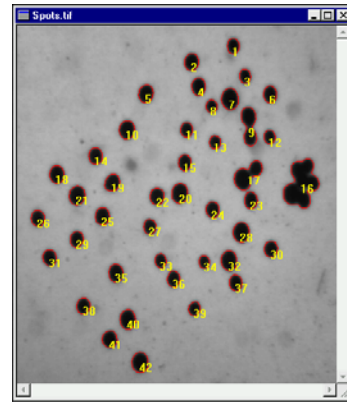
---

**Note:** You do not have to apply the segmentation to the image with **Make Mask** in order to count/measure the objects within it. Once you have segmented out the objects to your satisfaction (as evidenced by a segment Preview), the Segmentation dialog box can be closed, and you may proceed to count and measure the objects.

---

**Count:** Click this button to initiate a counting and measuring operation. When this button is clicked, *Image-Pro* will count and outline the objects within the active image or AOI, as shown in this example.

If either the **Automatic Bright Objects** or **Automatic Bright Objects** intensity option is enabled, *Image-Pro* will automatically analyze the image before performing the count, and identify the intensities that separate the objects from the background. Otherwise, it will count the objects as defined by the intensity range you have set manually.



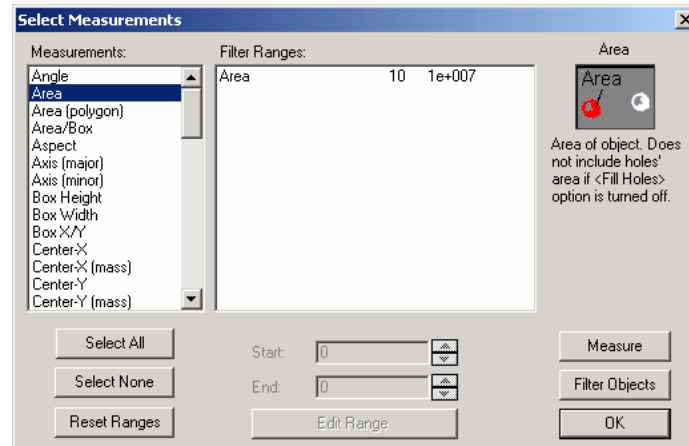
Before clicking the **Count** button, be sure you have specified your measurement and outline options. *Image-Pro* will measure as well as count if measurements have been selected with the *Select Measurements* command, and if the **Measure Objects** option is on. Otherwise, objects will simply be outlined and counted. You can control whether measurements replace or are added to existing data in the data sheet using the **Add Count** option. You can also count and measure only objects meeting certain criteria using the **Apply Ranges** option and **Set Ranges** command. See option and command descriptions below for more information about these capabilities.

The count operation will outline the objects it finds in your image in the manner you have specified in the **Options** menu. See **Options** below.

## Measure:Count/Size

**Measure:** This button can be used as a shortcut to update the measurements you have chosen on the *Count/Size:Measure: Select Measurements* dialog.

When you choose the *Measurements* command, the **Select Measurements** dialog box is displayed:



**Measurements:** Use this list box to select types of measurements. Click on the measure you want taken upon the counted objects. A measure will be highlighted when it is selected. Clicking on a selected measure will deselect it.

**Select All:** Click this button activate all the measurements for your image.

**Select None:** Click this button to clear all active measurements.

**Reset Ranges:** Clicking this button resets all the measurements to their “factory defaults.”

Click **OK** to return to the *Count/Size* menu, or **Measure** to perform the specified measurement upon the counted objects.

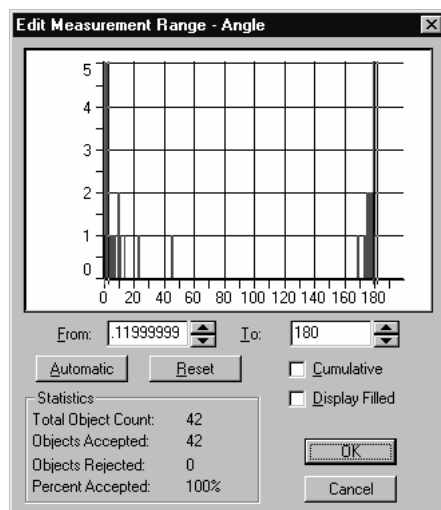
---

**Note:** Clicking **OK** will not perform the specified measurements. Measurements are not taken until you select **Count** in the **Count/Size** window, or either **Measure** or **Filter Objects** in the **Select Measurements** dialog box.

---

Clicking **Measure** will remeasure all the counted objects in your image or AOI and update the measurement data sheet with the selected measurements. If a measure has been deselected, its measurement values will be removed from the measurement data sheet. If the **Apply Ranges** option on the *Count/Size* menu is enabled, only objects meeting the criteria set forth in the **Set Ranges** dialog box will be counted and measured.

**Edit Ranges:** Use button to set or modify a measurement range, in order to restrict the values that are measured for an object. Clicking this button displays the **Edit Measurement Ranges** dialog box:



Use the settings on this dialog to adjust the values for the measurement range(s). **Cumulative** will add a series of range measurements together. **Display filled** will color in the objects in that range on your active image.

Click **OK** to return to the *Count/Size* menu, or **Filter Objects** to apply a filter to the measurements for the counted objects.

**Start/End:** Enter the minimum and maximum allowable values for the selected measure. *Image-Pro* will count and record data for only those objects whose measurements equal or fall between the two values you specify here.

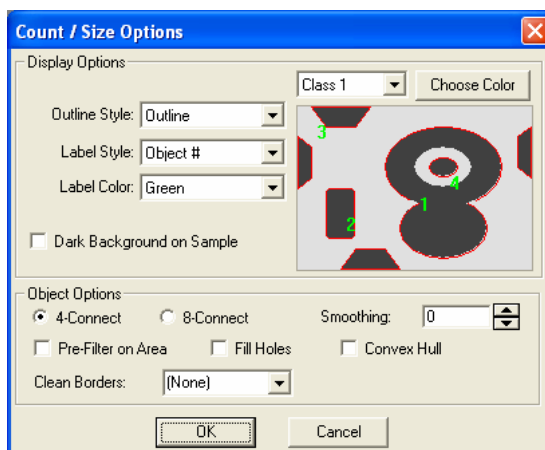
---

**Note:** Only measurements that have been specified with the **Select Measurements** dialog box can be selected and limited with **Set Ranges**. If the measure you want to limit is not listed by **Set Ranges**, you will need to enable it in the **Select Measurement** list box.

---

**Options:** Click this button to access and change options relating to the way counted objects are identified and outlined. These options can be used to change the outline color, or to display counted objects with a solid color instead of an outline. It also contains options that determine how holes and partial objects are treated.

When you click the **Options** button, the **Count/Size Options** dialog box is presented.



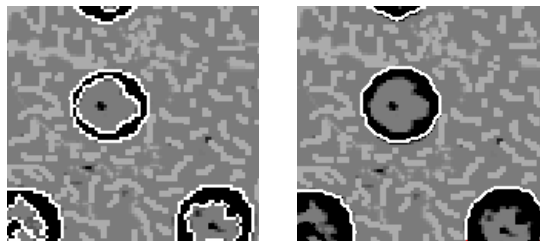
**Display Options:** Use the options in this group box to specify how you want your counted objects highlighted and labeled. The results of each option you select will be depicted in the sample image to the right.

**Outline Style:** This list box determines the way in which you want your counted objects distinguished. Choose one of the following options:

- ◆ **None** - counted objects will not be highlighted in any way (other than with their label number, if that option is enabled).
- ◆ **Outline** - counted objects will be highlighted with a colored trace around their perimeter.
- ◆ **With Holes** - counted objects will be highlighted with a colored trace around their perimeter *and* around any holes that are within the object (holes are defined as pixels



within an object that are not of the specified intensity). The objects on the left have been outlined using the **With Holes** option, and on the right with the **Outline** option.



---

**Note:** If the **Fill Holes** checkbox is selected, the **With Holes** outline style has no effect. Only a perimeter outline will appear. This is because the **Fill Holes** option treats all pixels encompassed by the perimeter as part of an object, regardless of their intensity value.

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- ◆ **Filled** - counted objects will be highlighted with a solid color. The entire object will appear in the color selected in the color option box.
  - ◆ **Ellipse** - counted objects will be highlighted with their ellipse equivalents (ellipse with same major and minor axes as object).
  - ◆ **Class** - counted objects will be colored according to their class. This option will color-code the counted objects according to the classifications created by the **Auto Classification** or **Single Variable Classification** commands. If there is only one object class, this selection will yield the same results as **Outline**.
  - ◆ **Class Filled** - counted objects will be colored according to their class, then highlighted with a solid color. This option will color-code the counted objects according to the classifications created by the **Auto Classification** or **Single Variable Classification** commands. If there is only one object class, this selection will yield the same results as **Filled**.
  - ◆ **Dot** -marks each counted object with a small cross hair positioned in the center of the object's bounding box.
- Label Style:** This list box determines the kind of label that will be displayed. Choose one of the following options:
- ◆ **None** - label numbers will not be displayed.

- ◆ **Obj #** - a counted objects will be displayed with the unique number that identifies it.
- ◆ **Class** - a counted object will be displayed with the number of the class to which it has been assigned by the **Auto Classification** or **Single Variable Classification** commands.
- ◆ **Measurement**- use the value for a specific measurement, such as “area”, as the label.

**Label Color:** The **Label Color** list box allows you to choose the colors for your labels. If you elect to show labels, all the labels will be in the same color, even if you have several ranges or several classes.

**Choose Color by Range:** If you have selected Multiple Thresholds, using the **Manual: Select Range** option on the **Count/Size** dialog box, the **(Range)** list box will contain the names of those ranges. Otherwise, only one range will be available to you. If you make no color changes, the colors you chose for the Multiple Threshold will be used.

If you have trouble distinguishing some ranges, you may want to change one or more colors. Note that you will be able to observe the distinction better if you select **Filled** for your **Outline Style**. From the list box, choose the range whose color you want to change.

Click the **Choose Color** button to change the color representation of the current range. The **Color** dialog box appears. **Colors**



To choose a different Basic Color, click the desired color, then click **OK**. For more information about the **Colors** dialog, see the *Process: Pseudo-Color* section earlier in this manual.

**Dark Background on Sample:** Check this box if your image has bright images on a dark background. This allows you to observe on the sample image the color selections you have made.

**Object Options:** The options in this group box affect how objects are recognized by the Count/Size module.

**Clean Border:** Select an option from the drop-down list to exclude from the count any objects that intersect the designated edges of your image or AOI.

**Pre-filter on area:** Select this option to include only those objects that are within the specified range in the **Total Count** display. When **Pre-filter** is not checked, the **Total Count** value represents all objects within the AOI or image, not just those meeting the range criteria.

**Fill Holes:** Select this option to exclude from the count any objects that are embedded within other objects. When **Fill Holes** is checked, *Image-Pro* will ignore any objects it finds that are totally encompassed by a larger object.

**Convex hull:** Select this option to create a smooth area around the perimeter of irregularly shaped objects. For example, if you have an area such as star or crescent shape, selecting this option will fill in the areas between the points before taking any measurements.

**Smoothing:** Enter a smoothing value from 0 (no smoothing) to 100 (Maximum smoothing). Any smoothing value greater than zero instructs *Image-Pro* to perform smoothing, and indicates to what degree smoothing should be performed. If perimeter and roundness are critical measurements, do not use smoothing.

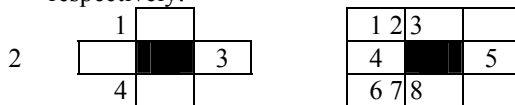
**4-Connect:** Check this box to use the 4-connect method of separating objects in the image. If the 4-connect method is used, only objects that share a corner pixel will be counted separately.

**8-Connect:** Check this box to use the 8-connect method of separating objects in the image. If the 8-connect method is used, objects that are connected in any way will be counted as one.

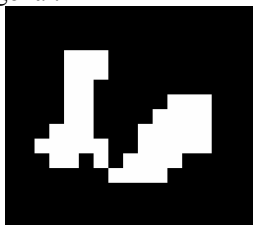
This property is used during the object extraction process. If the mode is set to 4-connected, objects that share only a corner pixel are counted as separate objects. When the mode is set to 8-connected, objects connected in any way will be counted as one.

## Measure:Count/Size

The following figures illustrate 4- and 8-connected joinings, respectively:

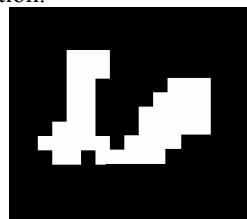


In the example below, two objects are connected by two pixels along a diagonal.



When 4-connected mode is selected, they will be counted as two objects. In 8-connected mode, they count as one.

In the example below, the same two objects are connected by two pixels in a vertical or horizontal direction.



In both 4- and 8-connected modes, the two objects are counted as one.

**Measure Objects:** Select this option on the **Count/Size** window if you want *Image-Pro* to record measurements for the counted objects. If this option is checked, *Image-Pro* will perform and record the measurements you have specified with the *Select Measurements* command. If this option is not checked, *Image-Pro* will only count and outline your objects.

**Apply Ranges:** Select this option if you want *Image-Pro* to count only those objects that fall within the measurement ranges you have specified with the **Set Ranges** command. If this option is unchecked, all objects are counted and measured; the **Set Ranges** criteria are ignored.

---

**Note:** The **Measure Objects** option must be checked when you use the **Apply Ranges** option. If **Measure Objects** is not enabled, *Image-Pro* will not have the data it needs to determine whether an object is within the specified range.

---

**Accumulate Count:** Select this option if you want *Image-Pro* to keep the current outlines and measurements when you perform a new count (i.e., when you click **Count**). Ordinarily, *Image-Pro* clears all previous measurements when it begins a new count;

however, if the **Accumulate Count** option is enabled, the previous measurements are maintained, and the new ones are simply added to it.

There are a couple of instances where the **Accumulate Count** option is useful. First, you might use it to add the measurements from one AOI to another. If you had collected measurements from one part of your image, then moved your AOI to collect additional measurements from another area, you might want to set **Accumulate Count** option to collect all the measurement in a single data set.

You might also want to use **Accumulate Count** if you were collecting data for more than one kind of object. If, for example, you had counted a set of objects defined by one intensity range, then redefined the intensity range for a new set of features, you would use **Accumulate Count** to combine the measurements of the two object sets.

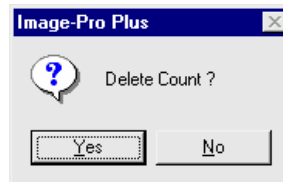
---

**Note:** If the new intensity range causes a new object to touch an existing outline, the new object will be counted as a part of the previous outline.

---

**Display objects:** Check this box to turn the display of the counted objects on or off.

**Delete:** Click this button to clear the existing object outlines and measurements. When you select this button, the following prompt will require you to confirm your request:



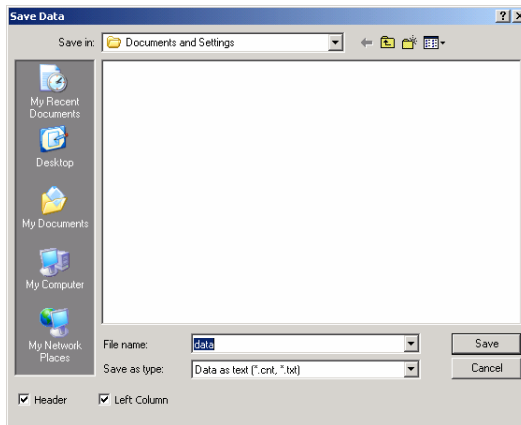
If you respond **Yes** or press **Enter**, *Image-Pro* will clear the outlines from your image, and clear the measurements from your data sheet. A response of **No** will leave your outlines and measurements unchanged.

## Count/Size File Menu

The commands on the *Count/Size* window's *File* menu are used to perform operations that load and save measurement, outline and environment settings to and from disk files. It also contains the command to close the *Count/Size* window.

- 
- ◆ **Data to File** : Select the *Data to File*. command to store the current measurement data sheet to disk. The measurement file can be saved to an ASCII file, or to a LOTUS 123™ WK1 spreadsheet file. In either case, the file will contain a measurement record for each object in the count. The saved data can be imported into a spreadsheet application or used as input to an external program.

When you select *Data to File*, the **Save Data** dialog box is displayed:



Specify the type of file you want to create in the **Save As Type** field. Then, enter the name of the file to which you want the measurement data saved and click **OK**.

**Header:** Check this box if you want *Image-Pro* to create a header for your file.

**Left Column:** Check this box if you want *Image-Pro* to place the data in the file starting with the far left column.

- 
- ◆ **Append Data to File** : Select the *Append Data to File*. command to store the current measurement data sheet as an attachment to an existing file.

The measurement file can be saved to an ASCII file, or to a LOTUS 123™ WK1 spreadsheet file. In either case, the file will contain a measurement record for each object in the count. The saved data can be imported into a spreadsheet application or used as input to an external program.

When you select *Append Data*, the **Save Data** dialog box is displayed (on preceding page). Specify the type of file you want to create in the

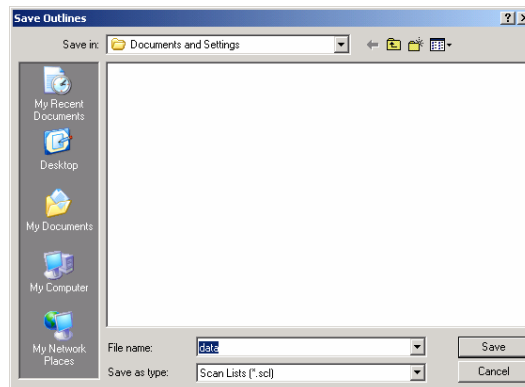
**Save As Type** field. Then, enter the name of the file to which you want the measurement data saved and click **OK**.

- 
- ◆ **Data To Clipboard:** Select the *Data To Clipboard* command to copy the current measurement data to the Windows Clipboard. From there, the data can be pasted into any application that accepts text from the Clipboard.
  - ◆ **Data to Printer:** Select the *Data To Printer* command to print out the current measurement data .
  - ◆ **Data Export:** Select the *Data Export* command to transfer data to Microsoft's Excel spreadsheet. For additional information about DDE, see the *Dynamic Data Exchange* discussion.

- 
- ◆ **Export Options :** Select the *Export Options* command to tell *Image-Pro* how and where to export the current data via DDE. For additional information about DDE, see the *Dynamic Data Exchange* discussion.

- 
- ◆ **Save Outlines :** Select the *Save Outlines* command to store the current outlines to disk. You may save your object outlines, as a list of polygonal shapes, to an ASCII or a binary file.

Saved outlines can be superimposed upon another image using the *Load Outlines* command. Outline files are required by the *Population Density* command, as they define the area from which object density will be derived. You might also use an outline file as input to an external program. When you select *Save Outlines* , the **Save Outlines** dialog box is displayed:



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Specify the name of the file to which you want the counted object outlines saved, choose “\*.SCL” for binary files or “\*.TXT” for ASCII files, then click **Save**.

---

**Note:** If you have highlighted your objects with the **Fill** option, *Image-Pro* will store the perimeter definitions to the outline file. No data will be included to describe the “fill.”

---

- ◆ **Load Outlines :** Select the *Load Outlines* command on the *Count/Size* window’s *File* menu to superimpose an outline file onto the active image. Outline files are created using the *Save Outlines* command from the *Count/Size* or *Measurements* command’s *File* menu. If you are preparing an image for a *Population Density* command, you must use this *Load Outlines* to superimpose the outlines encompassing your counted objects. You might also load an outline file for positional or alignment purposes.

When you select the *Load Outlines* command, the **Load Outlines** dialog box is presented.

Select the binary outline file (\*.SCL) you want to load, then click **Open**. When the outlines are loaded, their measurements (as currently specified in the *Select Measurements* command) are calculated and added to the measurement data sheet. If the outline file is one created by the *Measurements* command, and contains open outlines (e.g., lines, traces, angles), they will be closed when they are loaded (*Count/Size* works with polygons).

---

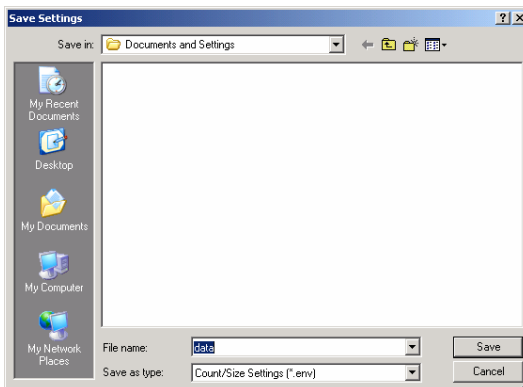
**Note:** An outline loaded from an outline file is simply “superimposed” upon your image. It is not actually written to your image bitmap.

---

- ◆ **Save Settings :** Select the *Save Settings* command on the *Count/Size* window’s *File* menu to store the current *Select Measurements*, *Set Ranges*, and *Options* specifications. The *Save Settings* command stores these values to a file so that, at some future point, you can simply reload these values instead of having to reestablish them manually. Your **Count/Size** settings are saved to an environment (.ENV) file.

When the *Save Settings* command is selected, the **Save Settings** dialog box is presented:





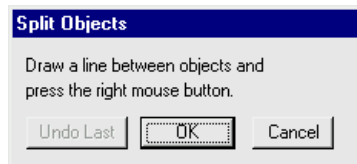
Specify the name of the file to which you want the count/size settings saved, then click **OK**.

- 
- ◆ **Load Settings :** Select the *Load Settings* command on the *Count/Size* window's *File* menu to set the **Select Measurements**, **Set Ranges**, and **Options** settings to the values defined in the *Image-Pro* environment file (an .ENV file). An environment file is created by saving these settings using *Image-Pro*'s *Save Settings* command.  
When the *Load Settings* command is selected, the **Load Environment** dialog box is presented. Select the environment file you want to load, then click **Open**.
- 
- ◆ **Record Environment:** Select this command if you want to record your current environment settings in a macro.
  - ◆ **Close:** Use this command on the *Count/Size* window's *File* menu to close the **Count/Size** window. Closing the **Count/Size** window will not affect active measurements and outlines, nor will it close the **Measurements** data sheet window if it is open.

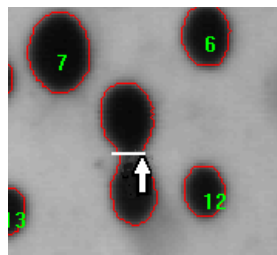
## Count/Size Edit Menu

The commands on the *Count/Size* window's *Edit* menu are used to modify counted objects and their outlines. *Note that you must select the objects in your image to apply these commands to them.*

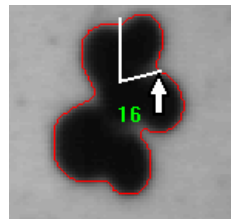
- ◆ **Split Objects :** Use the *Split Objects* command to manually split a single object (usually a cluster that *Image-Pro* has counted as one object) into two or more objects. When you select *Split Objects* the **Outline** dialog box and the **Split Objects** message box are both presented, the freeform AOI tool is activated, and the drawing-arrow cursor appears in your image:



Use the drawing arrow to draw a dividing line between your two objects. To draw a line, click the left mouse button at each endpoint. When both points have been defined, click your right mouse button. *Image-Pro* will display the line that you have defined. If you are satisfied with the line, click the right mouse button.

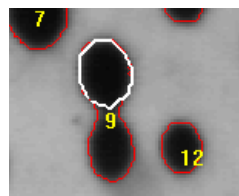


You may also split an object with a polyline (a continuous line with more than one segment). To create a polyline, simply click the left mouse button at each node. When the line has been entirely defined, click the right mouse button.



When placing the line to split your object, be sure the dividing line intersects the perimeter of the object that you are splitting (it's okay if the line extends outside the perimeter, as shown in the examples above).

You may also use the Freeform AOI Tool to segment the object. Simply click once on the **Outline** dialog box, then draw your AOI (or use the **Auto-Trace** feature). When the line has been entirely defined, click the right mouse button.

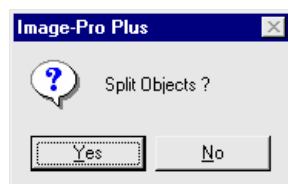


When you have finished splitting the object, using any of the above methods, click the right mouse button to finish the operation.

- 
- ◆ **Auto-Split:** Use the *Auto-Split* command on the *Count/Size* window's *Edit* menu to automatically analyze every counted object and split any detected clusters into individual objects (note that Auto-Split will evaluate *all* objects in your image or AOI—if you want to selectively split objects, use the *Split Objects* command above).

The *Auto-Split* command performs its splitting process by looking for concavities in objects. It works best on clusters created by round objects that overlap by no more than 1/3. Please refer to the **Measuring and Counting** discussion in *Section 1* for more about clustering criteria.

When you select the *Auto Split* command, the following message box is presented:

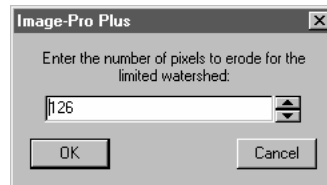


Click **Yes** to continue with the **Auto Split** operation, or **No** to cancel it.

- 
- ◆ **Watershed Split:** Use the *Watershed Split* command on the *Count/Size* window's *Edit* menu to automatically analyze every counted object and split clusters using the *Watershed* separation technique. The *Watershed* method erodes objects until they disappear, then dilates them again such that they do not touch.

The *Watershed Split* command may be more effective than the *Auto-Split* method if your objects are not convex.

- 
- ◆ **Limited Watershed Split:** Use the *Limited Watershed Split* command on the *Count/Size* window's *Edit* menu to set a limit on the number of pixels to erode for a watershed split. The *Limited Watershed* method erodes objects only up to a certain point. When you select this command, you will see the following dialog box:



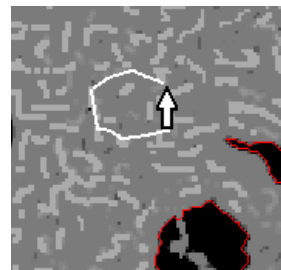
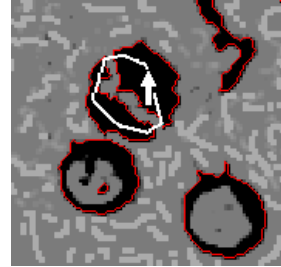
Enter the number of pixels to erode for the limited watershed split, and click **OK**.

- 
- ◆ **Draw/Merge :** Use the *Draw/Merge* command on the *Count/Size* window's *Edit* menu to manually create an object outline or merge individual counted objects into one. The basic steps involved when using the *Draw/Merge* command are:

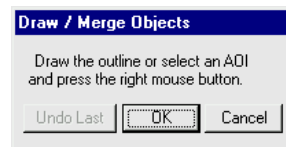
1. **Draw an AOI** that defines the object(s) you want to merge or create (you will probably want to use the **Freeform** AOI tool for maximum flexibility, but any of the AOI tools can be used).

You can combine objects by drawing the AOI to connect the perimeters of the objects (example, right). Or you can define an entirely new object by simply drawing an AOI over the background (lower example).

Please refer to the *Measuring and Counting* discussion in *Section 1* for more about defining AOI's to group objects for *Draw/Merge*.



2. Select the **Draw/Merge** command to create a counted object from the AOI you have defined. When you select the *Draw/Merge* command, the **Outline** dialog box and the **Draw/Merge Objects** message box are presented and the freeform AOI tool is activated.



If your AOI is already defined (as described in Step 1), click **OK**. If you have not yet defined an AOI, or are not satisfied with the one you have defined, define one now. Then click **OK**.

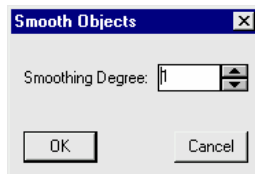
Once you click **OK**, *Image-Pro* will combine the objects, or add the new object to the count.

- ◆ **Convert AOI to Object:** The *Convert AOI to Object* command finds any AOIs or Multiple AOIs outlined in the image and converts them to objects. The AOI is then hidden to display the new objects. This command is only available when you have created AOIs before selecting the *Count/Size* function.

- ◆ **Smooth Objects:** Use the *Smooth Objects* command on the *Count/Size* window's *Edit* menu to smooth objects that have already

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been counted. When the *Smooth Objects* command is selected, the following dialog box is issued:

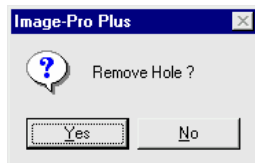


Enter a smoothing value from 0 (no smoothing) to 100 (maximum smoothing). This command performs the same smoothing as the **Smoothing** option in the **Count/Size Options** dialog box, but is done without recounting your objects (you won't lose any object edits, i.e., split, merge, hide, that have been done).

See also *Smoothing* in the discussion of the *Count/Size Options* dialog box.

- 
- ◆ **Remove Holes** : Use the *Remove Holes* command on the *Count/Size* window's *Edit* menu to remove counted objects that are embedded within other counted objects, *and* count as the area *all* pixels encompassed by the perimeter of an object, not just those falling within the selected intensity range.

When the *Remove Holes* command is selected, the following dialog box is issued:



Click **Yes** to proceed. This command performs the same process as the **Fill Holes** option in the **Count/Size Options** dialog box, but is done without recounting your objects (you won't lose any object edits, i.e., split, merge, hide, that have been done).

---

**Note:** The **Remove Holes** command does not literally remove, or fill, the holes in your objects. It does, however, remove embedded objects from the count and ensure that the entire area within an object is included in its area measurement. See also *Fill Holes* in the discussion of the *Count/Size Options* dialog box.

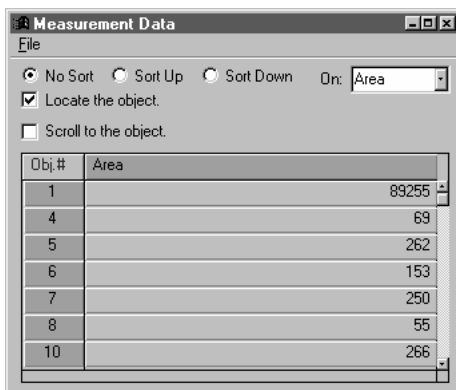
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- ◆ **Toggle Objects On/Off:** Use this command to show or hide objects in your image. When you left-click the mouse cursor on an object, its overlay is hidden, so that object will not be taken into account in any measurements or statistics. Left-click the mouse cursor again to display the object.
- ◆ **Delete Hidden Objects:** Use this command to remove objects that you have hidden, or that are out of the selected range. When you click this command, you will see a message asking you if you really want to remove these objects. Click **Yes** to remove the objects. The remaining objects in the image will be renumbered.

## Count/Size View Menu

Use the commands on the *Count/Size : View* menu to view the data and statistics associated with the counted objects in the active window.

- ◆ **Measurement Data:** Use the *Measurement Data* command to display the **Measurements** window. The **Measurements** window contains the measurement data sheet for objects in the active image window.



The **Measurements** window, like other windows, can be moved by dragging its title bar. It can be enlarged and reduced by dragging its borders. Use the scroll buttons along the bottom and left side to scroll through your data.

Only measurements for the active objects are listed in the data sheet. And, its contents are dynamic—the measurements will change as you activate/deactivate objects, or add/remove measurement types.

The **Sort** options on this dialog box allow you to leave it in object number order (No Sort), or sort your measurement information in ascending or descending order, using your choice of measurements for the sort (using the **On** list box).

**Locate the object:** As you examine the measurement data, you may want to view a specific object. To do so, click this check box, then select the object from the **Measurement Data** window (click anywhere on the row for that object's data). A radiating signal identifies the object if it is in view.

---

**Note:** If the **Measurement Data** window is covering up your image, you may need to drag it aside, in order to see the located object.

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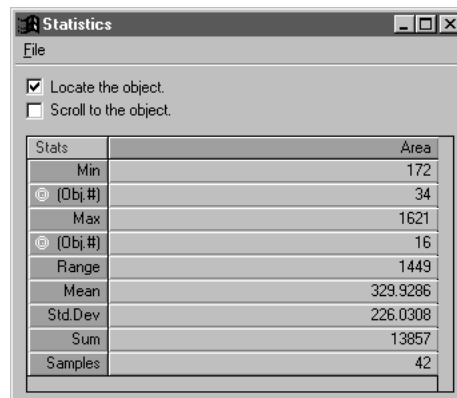
**Scroll to the object:** If you want to locate an object that may not be in view, click this check box before you select the object (see *Locate the object*, above). *Image-Pro* finds the objects, scrolls your image to center it in your view, and signals its location.

If you do not check the **Scroll to the object** check box and the object you are locating is not visible, *Image-Pro* will signal this fact with a beep and a radiating flag at the side or corner of the image to which you should scroll, manually, in order to see the object.

- 
- ◆ **View : Measurement Data : File Menu:** The options on this menu allow you to print, save, append, or copy this information to the Windows clipboard.
    - ◆ **Data to File :** Select the *Data to File* command to save the current measurement data sheet as an attachment to a file.
    - ◆ **Append Data to File :** Select the *Append Data to File* command to store the current measurement data sheet as an attachment to an existing file. The measurement file can be saved to an ASCII file, or to a LOTUS 123™ WK1 spreadsheet file. In either case, the file will contain a measurement record for each object in the count. The saved data can be imported into a spreadsheet application or used as input to an external program.
    - ◆ **Data To Clipboard** Select the *Data To Clipboard* command to copy the current measurement data to the Windows Clipboard. From there, the data can be pasted into any application that accepts text from the Clipboard.
    - ◆ **Data To Printer :** Select the *Data To Printer* command to send the current measurement data to your printer.
    - ◆ **Table To Clipboard:** Select the *Table To Clipboard* command to copy the current measurement data to the Windows Clipboard in table format. From there, the data can be pasted into any application that accepts text from the Clipboard.
    - ◆ **Data Export:** Select the *Data Export* command to transfer data to Microsoft's Excel spreadsheet. For additional information about DDE, see the *Dynamic Data Exchange* discussion.
    - ◆ **Export Options :** Select the *Export Options* command to tell *Image-Pro* how and where to export the current data

via DDE. For additional information about DDE, see the *Dynamic Data Exchange* discussion.

- ◆ **Statistics tics:** Use the *Statistics* command on the *Count/Size* window's *View* menu to display the statistic data sheet for the active window. The statistic data sheet contains a table of statistics for the measurements that have been made of the active objects. It contains information such as Minimum, Maximum and Mean values for each measurement type selected in the *Select Measurements* command. When you select the **Statistics** command, a **Statistics** window is opened. It contains the statistics data sheet for the objects in the active image window.



The screenshot shows a window titled "Statistics" with a menu bar containing "File". Below the menu bar are two checkboxes: "Locate the object" (checked) and "Scroll to the object" (unchecked). The main area contains a table with two columns: "Stats" and "Area".

Stats	Area
Min	172
Ⓞ (Obj.#)	34
Max	1621
Ⓞ (Obj.#)	16
Range	1449
Mean	329.9286
Std.Dev	226.0308
Sum	13857
Samples	42


The **Statistics** window can be moved by dragging its title bar. It can be enlarged and reduced by dragging its borders. Use the scroll buttons, if there are any, to scroll through your data.

The following statistics are listed for each measurement you have made:

- ◆ **Min:** This row lists the smallest value for each measurement you have specified. The object possessing the minimum measurement is identified, by number, below the stated minimum.
- ◆ **Max:** This row lists the largest value for each measurement you have specified. The object possessing the maximum measurement is identified, by number, below the stated maximum.
- ◆ **Range:** This row lists the difference between the maximum and minimum values for each measurement you have specified.

- ◆ **Mean:** This row lists the average (mean ) for each measurement you have specified.
- ◆ **Std. Dev:** This row lists the standard deviation for each measurement you have specified.
- ◆ **Sum:** This row lists the sum of the measurements for each measurement type (e.g., area, perimeter, etc.) you have specified.
- ◆ **Samples:** This row lists the total number of objects that have been included in the count from which these statistics were derived.

The contents of this window are dynamic—the statistics will change as you activate/deactivate objects, or add remove measurement types.

**Locate the object:** You can identify on your image any object that is the **Max** or **Min** object for a given statistic. To do so, click this check box, then select the object from the **Statistics** window (click on the object number in the **Obj.#** row; **Obj.#** rows are flagged with a symbol — ). A radiating signal identifies the object if it is in view.

---

**Note:** If the **Statistics** window is covering up your image, you may need to drag it aside, in order to see the located object.

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**Scroll to the object:** If you want to locate an object that may not be in view, click this check box before you select the object (see *Locate the object*, above). *Image-Pro* finds the objects, scrolls your image to center it in your view, and signals its location.

If you do not check the **Scroll to the object** check box and the object you are locating is not visible, *Image-Pro* will signal this fact with a beep and a radiating flag at the side or corner of the image to which you should scroll, manually, in order to see the object.

- 
- ◆ **View : Statistics : File Menu:** The options on this menu allow you to save, append, or copy this information to the Windows clipboard.
    - ◆ **Data to File:** Select the *Data to File* command to save the current statistics data sheet as an attachment to a file
    - ◆ **Append Data to File** Select the *Append Data to File*. command to store the current measurement data sheet as an attachment to an existing file. The measurement file can be saved to an ASCII file, or to a LOTUS 123™ WK1 spreadsheet file. In either case, the file will contain a

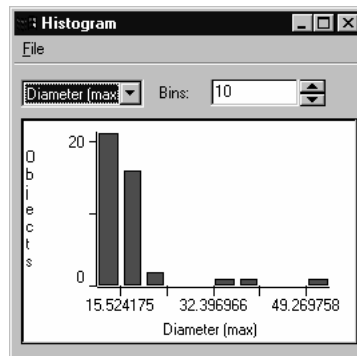
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measurement record for each object in the count. The saved data can be imported into a spreadsheet application or used as input to an external program.

- ◆ **Data To Clipboard** the *Data To Clipboard* command to copy the current measurement data to the Windows Clipboard. From there, the data can be pasted into any application that accepts text from the Clipboard.
- ◆ **Data To Printer :** Select the *Data To Printer* command to send the current measurement data to your printer.
- ◆ **Table To Clipboard:** Select the *Table To Clipboard* command to copy the current statistics data to the Windows Clipboard in table format From there, the data can be pasted into any application that accepts text from the Clipboard.
- ◆ **Data Export:** Select the *Data Export* command to transfer data to Microsoft's Excel spreadsheet. For additional information about DDE, see the *Dynamic Data Exchange* discussion.
- ◆ **Export Options :** Select the *Export Options* command to tell *Image-Pro* how and where to export the current data via DDE. For additional information about DDE, see the *Dynamic Data Exchange* discussion.

- ◆ **Histo gram:** Use the *Histogram* command on the *Count/Size* window's *View* menu to display measurement data in histogram form, where the X-axis represents the value of the measurement being graphed, and the Y-axis represents the number of objects with that measurement value.

When you select *Histogram*, a **Histogram** window is opened.



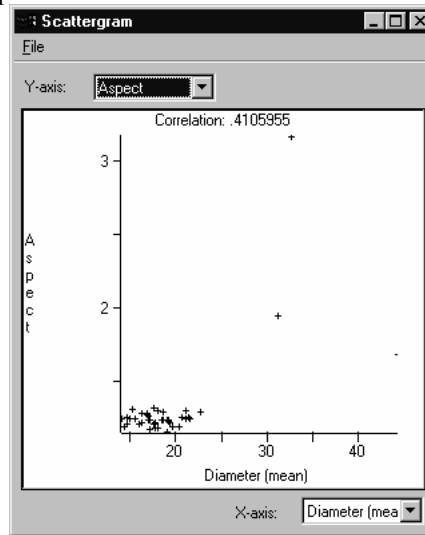
**Area:** From this list box, select the measurement type that you want to have graphed.

**Bins:** In this field enter the number of intervals you want the X-axis divided into.

- ◆ **View : Histogram : File Menu: Graph to Clipboard:** This menu allow you to copy this graph to the Windows clipboard.

- 
- ◆ **Scattergram:** Use the *Scattergram* command on the *Count/Size* window's *View* menu to plot two measurement types against each other.

When you select *Scattergram*, a **Scattergram** window is opened.



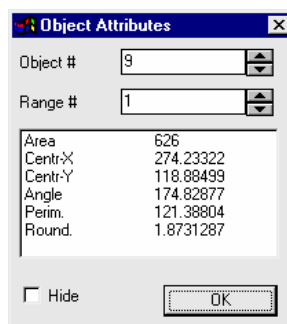
In the list box in the upper-left corner, select the measurement type you want plotted along the Y-axis.

In the list box in the lower-right corner, select the measurement type you want plotted along the X-axis.

- 
- ◆ **View : Scattergram : File Menu: Graph to Clipboard:** This menu allow you to copy this graph to the Windows clipboard.

- 
- ◆ **Object Window:** Use the *Object Window* command on the *Count/Size* window's *View* menu to view the measurements for a specific object. You can also use the *Object Window* command to deactivate an object (remove it from the list of measurements) and reactivate an object.

When the *Object Window* command is selected, the *Object Attributes* window is displayed:



**Object #:** In this field, enter the number of the object for which you want to view measurements. You may type the number in, or use the spin buttons to select it. When an object number has been entered, the measurements for that object are displayed in the window.

**Range #:** In this field, enter the number of the object's range for which you want to view measurements. You may type the number in, or use the spin buttons to select it. When a range and object number have been entered, the measurements for that object are displayed in the window.

---

**Note:** You will not be able to change this field if you did not select ranges or colors manually.

---

**Hide:** Check this box if you want to deactivate an object. When an object is deactivated, its measurements are removed from the measurement list (and any statistics or graphs that are derived from the active measurements). Its outline and label are cleared from the screen.

---

**Note:** This action does not delete an object, it merely deactivates it. The object can be reactivated by selecting it again with the **Object Attribute** command, and unmarking the **Hide** box.

You can invoke the **Object Attributes** dialog box, and you can show or

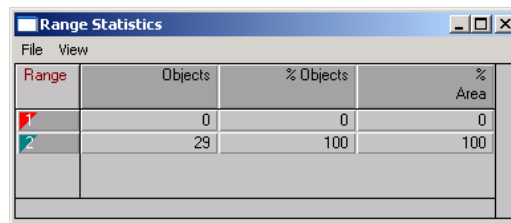
hide an object, even when the **Count/Size** dialog box is not open. After you have completed the count process, right-click on the object you want to query; a pop-up menu appears.



---

Select **Object Info** to bring up the **Object Attributes** dialog box. Select **Hide** or **Show** to hide or show the object.

---

- ◆ **Ranges Statistics:** If color intensity ranges are defined manually, the **Ranges Statistics** featured is enabled. You must define at least two ranges. The Range Statistics report each measurement for each range of colors, which define different objects, as shown here:



Range	Objects	% Objects	% Area
	0	0	0
	29	100	100

- ◆ **View : RangesStatistics : File Menu:** The options on this menu allow you to save, append, or copy this information to the Windows clipboard.
  - ◆ **Data to File:** Select the *Data to File* command to save the current statistics data sheet as an attachment to a file
  - ◆ **Append Data to File** Select the *Append Data to File* command to store the current measurement data sheet as an attachment to an existing file. The measurement file can be saved to an ASCII file, or to a LOTUS 123™ WK1 spreadsheet file. In either case, the file will contain a measurement record for each object in the count. The saved data can be imported into a spreadsheet application or used as input to an external program.
  - ◆ **Data To Clipboard** the *Data To Clipboard* command to copy the current measurement data to the Windows Clipboard. From there, the data can be pasted into any application that accepts text from the Clipboard.
  - ◆ **Data To Printer :** Select the *Data To Printer* command to send the current measurement data to your printer.
  - ◆ **Table To Clipboard:** Select the *Table To Clipboard* command to copy the current statistics data to the Windows Clipboard in table format From there, the data



can be pasted into any application that accepts text from the Clipboard.

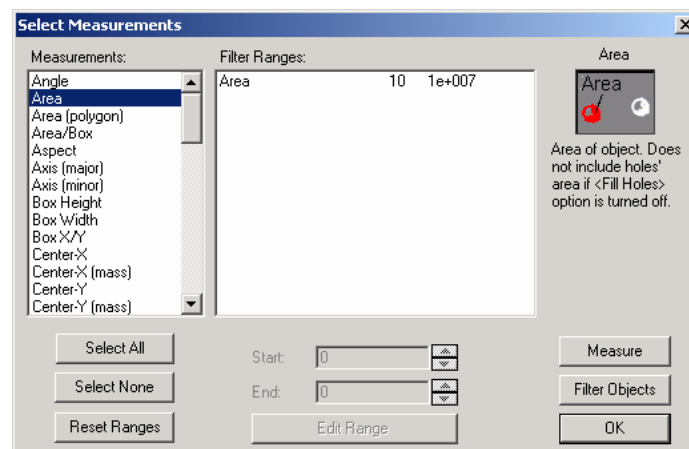
- ◆ **Data Export:** Select the *Data Export* command to transfer data to Microsoft's Excel spreadsheet. For additional information about DDE, see the *Dynamic Data Exchange* discussion.
  - ◆ **Export Options :** Select the *Export Options* command to tell *Image-Pro* how and where to export the current data via DDE. For additional information about DDE, see the *Dynamic Data Exchange* discussion.
- 
- ◆ **View : RangesStatistics : View Menu:** The options on this menu allow you to view a variety of measurements for the objects in your selected ranges. The available options change depending on which measurements you have selected in the main Count/Size dialog.

## Count/Size Measure Menu

Use the commands on the *Count/Size : Measure* menu to select and modify the kinds of measurements you want to make upon your counted objects. This menu is also used to perform population density and cluster analysis operations.

- ◆ **Select Measurements** : Use the *Select Measurements* command to specify the types of measurement data you want to record when your objects are counted. You may select as many of these measurement types as you need (they can all be selected simultaneously if needed). You also use the *Select Measurements* command to specify the limits by which *Image-Pro* will exclude objects from the count. When a range for a measurement is selected, and the **Apply Ranges** option in the *Count/Size* window is enabled, only objects that fall within the range (min and max inclusive) are included in the count and are recorded on the measurement data sheet.

When you choose the *Select Measurements* command, the **Select Measurements** dialog box is displayed:



**Measurements:** Use this list box to select types of measurements. Click on the measure you want taken upon the counted objects. A measure will be highlighted when it is selected. Clicking on a selected measure will deselect it..

**Select All:** Click this button activate all the measurements for your image.

**Select None:** Click this button to clear all active measurements.

**Reset Ranges:** Clicking this button resets all the measurements to their “factory defaults.”

Click **OK** to return to the *Count/Size* menu, or **Measure** to perform the specified measurement upon the counted objects.

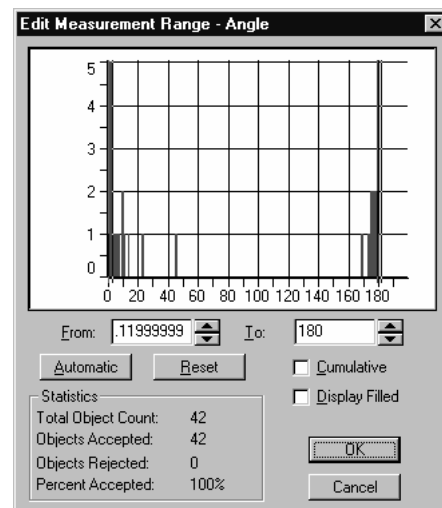
---

**Note:** Clicking **OK** will not perform the specified measurements. Measurements are not taken until you select **Count** in the **Count/Size** window, or either **Measure** or **Filter Objects** in the **Select Measurements** dialog box.

---

Clicking **Measure** will remeasure all the counted objects in your image or AOI and update the measurement data sheet with the selected measurements. If a measure has been deselected, its measurement values will be removed from the measurement data sheet. If the **Apply Ranges** option on the *Count/Size* menu is enabled, only objects meeting the criteria set forth in the **Set Ranges** dialog box will be counted and measured.

**Edit Ranges:** Use button to set or modify a measurement range, in order to restrict the values that are measured for an object. Clicking this button displays the **Edit Measurement Ranges** dialog box:



Use the settings on this dialog to adjust the values for the measurement range(s). **Cumulative** will add a series of range measurements together. **Display filled** will color in the objects in that range on your active image.

Click **OK** to return to the *Count/Size* menu, or **Filter Objects** to apply a filter to the measurements for the counted objects.

## Measure:Count/Size

**Start/End:** Enter the minimum and maximum allowable values for the selected measure. *Image-Pro* will count and record data for only those objects whose measurements equal or fall between the two values you specify here.

---

**Note:** Only measurements that have been specified with the **Select Measurements** dialog box can be selected and limited with **Set Ranges**. If the measure you want to limit is not listed by **Set Ranges**, you will need to enable it in the **Select Measurement** list box.

---

**Filter Objects:** Clicking **Filter Objects** will count only the objects meeting the specified criteria and update the measurement data sheet with the selected measurements. This **Filter Objects** button ignores the status of the **Apply Ranges** option in the main *Count/Size* window. When you click **Filter Objects** within this dialog box, *Image-Pro* always applies the specified limiting criteria to the count.

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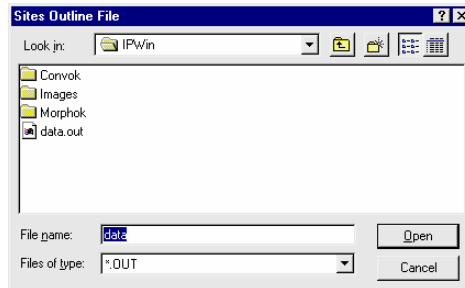
◆ **Population Density:** Use the *Population Density* command on the *Count/Size* window's *Measure* menu to overlay a set of previously stored outlines onto the current image, and calculate the object density within them.

Before selecting the *Population Density* command, you must have already performed the following steps:

- 1) **Created a set of site outlines** and stored them into an outline file. The site outlines define the areas for which population density will be determined.
- 2) **Counted the individual objects** for which density is being measured. This image must be the active image when the *Population Density* command is invoked.

If you are not familiar with the population density procedure, review the *Population Density Analysis* in *Chapter 9* of your *Image-Pro Plus Start-Up Guide*. It describes the entire population density process.

When you select the *Population Density* command, the **Sites Outline File** dialog box is displayed.



Select the outline you want to load, then click **OK**. The outlines from the selected file will be overlaid onto your active image, and the population density for each site will be displayed in the **Population Density** window.

Site #	Objects	Area	Raw Density	Cor. Density
1	2	82	.02439024	.02298045
2	2	125	.0160	.01459020
3	2	64	.031250	.02984020
4	2	122	.01639344	.01498364
5	2	131	.01526717	.01385738
6	2	102	.01960784	.01819805
7	3	218	.01376146	.01235167

Locate the site  
 Scroll to the site

The **Population Density** window can be moved by dragging its title bar. It can be enlarged and reduced by dragging its borders. Use the scroll buttons along the bottom and left side to scroll through your data. If you scroll to the bottom of the window, the following summary data are reported:

Site #	Objects	Area	Raw Density	Cor. Density
42	2	171	.01169590	.01028611
43	2	139	.01438849	.01297869
44	28	186	.15053764	.14912784
Mean	2.8181818	120.63636	.02336096	.02195117
Sum	124	5308	.02336096	.02195117
Backgnd	219	155342	.00140979	0
Total	343	160650	.00213507	.00072528

Locate the site  
 Scroll to the site

See *Population Density Analysis* in *Section 1* for a description of the values reported in the **Population Density** window.

## Measure:Count/Size

**Locate the site:** Select this check box if you want *Image-Pro* to signal the site location on the image. To do so, click this check box, then select the object from the **Population Density** window. A radiating signal identifies the object if it is in view.

*Note - If the **Population Density** window is covering up your image, you may need to drag it aside, in order to see the located object.*

**Scroll to the site:** If you want to locate an object that may not be in view, click this check box and the **Locate the site** check box before you select the object (see *Locate the object*, above). *Image-Pro* finds the objects, scrolls your image to center it in your view, and signals its location.

- 
- ◆ **Measure : Population Density : File Menu:** The options on this menu allow you to save, append, or copy this information to the Windows clipboard.
    - ◆ **Data to File:** Select the *Data to File* command to save the current statistics data sheet as an attachment to a file
    - ◆ **Append Data to File** Select the *Append Data to File* command to store the current measurement data sheet as an attachment to an existing file. The measurement file can be saved to an ASCII file, or to a LOTUS 123™ WK1 spreadsheet file. In either case, the file will contain a measurement record for each object in the count. The saved data can be imported into a spreadsheet application or used as input to an external program.
    - ◆ **Data To Clipboard** the *Data To Clipboard* command to copy the current measurement data to the Windows Clipboard. From there, the data can be pasted into any application that accepts text from the Clipboard.
    - ◆ **Data To Printer :** Select the *Data To Printer* command to send the current measurement data to your printer.
    - ◆ **Table To Clipboard:** Select the *Table To Clipboard* command to copy the current statistics data to the Windows Clipboard in table format From there, the data can be pasted into any application that accepts text from the Clipboard.
    - ◆ **Data Export:** Select the *Data Export* command to transfer data to Microsoft's Excel spreadsheet. For additional information about DDE, see the *Dynamic Data Exchange* discussion.

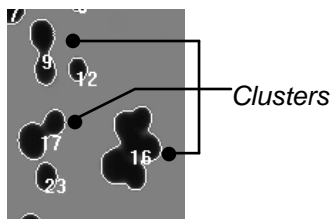
*Measure:Count/Size*

- ◆ **Export Options** : Select the *Export Options* command to tell *Image-Pro* how and where to export the current data via DDE. For additional information about DDE, see the *Dynamic Data Exchange* discussion.

- ◆ **Clusters:** Use the *Clusters* command on the *Count/Size* window's *Measure* menu to perform a cluster analysis of your counted objects. During cluster analysis, *Image-Pro* examines all of the counted objects, identifies objects that are clusters of single objects, and estimates the number of objects in the clusters.

**Important** - The **Cluster Analysis** command works by analyzing counted objects. Therefore, you must perform a **Count** before you perform **Cluster Analysis**. Your counted objects must include both the clusters you want analyzed and several typical single objects.

In the following example, objects 9, 16 and 17 are actually clusters of single objects. A cluster analysis of this image would provide an estimate of the number of single objects contained in these three clusters.



When you select the **Clusters** command, the counted objects are analyzed, and the analysis results are presented in the **Cluster Info** window:

Cluster Information	
File	
Typical object area in:	Pixels
Original count	42
Single objects	39
Clusters	3
Objects in clusters	9
Total objects	48
Typical object area	280.0256

This window provides the following information:

- ◆ **Original Count:** This value reports the number of objects detected by the **Count** process. This number reflects a cluster as a single object.
- ◆ **Single Objects:** This value reports the number of non-overlapping objects in the original count.

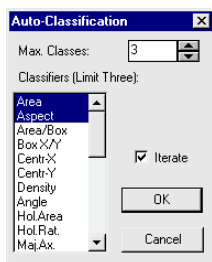


- ◆ **Clusters:** This value reports the number of groups of objects that have been counted as one, in the original count.
  - ◆ **Objects in Clusters:** This value reports the number of single objects encompassed within the clusters.
  - ◆ **Total Objects:** This value reports the total number of objects in the image, as determined by **Single Objects + Objects in Clusters**.
  - ◆ **Object Type Area:** This value reports the average area for a single object.
- 
- ◆ **Measure: Clusters: File Menu:** The options on this menu allow you to save, append, or copy this information to the Windows clipboard.
    - ◆ **Data to File:** Select the *Data to File* command to save the current statistics data sheet as an attachment to a file
    - ◆ **Append Data to File** Select the *Append Data to File* command to store the current measurement data sheet as an attachment to an existing file. The measurement file can be saved to an ASCII file, or to a LOTUS 123™ WK1 spreadsheet file. In either case, the file will contain a measurement record for each object in the count. The saved data can be imported into a spreadsheet application or used as input to an external program.
    - ◆ **Data To Clipboard** the *Data To Clipboard* command to copy the current measurement data to the Windows Clipboard. From there, the data can be pasted into any application that accepts text from the Clipboard.
    - ◆ **Data To Printer :** Select the *Data To Printer* command to send the current data to your printer.
    - ◆ **Table To Clipboard:** Select the *Table To Clipboard* command to copy the current statistics data to the Windows Clipboard in table format From there, the data can be pasted into any application that accepts text from the Clipboard.
    - ◆ **Data Export:** Select the *Data Export* command to transfer data to Microsoft's Excel spreadsheet. For additional information about DDE, see the *Dynamic Data Exchange* discussion.
    - ◆ **Export Options :** Select the *Export Options* command to tell *Image-Pro* how and where to export the current data

via DDE. For additional information about DDE, see the *Dynamic Data Exchange* discussion.

- ◆ **Auto Classification:** Use the *Auto Classification* command on the *Count/Size* window's *Measure* menu to automatically categorize your counted objects into groups with similar measurements. Before performing an *Auto Classification* you must have performed a count on the objects you want categorized.

When you select the *Auto Classification* command, the **Auto Classification** dialog box is presented. In it, select the measurement types to be used as classifiers, and the maximum number of categories into which you want the data divided. Then select **OK**.



**Classifiers:** In this list box, select the measurement types by which you want the objects classified. *Image-Pro* will analyze the data for clusters created when these measurement values are plotted against each other, and divide the data accordingly. Only *three* classifications are allowed at one time.

**Max. Classes:** In this field, enter the maximum number of categories into which you want the measurement data categorized. You may have a maximum of *16* classes. Depending upon the number of clusters *Image-Pro* identifies, fewer classes than the number specified here may actually be produced. However, the number of classes will never exceed the specified “Max. Classes” value.

**Iterate:** Check this box if you want *Image-Pro* to repeat and refine the classification process until there is no change in the results.

When you click **OK**, the selected object measurements are classified, and the results are presented in the **Classification** window:

*Measure:Count/Size*

Class	Objects	% Objects	Mean Area	Mean Aspect
1	25	22.32143	18.240	4.840847
2	87	77.67857	128.8506	1.381957

This window provides the information that is checked in the view menu (see below). If nothing is checked, only the object class and object count are given.

- ◆ **Class:** This number identifies the class.
- ◆ **Objects:** This value reports the number of objects in the class.

---

*Note:* Once objects have been classified, they can be color-coded by their class. To do this, select the *Class* outline option in the **Count/Size** dialog box.

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- 
- ◆ **Measure: Classification : File Menu:** The options on this menu allow you to print, save, append, or copy this information to the Windows clipboard.
  - ◆ **Data to File:** Select the *Data to File*. command to store the current measurement data sheet to disk. The measurement file can be saved to an ASCII file, or to a LOTUS 123™ WK1 spreadsheet file. In either case, the file will contain a measurement record for each object in the count. The saved data can be imported into a spreadsheet application or used as input to an external program.
  - ◆ **Append Data to File:** Select the *Append Data to File*. command to store the current measurement data sheet as an attachment to an existing file. The measurement file can be saved to an ASCII file, or to a LOTUS 123™ WK1 spreadsheet file. In either case, the file will contain a measurement record for each object in the count. The saved data can be imported into a spreadsheet application or used as input to an external program.
  - ◆ **Data To Clipboard:** Select the *Data To Clipboard* command to copy the current measurement data to the Windows Clipboard. From there, the data can be pasted into any application that accepts text from the Clipboard.
  - ◆ **Data To Printer :** Select the *Data To Printer* command to send the current data to your printer.
  - ◆ **Table to Clipboard:** Select the *Table To Clipboard* command to copy the current measurement data in table format to the Windows Clipboard. From there, the data can be pasted into any application that accepts text from the Clipboard.
  - ◆ **Data Export:** Select the *Data Export* command to transfer data to Microsoft's Excel spreadsheet. For additional information about DDE, see the *Dynamic Data Exchange* discussion.
  - ◆ **Export Options :** Select the *Export Options* command to tell *Image-Pro* how and where to export the current data via DDE. For additional information about DDE, see the *Dynamic Data Exchange* discussion.
  - ◆ **Close:** Select the *Close* command to close the **Classification** window.

- 
- ◆ **Measure: Classification : View Menu:** The options on this menu allow you to display your choice of measurements in the **Classification** window.
    - ◆ **Object Count:** This number identifies the class.
    - ◆ **Objects:** This value reports the number of objects in the class..
    - ◆ **%Object:** This value reports the percentage of objects in the class..
    - ◆ **Sum <measure>:** This value reports, for all objects in the class, the sum of each measurement you selected under *Count/Size : Measure : Select Measurements..*
    - ◆ **% <measure>:** This value reports, for all objects in the class, the percentage for each measurement you selected under *Count/Size : Measure : Select Measurements..*
    - ◆ **Mean <measure>:** This value reports, for all objects in the class, the mean for each measurement you selected under *Count/Size : Measure : Select Measurements..*
    - ◆ **Std.Dev <measure>:** This value reports, for all objects in the class, the standard deviation for each measurement you selected under *Count/Size : Measure : Select Measurements.*
    - ◆ **Min <measure>:** This value reports, for all objects in the class, the smallest object for each measurement you selected under *Count/Size : Measure : Select Measurements.*
    - ◆ **Max <measure>:** This value reports, for all objects in the class, the largest object for each measurement you selected under *Count/Size : Measure : Select Measurements.*
    - ◆ **Class Range:** This value reports, for all objects in the class, the range limits of each measurement you selected under *Count/Size : Measure : Select Measurements.* This option is available only in conjunction with the *Single Variable Class.* command.
    - ◆ **View All:** Check **View All** if you want to check all the measurement types for viewing.
    - ◆ **View Default:** Check **View Default** if you want to check just the default measurement types for viewing. The

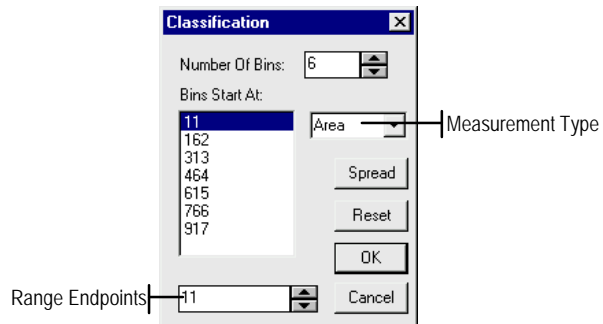
default selections depend on whether you have selected the *Single Variable Class.* or the *Auto Classification* command.

- ◆ **Single Variable Class.:** Use the *Single Variable Class* command on the *Count/Size* window's *Measure* menu to categorize your counted objects by a single measurement type (to classify by multiple measurements, see the *Auto Classification* command above).

The *Single Variable Class.* command lets you group objects, on an increasing scale, by their measurement values. For example, you might group classify your objects by their area measurement, dividing them into 4 categories.

Before performing a *Single Variable Class.*, you must have performed a count on the objects you want categorized.

When you select the *Single Variable Class.* command, the *Classification* dialog box is presented. In it, select the measurement type to be used as the classifier, and the maximum number of categories into which you want the data divided. Then select **OK**.



**Number of Bins:** In this field, enter the number of categories into which you want the selected measurement values divided. The beginning value for each category is displayed in the **Bins Start At** box.

**Measurement Type:** In this list box (located just above the **Spread** button) select the measurement type by which you want the objects categorized. Only measurement types for which you have actual measurement values will be listed here.

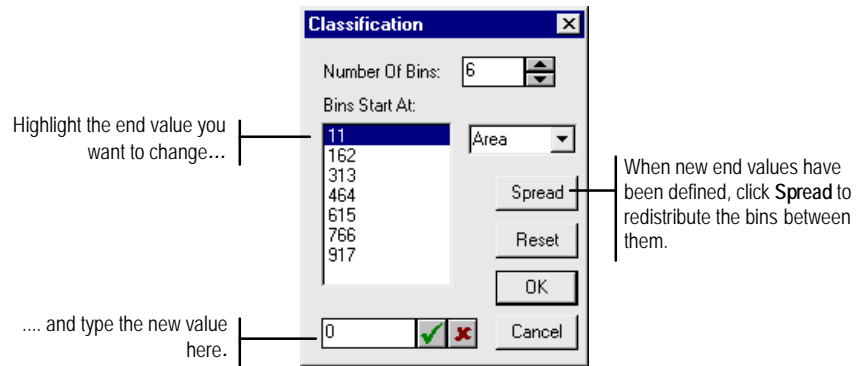
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**Note:** If the type of measurement by which you want to classify objects is not listed here, exit this dialog box, and select and measure it using the **Select Measurements** command.

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**Bins Start At:** This box will contain the equal intervals into which the measurement values will be divided for classification. The number of intervals contained in this box is determined by the value you have specified in the **Number of Bins** field. The first value in this box represents the beginning of the range (and the beginning of the first class), and the last value in this box represents the last value in the range. The values in between represent the beginning value for each class between these two values.

By default, the beginning and ending values in this box are derived from the minimum and maximum values contained in the set of measurement data you have chosen to classify. However, you may override these end values by highlighting the value you want to change, and typing a replacement value for it in field at the bottom of the dialog box (see illustration below). Once new beginning/ ending values have been selected, you can click **Spread** to distribute the bins equally between the new values.



You can also change any of the **Bins Start At** values by highlighting the value and entering a replacement for it in the box at the bottom of the dialog box.

**Spread:** Click this button to distribute the beginning bin values equally between the currently specified beginning and ending values (see illustration above).

**Reset:** Click this button to set the beginning and ending range values back to the min and max values in the set of measurement data to be classified (you would use this if you had manually changed these values in the **Bins Start At** list box, and wanted the original values back again).

---

**Measure: Classification : File Menu:** The options on this menu allow you to print, save, append, or copy this information to the Windows clipboard.

- ◆ **Data to File:** Select the *Data to File.* command to store the current measurement data sheet to disk. The measurement file can be saved to an ASCII file, or to a LOTUS 123™ WK1 spreadsheet file. In either case, the file will contain a measurement record for each object in the count. The saved data can be imported into a spreadsheet application or used as input to an external program.
- ◆ **Append Data to File:** Select the *Append Data to File.* command to store the current measurement data sheet as an attachment to an existing file. The measurement file can be saved to an ASCII file, or to a LOTUS 123™ WK1 spreadsheet file. In either case, the file will contain a measurement record for each object in the count. The saved data can be imported into a spreadsheet application or used as input to an external program.
- ◆ **Data To Clipboard:** Select the *Data To Clipboard* command to copy the current measurement data to the Windows Clipboard. From there, the data can be pasted into any application that accepts text from the Clipboard.
- ◆ **Data To Printer :** Select the *Data To Printer* command to send the current data to your printer.
- ◆ **Table to Clipboard:** Select the *Table To Clipboard* command to copy the current measurement data in table format to the Windows Clipboard. From there, the data can be pasted into any application that accepts text from the Clipboard.
- ◆ **Data To Printer :** Select the *Data To Printer* command to send the current data to your printer.
- ◆ **Data Export:** Select the *Data Export* command to transfer data to Microsoft's Excel spreadsheet. For additional information about DDE, see the *Dynamic Data Exchange* discussion.
- ◆ **Export Options :** Select the *Export Options* command to tell *Image-Pro* how and where to export the current data via DDE. For additional information about DDE, see the *Dynamic Data Exchange* discussion.
- ◆ **Close:** Select the *Close* command to close the **Classification** window.

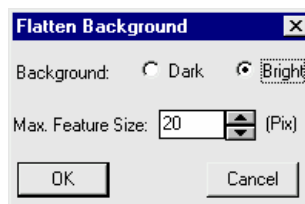


## Count/Size Image Menu

Use the commands on the *Count/Size* window's *Image* menu to perform pre-processing functions that can prepare your image for a more successful count/size operation. You might use the options on this menu to better distinguish objects from background, or to separate objects or particles that touch.

- 
- ◆ **Flatten Background:** Use the *Flatten Background* command to even out backgrounds that contain the same intensities as the objects you want to count. Backgrounds containing such intensities make it difficult to isolate just the objects you want to count. The *Flatten Background* command filters the background and reduces the intensity variations within it.

When you select the *Flatten Background* option, the **Flatten Background** dialog box is presented:



**Background:** Select the option, **Dark** or **Bright**, that describes the background of your image. Usually you will select the intensity that specifies the field upon which your objects lie. However, if your objects are large or numerous and they occupy most of the area within your image or AOI, this option should be set to describe your objects.

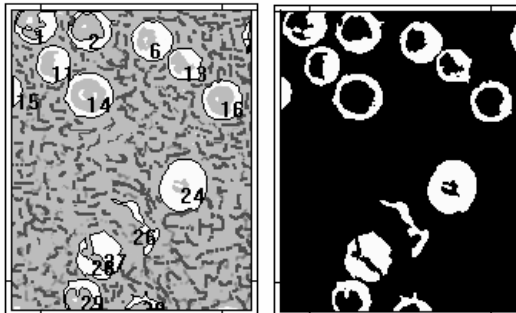
**Max. Feature Size:** Enter the approximate diameter of the largest object in your image or AOI. This value is used by the *Flatten Background* operation to distinguish objects from background. When selecting a **Max. Feature Size** value, it is better to estimate high rather than low. Selecting a value that is too large will increase processing time, but will not adversely affect the image. Selecting a value that is too low may produce unwanted results.

---

**Note:** Because of the large filtering kernel that is used by this operation, some distortion will be introduced at the edges of your image or AOI.

---

- 
- ◆ **Make Mask:** Use the *Make Mask* command on the *Count/Size* window's *Image* menu to create a gray scale image in which only the counted objects are shown. In the example below, an image containing just the counted spores has been created.



Before using the *Make Mask* command in the active image, you must have performed a count, or have loaded the saved outlines from a previous count. The mask will be created from the objects that the count describes. Pixels that are part of a counted object will be rendered in white; all others in black.

- 
- ◆ **Make Sorted Objects Image:** Use this command to generate an image of the counted objects sorted according to size (or other criteria). For details, please see the *Object Sorting* module described in the next section.

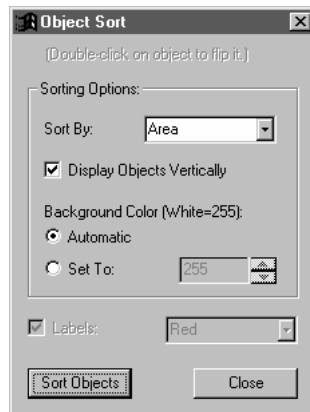
## Sort Objects

---

Use the *Sort Objects* command to order objects by size or any other available measurement. When the sorting operation is complete, objects are copied to a new image where they appear in the order selected.

To sort them, objects must first be counted and measured using a *Count/Size* operation. Any measurement type selected in *Count/Size* can be used to order them. During the sorting process, objects can be rotated automatically so that their major axes are aligned vertically. A new image is generated with the objects sorted according to the selected measurement and positioned in rows.

When you select *Sort Objects*, you will see the following dialog:



**Sorting Options:** The options in this group box allow you to specify how your objects will be sorted and displayed.

**Sort By:** Use the items in this drop-down list box to select which measurement to use for the sort. Only those measurements selected during the count/size phase will be presented.

**Display Objects Vertically:** Use this check box to select whether objects will be presented in vertical columns in the sorted image or in their original orientation.

**Background Color:** Use these two radio buttons and the spin box to set the gray scale index of the background of the sorted image. You are limited to 256 levels of grayscale, regardless of the image class. The values will be scaled for 12- and 16-bit images.

**Automatic:** If you click this button, the background index is calculated as the average gray level around the objects.

*Measure:Sort Objects*

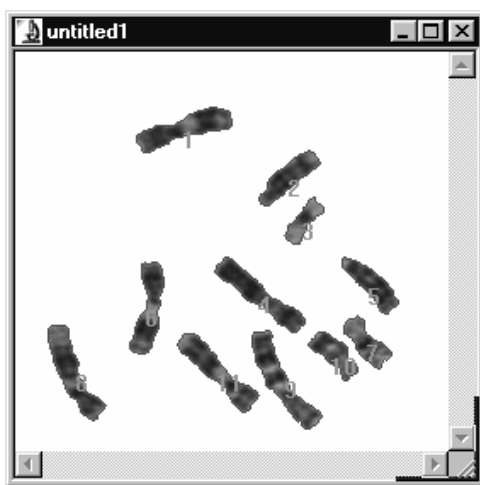
**Set To:** If you click this button, you can set the background index to a number that you choose by using the spin box.

**Labels:** Check this box to turn on and off object labels in the sorted image. Objects labels are the same as those assigned by Count/Size.

**Red:** Label color is red by default but a new color may be selected here.

**Sort Objects:** Click this button to start the sorting process.

**Close:** Click this button to close the dialog.



*Original Image*



*Sorted Image*

Double-clicking on an object in the sorted image will flip it (rotated 180 degrees) in case the sorting process positioned the object upside-down. Objects in the sorted image can be dragged to other locations in the sorted image or in other images. The shape of the cursor indicate whether an object can be dropped under the cursor or not. Objects are not allowed to overlap other sorted objects during drag and drop. Note that you cannot drag a sorted object of one class into a different class.

## Measurements

---

The *Measurements* commands and tools appear in two groups: *basic* and *advanced*. The *basic* measurements are identical to the *Measurements* module in previous versions of *Image-Pro Plus*. Two additional tools, the **Pitch** tool and the **Count** tool, have been added to the *advanced* measurements.

The basic steps involved in taking measurements are:

- 1. Selecting the measurement tool you need** to obtain your measurement. This is done by selecting the appropriate tool button (point, line, circle, etc.) .
- 2. Taking the actual measurement** in your image. Each tool has its own way of obtaining a measurement—you might stretch a defining line to obtain a straight-line length, select two existing features to measure the intervening distance, or trace an object to obtain an area. The process for each is defined in the tool descriptions below.
- 3. Collecting and saving the measurements** in the data sheet. When you take a measurement, the measurement is highlighted and numbered in the image, and the result is placed in the **Measurements** data sheet. You may take as many measurements as you need. The values of each will be recorded in the data sheet. You need to be in the advanced mode to see the **Measurements** page.

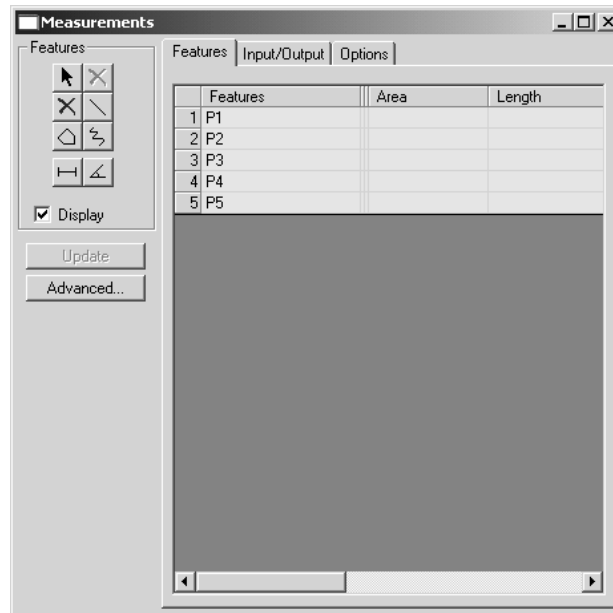
---

*Note:* To select more than one feature or measurement, press the <Ctrl> key while clicking on the feature (in the image) or row (on the Features or Measurement pages).

---

When you first choose *Measurements*, you will see the basic *Measurements* window:

## Measure:Measurements



There are three tabs or pages in the dialog box, which are organized in the order of workflow from left to right. The first tab is the **Feature** page. The second is the **Input / Output** page, and the last one is the **Options** page. This dialog is resizable, with a minimum size that accommodates the larger of the **Input / Output** and **Options** pages. When the dialog is opened, the last page used will be displayed.

Clicking the **Advanced** button displays additional tools and options, which are discussed later in this section.







Clicking the **Update** button recalculates all the measurements if the image calibration changes.

## Basic Measurements and Tools

The basic toolbar contains all the tools you will need to create the most common types of features and measurements:



*Note that Tool Tips for each of these tools will appear when you place your cursor on top of one of them.*

-  **Selector:** This button is used to select existing features and/or measurements, i.e. to move or delete them.
-  **Delete:** This button is used to remove all of the currently selected features or measurements from the image. All related measurements in the **Measurement** tab and the corresponding row(s) in the **Feature** tab will be removed. This button is enabled only when you have one or more features selected with the **Selection** tool.
-  **Delete All:** This button is used to remove all features and measurements from the image, whether they are selected or not. This button is enabled when you have one or more features on the image, and are using the selector tool.
-  **Straight Line:** This button is used to create a straight line feature on the current image. (This is the equivalent of the Length tool in previous versions of *Image-Pro Plus*.)
-  **Polygon:** This button is used to create a polygon feature on the current image. This will be created using the current trace/wand tool, and will always yield a closed figure. (This is the equivalent of the Area tool in previous versions of *Image-Pro Plus*.)
-  **Trace:** This button is used to create a trace on the current image. This will be created using the current trace tool, and will always yield a open figure. (This is the equivalent of the **Trace** tool in previous versions of *Image-Pro Plus*. See the **Trace** and **Magic Wand** tools earlier in this manual.)

## Measure:Measurements

Under these tool buttons, there are six measurement buttons. When one of these measurement tools are selected, you will be prompted to select the appropriate features. If two such features are already selected, the measurement will be added immediately, otherwise the measurement is added when the second of two appropriate features is selected. The selection is then cleared, and you can continue to select features and add more measurements.



**Distance Measurement:** This button creates a measurement of the distance between two features.



**Create Click and Drag Angle Measurement:** This button is used to create two new lines and take an angle measurement between these two lines. The two lines do not need to intersect. The angle is measured from the first line drawn to the second line, in a counter-clockwise direction. Note that this tool is different from the five other measurement tools in that rather than selecting two features for the measurement, you will create two features, and then create the measurement derived from them.



**Add Angle Measurement:** This button creates a measurement of the angle between two existing line features. The angle is measured from the first line drawn to the second line, in a clockwise direction. (This is the equivalent of the **Angle** tool in previous versions of *Image-Pro Plus*.)



**Horizontal Thickness:** Adds a thickness measurements between two primarily horizontal lines or traces.



**Vertical Thickness:** Adds a thickness measurement between two primarily vertical lines or traces.



**Curve Thickness:** This button creates a measurement of the minimum or maximum distance between any two features in any direction.

**Display:** Checking this box allows you to show or hide the measurements on the image.

When any tool is selected (other than the selection and deletion tools), a modeless dialog will be displayed, prompting you for the correct action, i.e. click and drag a line, select two features for distance measurement, etc. Similar text will be displayed in the *Image-Pro* status bar. This dialog will be updated automatically when another tool is selected, and closed when the dialog is closed or the selection tool is selected.



---

**Note:** When *Measurement* tools are active, these tools receive all mouse movements and clicks on the image first. This means that tools such as the AOI manager, Zoom, and Pan do not receive mouse movements or clicks. If you wish to manipulate the image using these tools, you must first de-select the **Measurement** tool by clicking it again. Clicking on a active tool button will release it, turning off the tools and allowing other tools to receive mouse events.

---

## Features

The **Features** page is visible in both the basic and advanced Measurements. It is illustrated below in the basic format:

	Features	Area	Length	Radi
1	L1		0	
2	L2		0	
3	L3		146.2395	
4	PG1	7256	699.1219	

The **Features** page includes every possible measurement for all the features and measurements that you have created for the current image. You can resize the columns for a better fit, or to hide measurements that are not of interest. In basic mode, all the position column are hidden (the default). In the following example, the **Area** column has been hidden:

	Features	Center Pos.	A	Length	Radius	Start Pos.	End Pos.
1	L1	292		378.5657		104	480
2	L2	322		374.1925		136	510
3	L3	246		260.0692		116	376

You can edit the name of a feature by clicking on the name in that feature's grid row and typing the new name. If you are using the selection tool or most of the measurement tools, you can select a feature by clicking on the row number. Multiple rows can be selected holding down the <Ctrl> or <Shift> keys while clicking on the row numbers, or clicking and dragging the mouse across the appropriate rows.

## *Measure:Measurements*

When you have selected one or more features on the image or the **Features** page, using the selection tool, you can delete the feature and all of its related measurements by pressing the <Del> key, or clicking the **Delete** button in the Measurements toolbox.

An optional second grid can display statistics for the measurements – a control on the **Options** page determines whether the statistics grid is displayed or not. When the statistics grid is enabled, it will track the **Features** grid as the columns are scrolled and/or sized. The sizing bar between the two grids allows the user to determine how much of the **Features** page is used by each grid. The statistics grid will include calculated minimum, maximum, and mean values for each type of measurement, the range of the measurement (max - min), the sum of the measurements, the standard deviation, and the number of values being analyzed.

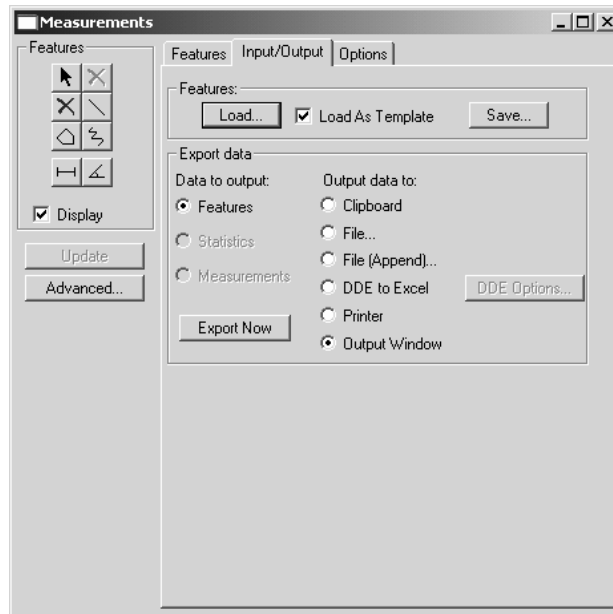
---

**Note:** All of the characteristics of the data overlay, including the font face and size, can be edited by double-clicking on the overlay itself. Please see the *Annotation* discussion for more information.

---

## Input/Output

The **Input / Output** page is visible in both the basic and advanced modes. It includes the ability to load or save measurements (the feature definitions and the selected set of measurements), to load outlines (features only), copy data to the clipboard, to file, and to export to Excel.



Each command on the **Input/Output** page is described below.

**Features:** The buttons in this group box allow you to load and save files containing feature outline, statistics, and measurement information.

**Load:** Click this button to load an outline or measurements file to the current image.

If you are loading an outline file ( \* . SCL ) the appropriate line, angle, trace, polygon, or thickness features will be created from the loaded outlines. Note that thickness features cannot be fully recreated from previously saved outline files. Line features will be created to represent the minimum and maximum distances originally measured.

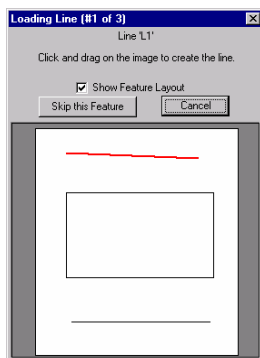
If you are loading a measurements file ( \* . msr ) the **Load as Template** option determines how the file will be loaded. **If Load as**

## Measure:Measurements

**Template** is NOT checked, the features and measurements from the file will be restored to the image. You would use this method to continue or reuse measurements from a previous session.

**Load as Template:** Click this button to load a file as template for future measurements. If you are loading a template file ( \*.tpl ) (**Load as Template** is checked) you will be prompted to make the measurements on the current image. You would use the Template option to create a set of template measurements on a new part of the sample. You could then reuse the template measurements in subsequent sessions.

The following dialog prompts you during the loading process:



This dialog indicates the type of feature to be measured, (i.e. “Line”), the feature’s name (‘L1’), instructions for its creation, and a representation of the features layout indicating the current feature in red. You can choose to **Skip this Feature**, which causes the load to continue with the next primary feature, or to **Cancel** loading. If loading is canceled, features already created and all dependent features and measurements will be retained – only the loading of the rest of the features will be canceled.

**Save:** Click this button to save the layout and measurements of the current image to a measurements file ( \*.msr ).

**Data to Output:** The selections in this area indicate the type of data you wish to save. Click the radio button to indicate your choice:

- ◆ **Features**
- ◆ **Statistics**
- ◆ **Measurements**

**Output Data to:** The selections in this area indicate where you want the output data to go. Click the appropriate radio button to indicate your choice:

**File:** Select this command if you want to save the measurement values (the results in the data sheet) to a file.

**File (Append):** Select this command if you want to append the measurement values to an existing file.

**Clipboard:** Use the *Measurement* window's **Copy To Clipboard** command to copy the current measurement data to the Windows Clipboard. Once on the Clipboard, the data can be pasted into any Windows application that accepts text from the Clipboard.

**Data Export:** Select the *Data Export* command to transfer data to Microsoft's Excel spreadsheet. Before selecting *Data Export*, you should use *Export Options* to determine the correct placement (within Excel) for the data. When you select *Data Export*, the transfer occurs with little visible evidence that it has happened. By switching to the Excel application, you can confirm that the transfer has occurred. For additional information about DDE, see the *Dynamic Data Exchange* discussion.

**Printer:** Click this radio button to send the data to your specified printer.

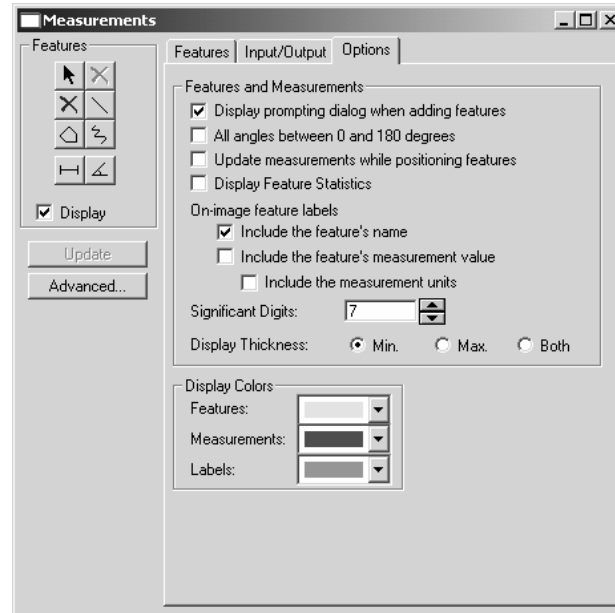
**Output Window:** This the button to send the data to the Output Window (see the Macro menu for a complete description).

**Export Now:** Outputs the data to clipboard, file or Excel, depends on the user's selection.

**Options :** Select the *Options* command to tell *Image-Pro* how and where to export the current data via DDE. For additional information about DDE, see the *Dynamic Data Exchange* discussion.

## Options

The basic **Options** tab includes a number of controls to set the default behavior of the *Measurements* module, and is illustrated below:



**Display prompting dialog when adding features** is used to determine whether the feature prompting dialogs are shown while creating new features (this does not affect the prompting during loading of a measurements file). The default for this option is checked, and will be unchecked if the user clicks **Do not display these prompts again** on the prompt dialog.

**All angles between 0 and 180 degrees** is used to set the reporting of angles. When checked, all angles will be reported between 0 and 180 degrees, otherwise the angles will be reported between 0 and 360 degrees.

**Update measurements while positioning features** is used to turn on updating of whichever grid is visible while objects are being positioned or resized. This involves recalculating measurements and possible updates of multiple rows, so for slower machines it is possible to suppress the update until the positioning is complete by leaving this box unchecked.

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**Display feature statistics** is used to control whether the *Feature Statistics* are shown on the **Features** page or are hidden.

**Include the Feature's Name:** Check this box to use the name of the feature as part of the label on the image.

**Include Feature's Measurement Value as its label on the image** creates a label on the image displaying the value of the measurements.

**Significant Digits:** Indicate the number of significant digits to use in the measurement values. The valid range is 3 – 20 digits.

**Include Measurement Units:** Check this box to include the measurement units as part of the label on the image.

**Display Thickness** is used to display minimum, maximum, or both thickness measurements on the image.





**Display Colors:** The **Feature**, **Measurement** and **Label** color combo boxes are used to set the respective colors used in drawing the overlay on the image. The possible selections in each combo box are Red, Green, Blue, Yellow, Cyan, Magenta, White and Black.

## Advanced Measurements and Tools

The advanced measurements add ten new tools to the basic set described in the previous section. The **Advanced** tools include best-fit measurements: line, circle, and arc; the rectangle, point, and circle measurement tools, as well as the pitch, count, data overlay, and count/size tools.





Note that best-fit features are defined by their points. If you need to move a best-fit feature, you will need to select all of the points in the feature to move it.


-  **Point:** This button is used to create a point feature on the current image.
-  **Circle:** This button is used to create a circle feature on the current image. Place your cursor on the image and drag it until the circle reaches the correct size.
-  **Rectangle:** This button is used to create a rectangle feature on the current image. Place your cursor on the image and drag it until the rectangle reaches the desired size.
-  **Best Fit Line:** This button is used to create a straight line that best fits several points on the current image. Click on the image to add up to 1000 points, and double-click to end the list of points. A setting on the **Advanced Options** page determines the limit to the number of points and the feature will be added when this limit is reached. For instance, to draw a two-point line (with two clicks) this option could be set to a user limit of 2. The end points of the





line will be the point where the line is perpendicular to the first and last points.


 **Best Fit Circle:** This button is used to create a circle that best fits several points on the current image. You should click on the image to add up to 20 points, and double-click to end the list of points, creating the best-fit circle. A setting on the **Advanced Options** page determines a user-defined limit to the number of points and the feature will be added when this limit is reached.

 **Best Fit Arc:** This button is used to create an arc that best fits several points on the current image. You should click on the image to add up to 20 points, and double-click to end the list of points. The points of the arc will need to be ordered, i.e. clicked in order from the beginning to the end of the arc. A setting on the **Options** page determines a user-defined limit to the number of points and the feature will be added when this limit is reached.

 **Pitch:** This button is used to measure the calibrated perpendicular distance between a set of user-defined points and a user-defined reference line. This tool asks you to create a reference line on the image. Then, clicking on points above and below this line will draw perpendicular lines from the reference line to the point.

 **Count Gray Spots:** Use this button to count selected objects based on the gray value of a selected spot. You can click on a gray object in an image, and all the objects in that image with a similar gray value will be outlined and counted. The result of this measurement indicates the number of objects found.

 **Launch Count Size:** Clicking this button invokes the **Count/Size** feature of *Image-Pro Plus*. (Please refer to the **Count/Size** descriptions earlier in this chapter.)

 **Data Overlay:** Click this button to place selected data onto the image as an overlay. After you click the right mouse button, the chosen features and data will be formatted into a table and placed in the upper right hand corner of the image. Using the selection tool, you can double-click the **Data Overlay** to change the color, font, or text size. The **Data Overlay** can be searched and deleted like any other feature.

**Display:** Check this box to show or hide the measurements on the image.

## Measure:Measurements

### Advanced Distance Measurements

The same distance measurement tools described in the basic mode are also available in the advanced mode.

The table below shows which distance measurements are available in the advanced mode:

Features	Measurement
point - point	ctr-to-ctr
point - line	ctr-to-ctr , min., max., perpendicular
point - circle	ctr-to-ctr , min., max.
point - rect.	ctr-to-ctr , min., max.
point - polygon or trace	ctr-to-ctr , min., max.
point - arc	ctr-to-ctr , min., max.
line - line *	ctr-to-ctr , min., max.
line - circle **	ctr-to-ctr , min., max., perpendicular (ctr. pt.)
line - rect **	ctr-to-ctr , min., max., perpendicular (ctr. pt.)
line - polygon or trace **	ctr-to-ctr , min., max., perpendicular (ctr. pt.)
line - arc	ctr-to-ctr , perpendicular (ctr. pt.)
circle - circle	ctr-to-ctr , min., max.
circle - rect	ctr-to-ctr
circle - polygon or trace	ctr-to-ctr
circle - arc	ctr-to-ctr
rect - rect	ctr-to-ctr
rect - polygon or trace	ctr-to-ctr
rect - arc	ctr-to-ctr
polygon or trace - polygon	ctr-to-ctr
polygon or trace - arc	ctr-to-ctr
arc - arc	ctr-to-ctr

For line-to-line distance measurements, the minimum and maximum distances are evaluated between the end points, using the two pairs of end points where the distances evaluated do not intersect.

\*\* For these distance measurements, the minimum and maximum distances reported are the minimum and maximum perpendicular distances between the line (extended if necessary) and the other object. The minimum distance may be zero if the object intersects the extended line.

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Note that the ctr-to-ctr distance measurement is the ONLY distance measurement that is guaranteed to be available for all feature combinations.

The following table lists the types of features available and the measurements which each can generate, with the initial default measurement indicated (the last measurement used for each feature type will be retained for the next session):

Feature / Meas.	Pos	Len	Area	Ang	Rad	Start Pos	End Pos	Ctr. Dist	Min. Dist	Avg Dist	Max Dist	Perp Dist
Point	D											
Line	*	D		*		*	*					
BF Line	*	D		*		*	*	* *				
Rectangle	*	*	D									
Circle	*	*	D		*							
BF Circle	*	*	D		*			* *				
BF Arc	*	*		*	D	*	*	* *				
Trace	*	D				*	*					
Polygon	*	*	D									
Distance	*							D	* *			*
H / V / C	*								* D *			
Thick.												

*D = Default*

## Advanced Measurements

The **Measurements** page is visible only in the advanced mode. It contains the following measurement information:

	Features	Measurement	Value
1	L1	Length	0
2	L2	Length	0
3	L3	Length	146.2395
4	PG1	Area	7256

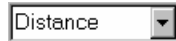
The **Measurements** grid includes measurement data for the selected measurements. As with the **Features** grid, you can resize the columns for a better fit to the displayed values or to hide columns that are not of interest. If tolerance testing is enabled (see the **Advanced Options** page) additional columns will report the pass/fail status along with either the minimum and maximum acceptable values (as shown above), or the target value and minimum / maximum tolerances, depending on the type of tolerance testing selected on the **Advanced Options** page.

By default, the target value will be identical to the measured value when first measured. Double-clicking in the **Target Value** cell allows you to adjust the target values. This affects the pass/fail tolerances.

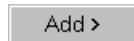
The **Measurement** grid is also used to set or adjust the type of measurement, tolerances, etc. You may edit the name of a feature by clicking on the name in that feature's grid row and typing the new name. Clicking on the measurement type reveals a combo box that can be used to change the particular measurement to report and test for tolerance; as with the *Select Measurement* combo box, only the valid measurements will be listed. You can change either tolerance by clicking on the current value and editing. It is possible to delete a measurement by clicking on the row number (which selects the row) and pressing the <Del> key, or clicking the **Delete** button in the

Measurements toolbox. Multiple rows can be selected holding down the <Ctrl> or <Shift> keys while clicking on the row numbers.

The following controls along the left side of the **Measurements** dialog are used to select, add and remove measurements from the **Measurements** page.



The **Select Meas.** combo box contains the available measurements for the currently selected feature or measurement, as determined by the table shown previously. This combo box determines the current measurement for each feature type. This control will be disabled under the following conditions: no features or feature creation tools are selected, or the set of selected features includes different feature types.



The **Add** button is used to insert measurements of the features to the **Measurements** page's grid. Whenever one or more features are selected on the image, the **Add** button will be enabled. When the **Add** button is clicked, the current measurement for each of the selected features will be inserted in the Measurement grid.



The **Auto Add** check box is to indicate that the currently selected measurement for each feature type should be added to the **Measurements** grid whenever a new feature is created. The default setting of the check box is checked, and the setting is remembered from session to session.



The **Remove** button is used to remove the selected measurement from the grid of the **Measurements** page. The **Remove** button will be enabled whenever one or more rows in the **Measurements** grid are selected. The features on the image and the measurements on the **Features** page are unaffected.

There are several ways to add a measurement to the grid:

1. When a new feature is created and **Auto Add** is checked, the currently selected measurement for that type of feature will be added to the grid at this time.
2. Double-clicking a feature on the image will add the currently selected measurement for that type of feature.
3. After selecting one or more features, clicking **Add** will add for each feature the currently selected measurement for that type of feature.

If your screen is very crowded, and you want to make sure that you are selecting the correct features, follow these instructions:

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1. Select the tool you will be using (i.e. distance).
2. Select the measurement that you want to **Auto-Add** (these first two steps only apply if you have **Auto-Add** selected).
3. Select the two features between which you want to measure.
4. Select the measurement tool.

The correct measurement feature will then be added measuring between the correct two features.

---

**Note:** If multiple features of different types are selected, the **Select Meas.** combo box is disabled, since it can only select the measurement type for one type of feature at a time.

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## Advanced Options

The **Advanced Options** page contains the best-fit and pass/fail measurement statistics:

The screenshot shows the 'Advanced Options' dialog box. It has a tabbed interface with 'Advanced Opt.' selected. The 'Best-Fit Features' section includes a checkbox for 'Display points for best-fit features' (unchecked) and three spinners for 'Maximum Feature Points': Lines (10), Circles (3), and Arcs (3). The 'Pass/Fail Measurements' section has radio buttons for 'Do not test pass/fail tolerances', 'Test tolerances' (selected), and 'Test min/max value'. There are also radio buttons for 'Default Tolerance' (Percent selected, Absolute unselected) and a spinner for 'Test min/max value' (0.0010). The 'Count' section has a checked checkbox for 'Display Count minimum object size dialog'.

**Display points for best-fit features** determines whether the points added to define best-fit features are listed on the **Features** page or not, and if they will be displayed on the image.

The **Maximum Feature Points** edit boxes for **Line**, **Circle**, and **Arc** features are used to set the maximum number of points for best fit lines, circles, and arcs. Lines will allow from 2 to 1000, while circles and arcs

will allow from 3 to 20. When creating a best-fit feature, the feature will be automatically created when the current maximum is hit. This makes it possible to set feature creation to accept 2-point lines and 3-point circles and arcs (without requiring double-click on the last point) while still supporting multiple-point fits when desired.

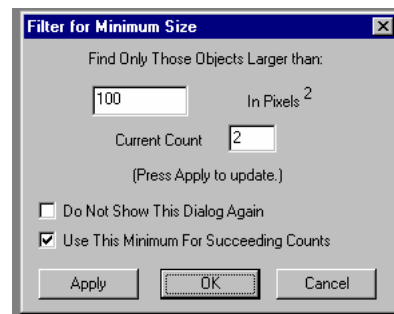
**Do not test pass/fail tolerances** is used to select display of measurements only on the **Measurements** grid.

**Test tolerances** is used to select display of minimum and maximum tolerances, a target value, and a pass/fail indication.

**Test min/max value** is used to select display of minimum and maximum measurement values and a pass/fail indication.

**Default tolerance** is used to set the default tolerance that is used when a measurement is added. This will be specified either in **Percent** or as an **Absolute** value of the value measured.

**Display Count minimum object size dialog:** Checking this box indicates that the **Filter for Minimum Size** dialog will be displayed after you select the object intensity.



**The [size] In Pixels<sup>2</sup>:** The number in this box indicates the minimum size of the objects to count. To ignore small noise features, set the minimum size larger.

**Current Count:** This field indicates the number of objects counted using the current threshold and minimum size.

**Apply:** Clicking this button removes those objects which do not meet the minimum size requirement and updates the current count in the **Features** table. The dialog remains open.

**Do not show this dialog again:** Checking this box will prevent the dialog from being displayed after the trace or wand tool is used to select the threshold.

*Measure:Measurements*

**Use this minimum for succeeding Counts:** Checking this box ensures that the minimum size that has been selected is used for all subsequent counts (and as the default for this dialog if shown).

**OK:** Selecting **OK** also removes those blobs which do not meet the minimum size requirement and updates the current count, and closes the dialog.

**Cancel:** Selecting **Cancel** closes the dialog and discards the count. Since the **Count** tool is still active, you can select a new object intensity.

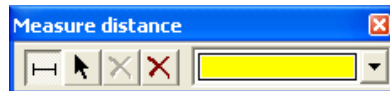
The object count will be displayed in the **Features** table on the **Features** tab of the **Measurements** dialog.



## Measure Distance

---

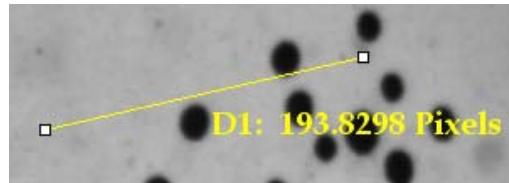
The **Measure Distances** function allows you to measure distances by drawing a line between two features on your image. When you select **Measure Distances** from the *Measure* menu, the **Measure Distance toolbar** appears:



The **Distance** tool is automatically selected so that you can begin to measure distances right away.

To measure distance, click and drag to create a line on your image. You can draw as many lines as you like.

The distance will be displayed on the line, as shown here:



This picture illustrates a distance feature using the default name and display settings.

The other tools are:

**Selection:** Click this button to use the normal Windows selection options (e.g. Control and Shift keys) to select one or more features to be moved, deleted or edited.

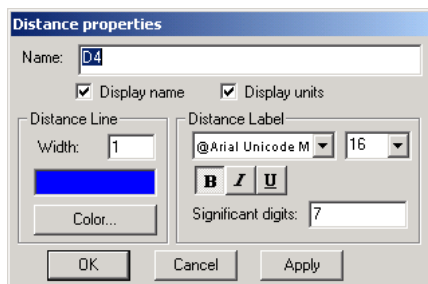
**Delete:** The **Delete** tool is only enabled when the selection tool is active. When clicked, an “are you sure” prompt will be displayed, and the selected distances will be deleted if you agree.

**Delete All:** When clicked an “are you sure” prompt will be displayed, and all distances will be deleted if you agree.

**Distance Color:** The **Distance Color** drop-down list allow you to select of one of 6 standard display colors. If the color is changed, the distance features on all open images will be updated to the selected color. This assures that distance features are always distinguishable from other annotation and measurement features.

## Measure:Measure Distance

Double-click on a distance feature with the selection tool to change the distance properties. You will see the following dialog:



**Name:** The **Name** edit box will be disabled if more than one feature is selected when the properties are edited. When enabled, you will be able to edit the default name.

**Display Name/Display Units:** The **Display name** and **Display units** settings determine whether the name prefix and units suffix are included in the on-image display of the values (“D4” and “Pixels” respectively in the example above).

**Distance Line:** Use these setting to modify the size and color of the distance line.

**Width:** Indicate the pixel size of the distance line on your image.

**Color:** The **Color...** button changes the current distance color.

Additional colors beyond the 6 available from the drop-down list can be selected using this method.

**Distance Label:** The **Distance Label** section allows you to select or change the format of the label font, size, and attributes such as bold and underline. Note that editing these properties changes both the default properties of all future distance features, as well as the display of all existing distance features on all open images.

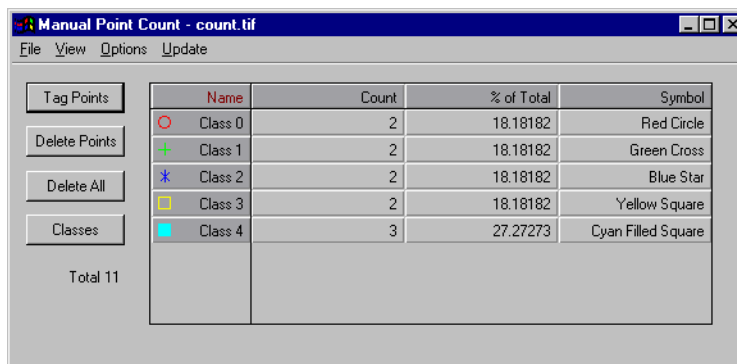
**Sign Digits:** The **Significant Digits** edit box allows you to specify how many significant digits will appear on the image next to the measurement line. The software can carry up to eight digits internally but you may not want that many digits shown on the image, as it can get cluttered. You may specify as many (up to 8) as you find appropriate.

*Measure:Measure Distance*

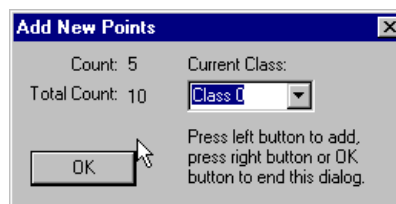
**Distance** features drawn on an image will be saved along with the image when the image is saved to a TIFF file, and restored when the TIFF file is re-opened. Note that if a TIFF file is opened that contains distance features of a different color than the current setting, the color setting will be updated to the file's color. This is another measure to assure that distance features are always distinguishable from other annotation and measurement features.

## Manual Tag

Use the *Manual Tag* command to select individual objects or groups of objects in an image with your mouse. You can create different classes of objects for analysis, and “tag” each object with a specific marker. Clicking on the **Manual Tag** command displays the **Manual Point Count** dialog box:

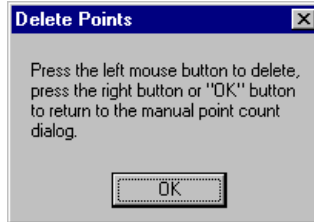


**Tag Points:** Clicking on this button activates the manual tagging mode. Manual tagging mode allows you to use the mouse to place markers on various classes of items in the image, and to switch among different classes of items for analysis. You will see the following dialog box:



Select the name of the current class from the list box. Then, using your left mouse button, click on the items in the image that belong to this class. There is a limit of 255 class marker types for any given image. When you have finished, click on the right mouse button or click **OK**.

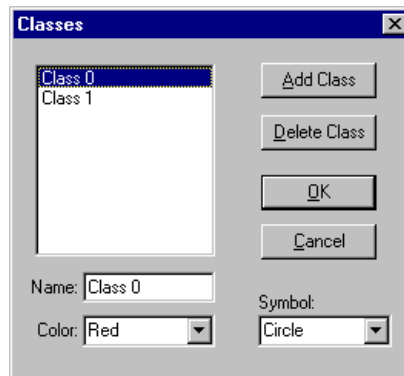
**Delete Points:** Clicking on this button allows you to delete one or several of the markers in the image. You will see the **Delete Points** dialog box:



Use the left mouse button to select the markers to be deleted. When finished, click the right mouse button or click **OK**.

**Delete All:** Clicking *Delete All* will erase all the tagged markers from your image.

**Class:** Clicking on the *Class* button allows you to define the classes used to identify the different objects in your image. Clicking on the button displays the following dialog box:



You may select the name, color and symbol to identify the class of object.

- ◆ **Add Class:** Adds a new class with a default name to the list of available classes. There is a limit of 255 possible classes per image.
- ◆ **Delete Class:** Removes the highlighted class from the list of available classes.
- ◆ **Name:** Allows you to change the name of the highlighted class.
- ◆ **Color:** Allows you to select a color to identify each class of objects.
- ◆ **Symbol:** Allows you to select a symbol to identify each class of objects.

## Measure:Manual Tag

- ◆ **OK/Cancel:** Click **OK** to save the class information; click **Cancel** to abandon the changes.

## Manual Tag File Menu

The commands in the *Manual Tag* window's *File* menu are used to save measurement data (the measurement values) to a disk file. You can also save the points and sets of points you have generated in the image. You may use the files as input to an external program, or you can reload them into *Image-Pro* for future use with this or another image.

Each command on the *Manual Tag : File* menu is described below.

- 
- ◆ **Data to File:** Select this command if you want to save the measurement values (the results in the data sheet) to a file. Measurement data are saved in an *Image-Pro* count file (.CNT) in ASCII format. The file will contain the data sheet, exactly as it appears in the window. The saved data can be imported to a spreadsheet application or used as input to an external program.
- 
- ◆ **Append Data to File:** Use the *Append Data* command on the *Manual Tag: File* menu to add the current measurement data to an existing file. Select the file to which you want the current data appended, then click **OK**. The current measurement data will be written to the end of the specified file. If the file does not already exist, *Image-Pro* will create it.
- 
- ◆ **Data To Clipboard:** Use the *Manual Tag* window's **Data To Clipboard** command to store the current data to the Windows Clipboard. Once on the Clipboard, the histogram data can be pasted into any Windows application that accepts text from the Clipboard.
- 
- ◆ **Data to Printer:** Select the *Data To Printer* command to print out the current tag data.
- 
- ◆ **Save Options:** This selection offers you a choice of saving the data in a data sheet with or without a top line and/or left-hand column.

- 
- ◆ **Data Export:** Select the *Data Export* command to transfer data to Microsoft's Excel spreadsheet. Before selecting *Data Export*, you should use *Export Options* to determine the correct placement (within Excel) for the data. When you select *Data Export*, the transfer occurs with little visible evidence that it has happened. By switching to the Excel application, you can confirm that the transfer has occurred. For additional information about DDE, see the *Dynamic Data Exchange* discussion in *Part One* of this manual.
  - ◆ **Export Options:** Select the *Export Options* command to tell *Image-Pro* how and where to export the current data via DDE. For additional information about DDE, see the *Dynamic Data Exchange* discussion in *Part One* of this manual.

- 
- ◆ **Save Points:** Use the *Save Points* command on the *Manual Tag* window's *File* menu to save the locations of your manual tags to a file.

- 
- ◆ **Load Points:** Select the *Load Points* command on the *Manual Tag* window's *File* menu to superimpose a tagged file onto the active image, and add the measurements it produces to the data sheet.

---

**Note:** A tagged image loaded from a file is simply "superimposed" upon your image. It is not actually written to your image bitmap.

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- ◆ **Save Settings :** Select the *Save Settings* command on the *Manual Tag* window's *File* menu to store the current tagged points specifications. The *Save Settings* command stores these values to a file so that, at some future point, you can simply reload these values instead of having to reestablish them manually.

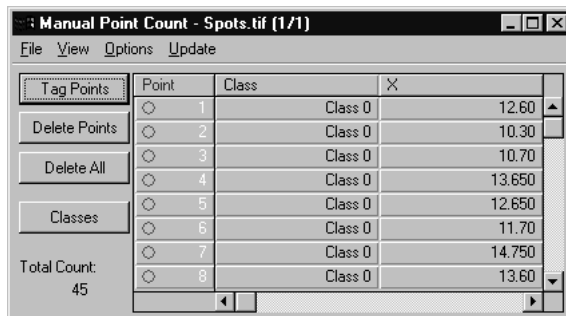
- 
- ◆ **Load Settings :** Select the *Load Settings* command on the *Manual Tag* window's *File* menu to set the tagged points settings to the values defined previously.

- 
- ◆ **Close:** Use this command on the *Manual Tag* window's *File* menu to close the *Manual Tag* window. Closing the *Manual Tag* window will not affect active measurements and outlines.

Measure:Manual Tag

## Manual Tag View Menu

Use the commands on the *Manual Tag : View* menu to view the data and statistics associated with the tagged objects in the active window.



The screenshot shows a dialog box titled "Manual Point Count - Spots.tif (1/1)". It has a menu bar with "File", "View", "Options", and "Update". Below the menu bar is a table with columns "Point", "Class", and "X". The table contains 8 rows of data. To the left of the table are buttons for "Tag Points", "Delete Points", "Delete All", and "Classes". At the bottom left, it says "Total Count: 45".

Point	Class	X
1	Class 0	12.60
2	Class 0	10.30
3	Class 0	10.70
4	Class 0	13.650
5	Class 0	12.650
6	Class 0	11.70
7	Class 0	14.750
8	Class 0	13.60

When you select the *View* menu, you have the option to display **Counts**, **Points**, or **Class Statistics** in the table (example above). You may also hide or show the **Area**, **Label**, and **Point** symbol on the image. Each of these choices is described below:

- ◆ **Counts** - displays the name of each class, the number of objects, the symbol for each class of objects, and the percent of the total number of objects for each class of objects in the image.
- ◆ **Points** - displays the marker number, class, x and y position, and color identifier for each class of objects in the image.
- ◆ **Class Stats** - displays the statistics (min, max, mean, standard deviation, total) associated with each class of objects in the image.
- ◆ **Area** - draws a circle around the selected markers. The radius of the circle is specified in the **Options** dialog box. This option allows you to zoom in/out to examine a selected tagged point. This option is only available when intensity calculations for the area or radius are active.
- ◆ **Label** - displays a label (number) for each marker, when selected..

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*Note:* If the marker is hidden (see Hide/Show Marker, below), the label will not be visible.

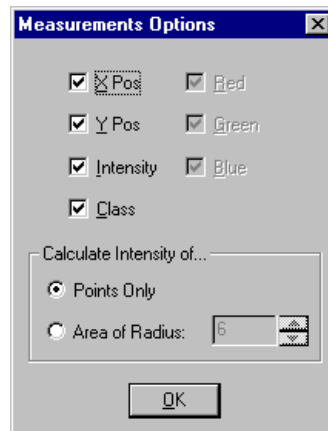
---

- ◆ **Hide/Show Points** - hides or shows the class symbol associated with each tagged point.



## Manual Tag Options Menu

Use the **Options** menu to select which characteristics in each class of objects will be displayed. Options set in this dialog affect the display of the image on the screen when the **Points** option from the *Manual Tag : View* menu is selected. Select one or all of the choices from the following dialog box.



- ◆ **X Pos:** Displays the x position of the points.
- ◆ **Y Pos:** Displays y position of the points.
- ◆ **Intensity:** Displays intensity of the points.
- ◆ **Class:** Displays the class name of the points..
- ◆ **Red, Green, Blue:** Allows the user to choose red, blue, or green values for the points in the image. If you have a monochrome image, these checkboxes are dimmed.

**Calculate Intensity Of:** This selection offers you a choice of analyzing the intensity of the tagged points only, or within given area.

- ◆ **Points Only:** Calculates the grayscale or RGB intensity of the pixel(s) under the marker. When you select this option, *Image-Pro* resets the image to its original point size.
- ◆ **Area of Radius:** Displays the grayscale or RGB intensity of a circular area of a certain radius from 1-15 pixels. Use the scroll arrows to select the length of the radius or type in a number. When this selection is activated, the points displayed on the image change in diameter as the radius setting changes.

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## **Manual Tag Update Menu**

Select the **Manual Tag** window's *Update* menu to refresh the values in the data sheet following a change to the image data. For example, if you added a class while the *View* options were displayed, you would need to update the **Manual Tag** window to add the new class information.

The **Manual Tag** data sheet is updated automatically as points are deleted or selections from the *Options* menu change the display. However, you will need to use the *Update* menu to refresh the values if the image bitmap changes during a **Manual Tag** session.

To update the **Manual Tag** data sheet, simply click the *Update* menu. The values in the data sheet will be rewritten based upon the contents of the active image or AOI.

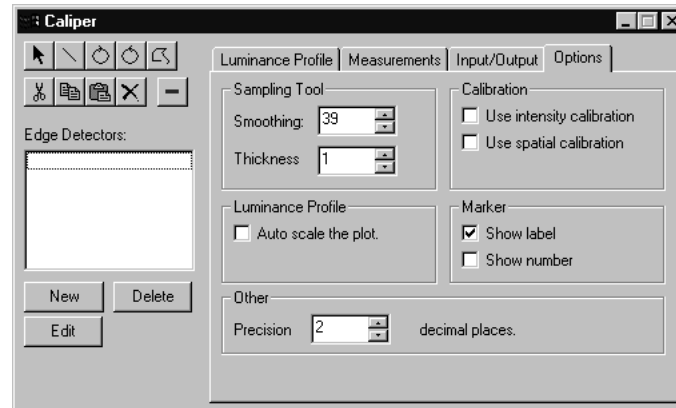
## Caliper

---

The *Caliper*, or *Edge Detection* tool can be used to:

- ◆ define a line or shape called a *sampling tool* and place it on the image
- ◆ create *edge detectors* that will find edges along the length of the sampling tool
- ◆ derive distance *measurements* based upon the detection and location of the edges.

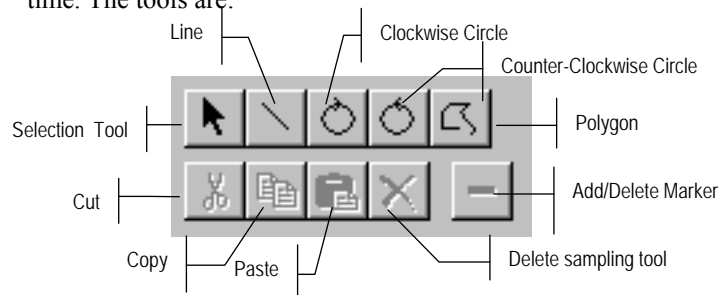
When you first select *Caliper* from the *Measure* menu, you will see a tabbed dialog:



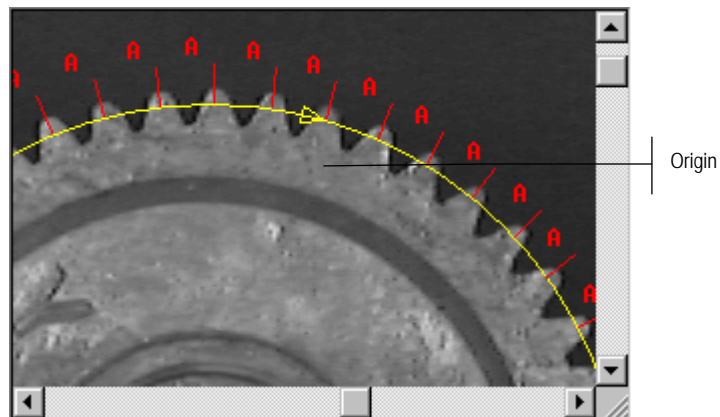
The sampling tools appear at the left side of the **Caliper** window. The tabbed dialog pages on the right side contain selections that allow you to refine the edge detection and measurement operations, and direct the output to a file, printer, or spreadsheet.

## Sampling Tools

The first step in the edge-detection process is to create a sampling tool using the buttons in the toolbar. The sampling tools are used to create the **Luminance Profile** (described later in this section). You may use more than one tool per image, but you may only select one tool at a time. The tools are:



The picture below shows an image with a clockwise circle applied to it.



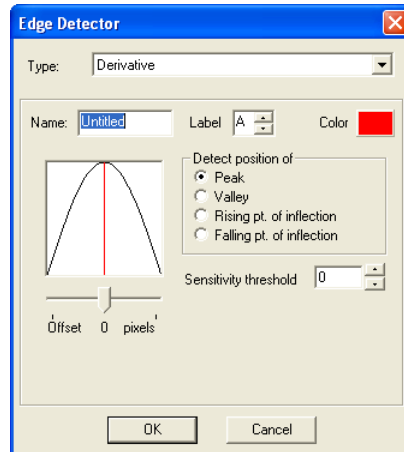
---

**Note:** You can move the location of the origin of the circle tool with your cursor. This may be important for obtaining accurate **measurements**.

---

**Important:** If you have more than one tool drawn on your image, the smaller one may be hidden by the larger one. To “stack” the tools, select the larger image and click the right mouse button. The pop-out menu contains a *Caliper* selection, which lets you send the selected tool to the back, making the smaller tool accessible again.

To select an edge-detection method, click the **New** button. You will see the following dialog:



**Type:** The *Caliper* tool offers two different types of edge-detection methods: **Derivative** and **Pattern Match**. Choose either method from the drop-down list:

**Derivative:** Click this radio button to use the **Derivative** method of edge-detection. This method uses the first- and second-order derivatives of the luminance profile to detect peaks, valleys, rising points of inflection, and falling points of inflection.

**Pattern Match:** Click this radio button to use the **Pattern Match** method. This method uses a pattern-matching template of luminance values ranging from 0 (black) to 255 (white) as the basis for placing markers. The *Caliper* compares the luminance values in the image to the template. Markers are placed at those locations in the image that most closely match the pattern in the template.

**Name:** Enter a name for the edge detector.

**Label:** Use the spin buttons to select a letter to identify the markers.

**Color:** Use the **Color** button to choose a color for the markers.

If you choose the **Derivative** method:

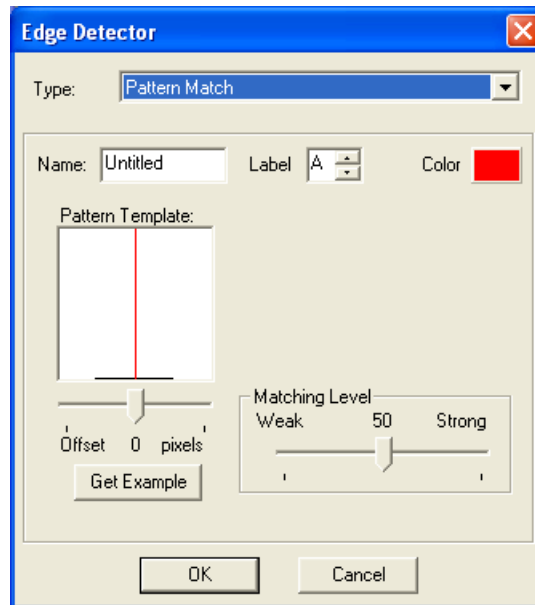
**Detect Position of:** If you are using the **Derivative** method, you may use the radio buttons to choose the features that you want to select. Click the appropriate radio button next to **Peak**, **Valley**, **Rising point of inflection**, or **Falling point of inflection**. These selections are previewed in the pattern template window.

## Measure:Caliper

**Offset:** The offset slider lets you choose how many pixels to offset the markers from the detected features.

**Sensitivity Threshold:** The **Sensitivity Threshold** defines the minimum difference between an extreme point and neighboring opposite extremes. If the difference between the extremes is smaller than the sensitivity threshold, the marker is ignored. The **Sensitivity Threshold** is defined in percentage from 0 to 100 from the dynamic range of the *Caliper* profile. The default value is 0, so that all peaks and valleys are detected, even when deviations of intensities are very small.

If you choose the **Pattern Match** method:

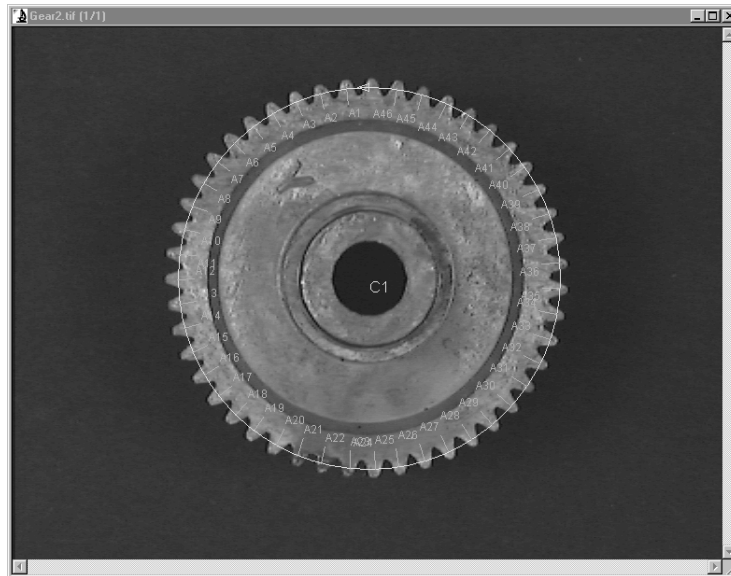


**Matching Level:** If you are using the **Pattern Match** method, you can use the slider to select the degree of “closeness” of the pattern match. Strong means that the matches are nearly perfect; as the slider moves from right to left, the matches are less identical.

**Get Example:** In pattern matching, clicking this button allows you to draw a line on the image over a sample of an edge that you wish to detect. The pattern detected by this sample will be used to identify other edges of this type in the image.

*Measure:Caliper*

After you have selected your edge-detection method, click the **OK** button. The markers will appear in the image, as shown here:



In this example, peaks were selected for edge detection, so the top of each gear tooth is marked.

---

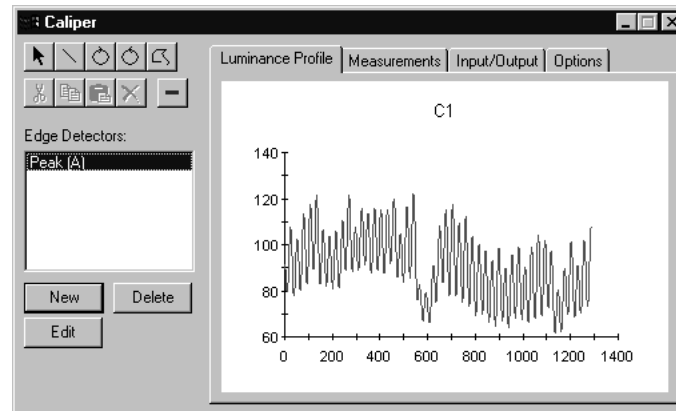
**TIP:** To use edge detection manually, follow these steps:

1. Click **New** and select **Pattern**.
  2. Do not select a default pattern.
  3. Create as many detectors as you want this way.
  4. Then select the first detector type and click "-" (Toggle Marker). Since there aren't any, this tool adds markers.
  5. Then, the select the rest of the detectors you've defined and add markers to each detector.
-

Measure:Caliper

## Luminance Profile

This page displays the **Luminance Profile**, as shown here:

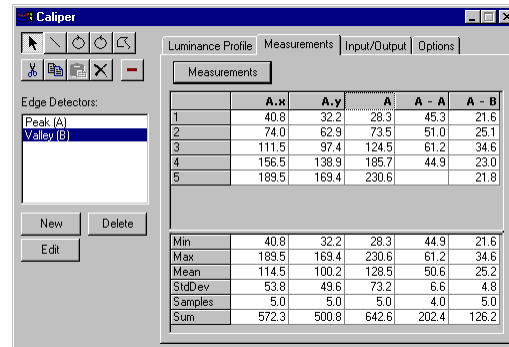


The scaling and display of the **Luminance Profile** are controlled from the **Options** page. The **Luminance Profile** can display luminance values from the minimum and maximum values of the image class, or automatically scale the graph to the minimum and maximum values in the profile. The peaks in the **Luminance Profile** correspond to the peaks detected in the gear image. The valleys in the profile correspond to the valleys detected in the gear image.



## Measurements

This page displays the measurements associated with the edges detected.



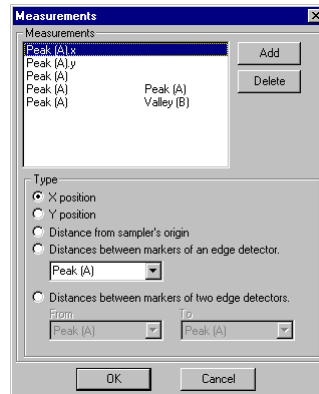
	A.x	A.y	A	A - A	A - B
1	40.8	32.2	28.3	45.3	21.6
2	74.0	62.9	73.5	51.0	25.1
3	111.5	97.4	124.5	61.2	34.6
4	156.5	138.9	185.7	44.9	23.0
5	189.5	169.4	230.6		21.8
Min	40.8	32.2	28.3	44.9	21.6
Max	189.5	169.4	230.6	61.2	34.6
Mean	114.5	100.2	128.5	50.6	25.2
StdDev	53.8	49.6	73.2	6.6	4.8
Samples	5.0	5.0	5.0	4.0	5.0
Sum	572.3	500.8	642.6	202.4	126.2

After the measurements are defined, the **Measurements** page shows the measurement in each column and statistics are given at the bottom of the table. The example above shows the following columns:

- A.x** The X positions of the markers in edge detector A.
- A.y** The Y positions of the markers in edge detector A.
- A** The distance of the markers along the sampling tool from the origin of the sampling tool.
- A-A** The distances between two consecutive markers.
- A-B** The distances between the markers in edge detector A and edge detector B.

## Measure:Caliper

To display the measurements on this page, click the **Measurements** button. You will see the following dialog:



**Measurements:** This box lists the defined measurements. There are five types of measurements:

**X-position** shows the position of the markers in the image. Values are in image coordinates.

**Y-position** shows the position of the markers in the image. Values are image coordinates.

**Distance from sampler's origin** shows the distance along the sampling tool from the origin of the tool.

**Distance between markers within a detector** shows distances between two adjacent markers of the same edge detector type. This measurement is useful for finding aberrations in distances between the teeth of a gear, for example.

**Distances between markers of two edge detectors** calculates distances between two markers of two different edge detectors. This distance measurement always produces positive values. In other words, an A-B (A to B) measurements measure the distance between B marker positioned after the A marker. This type of measurement is useful for finding bars and spaces in a barcode. A-B can be arranged to measure the bar, and B-A can be arranged to measure the spaces between the bars.

**Add:** To add a measurement to the list, highlight your selection, and click the **Add** button.

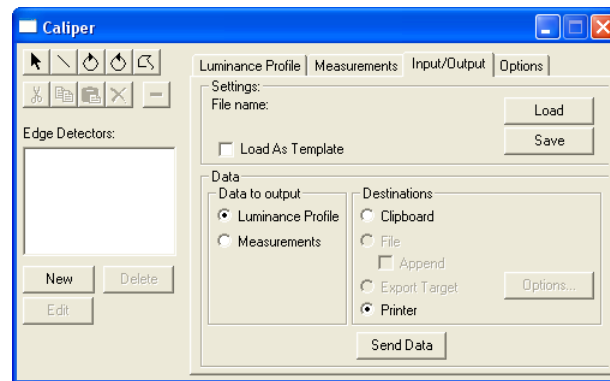
**Delete:** To remove a measurement from the list, click the **Delete** button.

**Type:** The selections in this area allow you to defined the type of measurement to display (see previous page). Click the appropriate radio button:

When you have decided which measurements to display, click **OK**. To quit, click **Cancel**.

## Input/Output

The **Input/Output** page displays the options for loading and saving *Caliper* settings files, and saving/printing the measurements and luminance profile.



**Load:** Click the **Load** button to use *Caliper* settings that you have defined previously. Settings include your sampling tools, edge detectors, and measurement definitions. *Caliper* settings are stored in files with the extension **\*.cps**.

**Load as Template:** When checked, the detector(s) that are loaded will automatically detected edges on the image, and the marker positions will be recreated from the detector(s). When this option is not checked, the final detector positions as saved in the file (which may have been edited) will be restored.

---

**Note:** The behavior of the **Load as Template** tool in this feature *does not* correspond to similarly-named options in other tools. In this case, the **Load as Template** tool allows you to apply a pre-configured caliper to a new image, auto-detecting edges on the new image and recalculating measurements on the basis of the markers found. In contrast, loading caliper settings with **Load as Template** un-selected is intended to allow you to reload your caliper markers and measurements on to the original image.

---

## Measure:Caliper

**Save:** Click the **Save** button to save and reuse *Caliper* settings that you have just defined. Caliper settings are stored in files with the extension **\*.cps**.

If you are loading a caliper settings file (**\*.cps**) the **Load as Template** option determines how the file will be loaded. **If Load as Template** is NOT checked, the features and measurements from the file will be restored to the image. You would use this method to continue or reuse measurements from a previous session.

**Data to Output:** Click the appropriate radio button to indicate if you are sending the measurements or the luminance profile as output.

**Destinations:** Click the appropriate radio to indicate the destination for your output.

**Clipboard:** Select **Clipboard** to copy the caliper data to the *Windows Clipboard*. When the *Luminance Profile* is selected, a picture of the luminance profile plot will be placed on the clipboard; when the *Measurements* are selected, the measurement data in text format will be copied to the clipboard. From there, the data can be pasted into any application that accepts the types of clipboard data that you have copied.

**File:** Select **File** to save your data in a file. When you click the **File** button, you will be asked to supply a file name.

**Printer:** Select **Printer** to print out the current measurement data.

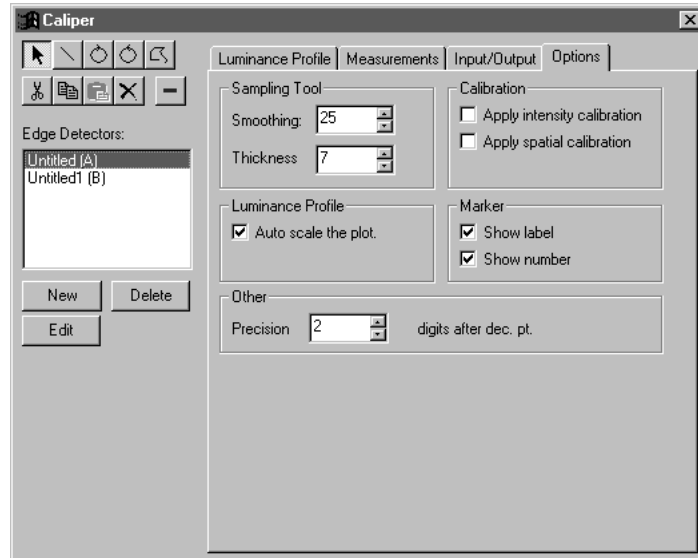
**Data Export:** Select the **Data Export** command to transfer data to Microsoft's Excel spreadsheet. For additional information about DDE, see the *Dynamic Data Exchange* discussion.

**Options :** Click the **Options** button to tell *Image-Pro* how and where to export the current data via DDE. For additional information about DDE, see the *Dynamic Data Exchange* discussion.

**Send Data:** Click this button to send the output data to the selected destination.

## Options

The **Options** tab contains settings for various areas in the *Caliper Tool*.



The **Sampling Tool** group box offers two selections to smooth the luminance profile. **Smoothing** and **Thickness**.

**Smoothing:** Use the spin buttons to apply the Gaussian smoothing filter to the luminance profile. The numbers indicate the degree of smoothing that the filter uses. **Smoothing** of 1 means no smoothing. If smoothing value is more than 1, a Gaussian smoothing kernel will be applied to the luminance profile.

**Thickness:** The **Thickness** control specifies the thickness of the sampling tool in pixels. The thicker the sampling tool, the more pixels used to generate a pixel value. Use the spin buttons to select the thickness of the sampling tool. Thicker lines cover more pixels, therefore averaging in the values of the surrounding pixels.

The **Smoothing** and **Thickness** options adjust the behavior of the sampling tool. By increasing the **smoothing** value, you can reduce the sensitivity of the sampling tool to small changes in the luminance profile. If you find large numbers of markers on your sampling tool, increase the smoothing. Values in the range of 10 - 30 or more are good values to try.

## Measure:Caliper

Similarly, the **Thickness** controls how much data is sampled by the tools. Increasing the thickness value causes more pixel data to be sampled. This will reduce the effect of small variations in image luminance.

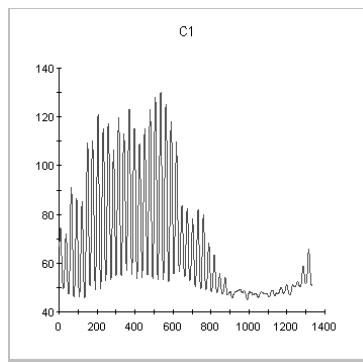
The **Calibration** group box allows you to use intensity calibration to calculate the luminance profile, or use the spatial calibration to report measurements in spatially calibrated values. Check the appropriate box:

**Apply Intensity Calibration:** Check this box to apply intensity calibration.

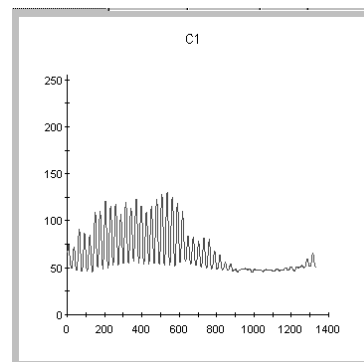
**Apply Spatial Calibration:** Check this box apply the spatial calibration.

The **Luminance Profile** group box allows you to select **Auto-Scaling** for displaying the profile. The **Luminance Profile** can display luminance value from the minimum and maximum values of the image class, or automatically scales the graph to the minimum and maximum values in the profile. .

**Auto-Scale the Plot:** Check this box to activate **Auto-Scaling**, which automatically adjust the display of the luminance profile to include only those values actually detected. An example appears here:



*Luminance Profile with Auto-Scaling On*



*Luminance Profile with Auto-Scaling Off*

The **Marker** group box controls the display of the edge-detection markers (label and sequence number) on the image:

**Show Label:** Check this box to display the marker label (A,B, C, etc.) on the image.

*Measure:Caliper*

**Show Number:** Check this box to display the marker number (1, 3, 11 etc.) on the image.

The **Other** group box contains additional selections for the display.

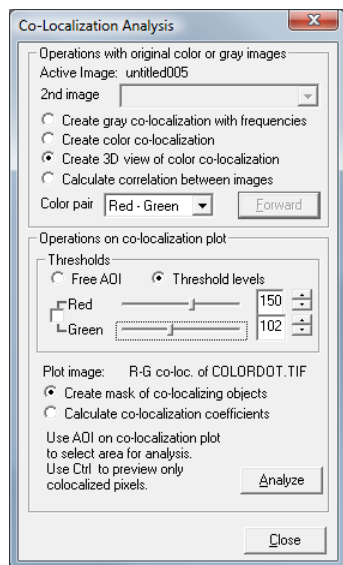
**Precision:** Use the spin buttons to indicate how many numbers after the decimal point you want to display on the measurements page.

## Co-Localization

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Co-localization describes the existence of two or more molecule types in precisely the same space. The material under examination may be treated with different types of dyes to indicate the presence or absence of areas of co-localization or overlap. The *Co-Localization* command can be used to measure the amount of co-localization in a color image, in a pair of gray images, in an image sequence, or in an AOI on a color image.

Selecting *Co-Localization* from the *Measure* menu displays the following dialog box:



**Operations with original color or gray images:** Co-localization operates on two channels of a color image or a pair of identically acquired grayscale images. When a grayscale image pair is used, the two images must be the same image type and size, and they are typically acquired using the same magnification, camera, capture sub-region, etc. so that the locations of individual pixels can be compared for co-localization. An AOI on a grayscale image will be ignored; however if there is an AOI on a color image, the calculations will be limited to the area of the AOI. Otherwise, calculations will be performed on the whole image.



**Active Image:** Indicates which image is currently in the active workspace. This image will be used as the baseline for the co-localization calculations.

**2nd image:** Indicates the second image in the pair of grayscale images for co-localization. This control will be disabled if the active image is color, or if there are no matching grayscale images to act as the second image of the pair.

If the original images are grayscale, the next four options will create a color image showing the two source images merged according to the Color Pair that has been selected, in addition to any Co-Localization plots that are specified.

**Create gray co-localization with frequencies:** Choosing this option will create a new, untitled grayscale image that displays the co-localization areas of the two channels selected (these can either be two grayscale images, or two channels of a color image) when the **Forward** button is clicked.

**Create color co-localization:** Choosing this option will create a new, untitled color image that displays the co-localization areas of the two channels selected (these can either be two grayscale images, or two channels of a color image) when the **Forward** button is clicked.

**Create 3D view of color co-localization:** Choosing this option will create both a gray scale and a color Co-Localization plot of the selected area of the active image and uses *Image-Pro's Surface Plot* feature to create a three-dimensional image of the color co-localization when the **Forward** button is clicked.

**Calculate correlation between images:** Choosing this option will use the *Pearson Correlation Coefficient* to measure the amount of co-localization in the selected image or AOI when the **Forward** button is clicked. The results are displayed in the **Output Window**, which can be accessed from the *Macro* menu.

**Color pair:** Use this drop-down list box to select the two colors that *Image-Pro* will use to define the co-localization area. When using gray scale images as the data source, this drop-down list determines the colors that will be assigned to each source image in making the color composite image and the color co-localization image. When a color image is the data source, this drop down determines which two of the three RGB planes will be used for the co-localization calculations.

**Forward:** Click this button to begin the co-localization analysis process.

## Measure:Co-Localization

**Operations on the co-localization plot:** These selections affect the way in which co-localization is displayed on the image that *Image-Pro* created.

**Thresholds:** These options describe the way in which the co-localization area is defined.

**Free AOI:** When the **Free AOI** option is selected the area on the co-localization plot can be defined using a free form AOI.

**Threshold Levels:** With this option, the co-localization threshold can be defined using the sliders or edit controls corresponding to the color channels. You can lock or unlock two channel thresholds using the checkbox.

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**Note:** The co-localization pixels on the source image are highlighted when the slider is dragged or one of the threshold edit controls has focus.

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**Use an AOI on co-localization plot to select area for analysis:** Create an AOI on the co-localization image to define the range of intensities for each color on which the co-localization calculations should be performed. Also, notice that as the AOI is drawn, or moved on the co-localization plot, a three-color mask, based on the bounding box of the AOI, is applied to the source color image. The two colors from the imaging show non-co-localized pixels within the area selected by the AOI range of intensities. The third color, a summary of the channel colors, represents the co-localized pixels. For example, if the channels are red and green, the co-localized pixels are shown in yellow.

**Note:** To display the co-localized pixels only, press and hold the <CTRL> key while moving the AOI.

**Create mask of co-localizing objects:** Click this radio button to create an image mask using Image-Pro's Grid/Mask feature. The image mask will show the areas of co-localization, as defined by the AOI on the co-localization plot.

**Calculate co-localization coefficients:** Clicking this radio button uses the *Pearson Correlation Coefficient* to measure the amount of co-localization in the selected image or AOI. The mathematical results are displayed in the Output Window.

**Analyze:** Click this button to execute the operation selected above.

**Close:** Click this button to close the **Co-localization** dialog.

*Measure:Co-Localization*

**Co-Localization Plot:** The Co-Localization plot is a 256 x 256 pixel image displaying a scatter plot, with the origin in the **bottom left** corner, in which the intensity of each pixel represents the frequency with which that color pair exists in each image. For example, if pixel 10, 12 has an intensity of 2013, that means that there are 2013 pixels with an intensity of 10 in the first channel that also have an intensity of 12 in the second channel.

## Track Objects

The *Tracking* feature of *Image-Pro Plus 7.0* allows you to follow an object in an image sequence ( \*.seq ) as it moves through time and space. This new feature provides the ability to track objects automatically, semi-automatically, or manually.

In order to use object tracking, you must have an image sequence open in your *Image-Pro* workspace. When you select *Track Objects* from the *Measure* menu, you will see the **Tracking Data Table**, as shown here:

#	Name	Distance(1)	Distance(2)	Distance(3)
1	Track2	6.0000	7.0711	8.0000
2	Track3	14.0357	15.0000	13.0000
3	Track4	0.0000	1.0000	1.0000
4	Track5	10.0000	9.0554	9.0554
5	Track6	0.0000	1.0000	1.0000
6	Track8	8.5440	9.0554	9.0554
7	Track9	37.2156	27.0740	39.2046
8	Track10	9.0000	9.0000	9.0554
9	Track12	0.0000	1.0000	0.0000
10	Track13	1.0000	1.0000	1.0000
11	Track14	0.0000	1.0000	1.4142
12	Track15	1.0000	0.0000	1.4142
Mean value		5.6600	5.8273	5.8903
Standard ...		6.2482	5.6225	6.1405
Minimum		0.0000	0.0000	0.0000
Maximum		37.2156	27.0740	39.2046
Range		37.2156	27.0740	39.2046
Sum		509.8216	506.3778	511.5841
Index of Min		3.0000	12.0000	9.0000
Index of ...		7.0000	7.0000	7.0000
Total obje...		87.0000	87.0000	87.0000
Shown ob...		87.0000	87.0000	87.0000

The data table contains information about tracks on the active image and provides tools to create, measure, and visualize tracks.

The toolbar contains the following buttons:



**Load tracks:** Clicking this button loads saved tracks from \*.TRC files, and appends these tracks to the existing list.



**Save tracks:** Clicking this button saves the tracks in \*.TRC files.



**Save table:** Clicking this button saves the data table in text, tab-delimited, comma-delimited, or HTML format.



**Send table to Excel:** Clicking this button sends the tracking data to Excel, using regional settings as the decimal separator.



**Tracking options:** Clicking this button opens the **Tracking Options** dialog. In this dialog, you can select measurements, set default a prefix for names, set colors, graphic size, font size, and label type. See the **Tracking Options** section for more information.



**Load tracking options:** Clicking this button loads saved tracking options from a file.



**Save tracking options:** Clicking this button saves the tracking options to a file.



**Show statistics:** Clicking this button shows or hides the tracking statistics.



**Select tracks:** Clicking this button activates the selection mode on the active image.



**Add track manually:** Clicking this button enables you to create a new manual track on the active sequence. You will be prompted to click on an object's position on every frame of the sequence.



**Add intensity track:** Clicking this button creates an intensity track based on the active AOI. The intensity track is linked to the first selected track (if one or more tracks are selected). If no tracks are selected, the AOI position does not change from frame to frame.



**Add correlation track:** Clicking this button adds a new correlation track based on the active AOI. The AOI have to be placed around the object on the first (Start) frame of the sequence. The object will be tracked from the Start frame to the End frame. Please refer to the **Correlation** tab in the *Tracking Options* section for settings.

## Measure:Track Objects



**Add track automatically:** Clicking this button creates a new track on the active sequence. You will be prompted to click on an object's position on the first frame, the other track positions will be found automatically. If the program can not find the position of the object within the search radius, you will be prompted to locate that object manually.



**Find all tracks automatically:** Clicking this button finds all tracks in the active sequence. The program tries to find tracks for all objects located in the first frame. If a track is not complete, that track is canceled.



**Show graph:** Clicking this button shows the tracking graph.



**Merge selected tracks:** Clicking this button merges the selected tracks by interpolating the point positions found in the gaps between the tracks, and averaging the coordinates where the tracks overlap.



**Split track:** Clicking this button splits selected the track at the active frame point. After the split, one new track is created from the second part of the active track, and the active track is split at the active frame point. The active frame is included in the first track



**Show all tracks:** Clicking this button shows all tracks (undoes the hide operation).



**Hide unselected tracks:** Clicking this button shows the selected tracks only, and hides the rest.



**Hide selected tracks:** Clicking this button hides the selected tracks, and shows the unselected ones.

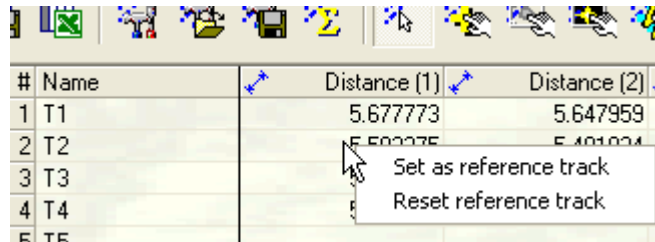


**Delete all tracks:** Clicking this button deletes all the tracks.



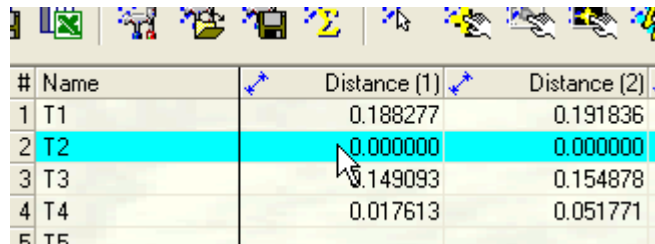
**Delete selected tracks:** Clicking this button deletes the selected tracks.

In addition, any track can be designated as the reference track by selecting **Set as Reference Track** from the context menu, as shown below. (The context menu appears when you click the right mouse button on the tracking table.)



#	Name	Distance (1)	Distance (2)
1	T1	5.677773	5.647959
2	T2	0.000000	0.000000
3	T3	0.149093	0.154878
4	T4	0.017613	0.051771
5	T5		

The reference track will be highlighted:



#	Name	Distance (1)	Distance (2)
1	T1	0.188277	0.191836
2	T2	0.000000	0.000000
3	T3	0.149093	0.154878
4	T4	0.017613	0.051771
5	T5		


When a reference track is set, all tracking parameters are calculated relative to the reference track. *Reference tracks* can be used to:

- Correct the geometric position (velocity, acceleration) of test tracks, assuming that the positions of objects on the reference tracks are static. The correction is done by subtracting the position of a reference object on every frame from all test objects. It can be used to correct for camera shifts during sequence acquisition.
- Correct the optical density (or any *Count/Size* parameter), where the reference track is placed on the background area, and all density-related parameters of the test tracks will be corrected by the intensity of the reference track. This technique can be used for background intensity correction.

Clicking the **Reset reference track** menu item resets the reference track.

The values in the **Tracking Data Table** can be sorted by clicking on that column's header.


## Measure:Track Objects

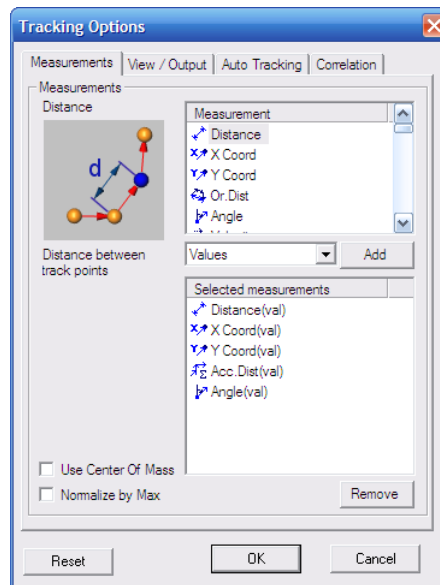
Double-clicking on the color icon  1 opens the color edit dialog that allows you to change a track's color.

The **Name** of each track can be modified by double-clicking on the cell containing the selected name and editing the text.

## Tracking Options

The list of measurements and other parameters are defined in the **Tracking Options** window, which can be opened from the **Tracking**

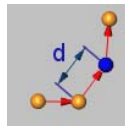
**Data Table** by clicking the **Tracking Options**  button. The **Tracking Options** dialog has been expanded to four pages, as shown here:



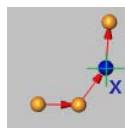


### Measurements Page

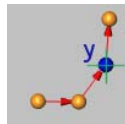
The *Measurements* page (above) allows you to select the options for measuring your selected tracks. This page includes the following items:



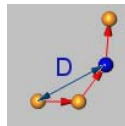
**Distance:** Distance between tracking points



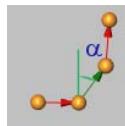
**X Coord:** X coordinates of tracking points



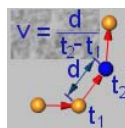
**Y Coord:** Y coordinates of tracking points



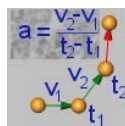
**Or. Dist:** Distance from the starting point to the selected object



**Angle:** Angle of the direction

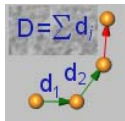


**Velocity:** Velocity of object

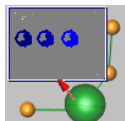


**Acceleration:** Acceleration of object

## Measure:Track Objects



**Acc. Dist:** Accumulated traveled distance from the starting point



The complete list of more than 50 density and morphological measurements, available in the Count/Size module, is also included into the tracking measurements. These measurements are available for tracks created in automatic and semi-automatic modes, and also for density tracks. Note that the measurements must be selected before creating tracks.

The following statistical parameters are included in the statistics combo-box:

- **Mean value**
- **Standard deviation**
- **Minimum**
- **Maximum Range**
- **Sum**
- **Index of Min**
- **Index of Max**
- **Total number of objects**
- **Values.**

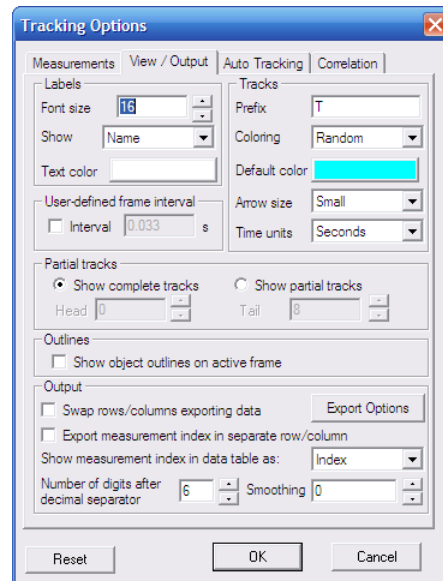
**Add:** Clicking the **Add** button adds the current measurement with a selected statistical field into the **Selected measurements** list.

**Values:** If the **Values** field is selected, the data table will include values for every vertex of the track. Otherwise, the corresponding statistical parameter will be calculated.

**Remove:** Clicking the **Remove** button deletes the selected measurement from the list.

## View/Output Page

The *View/Output* page contains options for displaying your tracks and measurements, as shown here:



**Labels:** The **Labels** group contains controls for the tracking labels displayed on the image.

**Text color:** This button set the text color.

**Font size:** This button sets the font size.

**Show:** The **Show** combo-box displays the type of labels. The labels can be one of the following:

**Name:** the track's name is displayed.

**First measurement:** value of the first selected measurement will be displayed as the label.

**None:** no labels are displayed.

**Interval:** The **Interval** control defines the way the interval between frames is measured, which affects the velocity and acceleration measurements. When the option is *off* (default), the frame interval is calculated as the difference between the capture times of successive frames. If the option is *on*, the user-defined interval is used. The interval is in seconds.

## Measure:Track Objects

Use this option if the analyzing sequence is captured by an external program (not *Image-Pro*). In that case, the capture frame time is not saved in the sequence file, and you may define the correct frame interval manually.

**Tracks:** The **Tracks** group contains other choices for displaying the tracks.

**Prefix** defines default prefix for track names.

**Color:** The **Coloring** control defines what color the tracks will have. Two choices are available: **Fixed color** and **Random**. If **Fixed color** is selected, new tracks will have the **Default color**. If **Random** color is selected, the colors for tracks are generated randomly, so every track has different color. Note that the colors of tracks can be edited later from the data table.

**Arrow Size:** The **Arrow size** defines the size of the line ends of the tracks. This selection also affects the size of any points on the **Tracking graph** and the size of the crossed circle that shows the track position on the current frame. The choices are: **None**, **Small**, or **Large**.

**Time Units:** The **Time Units** setting defines units for relative time display on the **Tracking Graph**, and time-related measurements, such as **Velocity** and **Acceleration**. It can be *Seconds*, *Minutes* or *Hours*. For example, if the calibration units for spatial calibration is in mcm and **Time Units** is in **minutes**, the **Velocity** values will have mcm/minute units and acceleration in  $\text{mcm}/\text{minute}^2$ .

**Partial Tracks:** The **Partial tracks** group defines how the tracks are shown on the image.

**Show Complete Tracks :** If **show complete tracks** is active the tracks are shown completely, from the beginning to the end, this option can be used with relatively short sequences.

**Show Partial Tracks:** If the option **Show partial tracks relatively to the active frame** is active, only the part of the track starting from **Active frame – Tail to Active frame + Head** is shown. This option should be used on long sequences.

**Outline:** If the **Show object outlines on active frame** is selected, the object outlines are shown on the image.

**Output:** The **Output** group determines the destination for the measurement data.

**Swap rows/columns for Excel:** When the **Swap rows/columns for Excel** option is on, rows and columns are swapped when data table is exported to *Excel*. The maximum number of columns in Excel is 256, so if you have more than 256 columns in the data table you have to activate this option to export the data correctly.

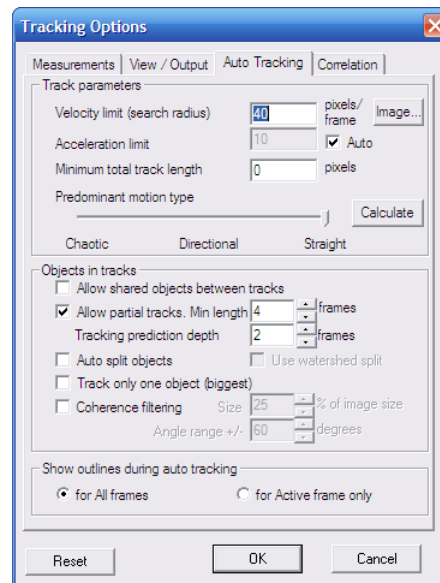
**Digits after decimal:** This option defines the number of digits after decimal point that will be displayed in the data table and other data windows.

**Export Options :** Select the *Export Options* command to tell *Image-Pro* how and where to export the current data via DDE. For additional information about DDE, see the *Dynamic Data Exchange* discussion.

**Smoothing:** The **Smoothing** option defines whether the point coordinates will be filtered using a running average filter. If the value is 0 (default), no filtering is applied. In other cases, a running average filtering is applied with filter size of **Smoothing\*2 +1**. The smoothing can be used when objects move from frame to frame by a very small distance (0-2 pixels). Because of rounding to nearest pixel position small movements produce a very jagged graph of velocities and accelerations. The filtering smoothes the and improves readability of the graphs.

## Auto-Tracking Page

The **Auto-Tracking** page defines the options used with automatic and semi-automatic tracking.



**Track Parameters:** The **Track Parameters** group defines the limits of the tracks you are creating.

**Velocity limit/Search radius:** The **Velocity limit (Search radius)** defines maximum distance in calibrated units between object positions on the neighboring frames. The parameter is used with automatic tracking to find tracks on the first two frames (on all frames with *Chaotic* movement type). If no object is found in the search radius, you will be prompted to click on the expected position manually. In automatic mode the track is closed at this point.

**Acceleration limit :** The **Acceleration limit** defines the maximum change in velocity of an object between the frames. The value is in calibrated units. Starting from the third frame, the estimated position of object is calculated based on the movement vector in the first two frames. The search area is defined as a circle with center in the estimated position and the radius of the *acceleration limit*. If no object is found within the search area, you are prompted to click on the expected position manually. In automatic mode the

## Measure:Track Objects

track is closed at this point. If **Auto** option is **on**, the acceleration limit is taken as half of the *velocity limit*.

**Image:** Clicking the **Image** button allows you to set the search radius directly on the image.

**Minimum total track length:** The **Minimum total track length** defines the minimum track length in calibrated units. The tracks that are shorter than this value will not be accepted during automatic and semi-automatic tracking. The default value is 0.

**Predominant motion type:** The **Predominant motion type** defines the type of objects' movement:

**Chaotic:** Select **Chaotic** if there is no directional movement of the objects, and the direction of the movement can be changed abruptly. In this case, the search area on all frames is defined only by the *speed limit*, the *acceleration limit* is ignored.

**Straight:** If the objects move following straight monotonous trajectories, choose the **Straight** type of motion.

**Directional:** Select the **Directional** type of motion if the object's movement is predominantly directional, but not always straight.

**Calculate:** Clicking the **Calculate** button will then analyze the existing tracks and calculate the *Velocity limit*, *Acceleration limit* and *Predominant motion type*, which will be used for further automatic and semi-automatic track detection.

**Objects in Tracks:** This group determines how the objects in the tracks will be defined.

**Allow shared objects between tracks:** If some objects on the image, which belong to different tracks, can not be separated (overlapped, clustered, etc) the **Allow shared objects between tracks** option can be checked. This will include merged objects in several tracks. If this option is unchecked, a single object can belong to only one track.

**Allow partial tracks:** If the track length is less than the number of active frames in the sequence, the sequence may have entries and exits, where some objects appear in the field of view after the first frame and leave the area before the last frame. These tracks can be taken into account by checking the **Allow partial tracks** option.

## Measure:Track Objects

**Minimum track length:** The tracks that are shorter than the **Minimum track length** are ignored when you select automatic or semi-automatic tracking.

**Tracking Prediction Depth:** Tracking Prediction Depth defines the number of frames ahead of the current, which are used to find most suitable track for the given object (default is 2). This parameter also defines the maximum number of consecutive occlusions on track. The occlusions number is less than the Prediction Depth by 1. For example, if the Prediction Depth of 3 the maximum number of consecutive object occlusions is 2, it means that if the tracking object in sequence disappears (or gets hidden) for 1 or 2 consecutive frames it will still be detected as a part of the track. The estimated position will be used for missed objects. The track points of missed objects do not have outlines and Count/Size measurement values. High prediction depth values increase computation time significantly.

**Auto split objects:** When **Auto split objects** is checked, the objects are automatically separated after the Count operation.

**Use Watershed split:** If **Use Watershed split** is checked, the watershed split is performed. If this option is unchecked, then *Image-Pro* will use the **Auto-split** option. This option is recommended when the active image has many objects that touch each other.

**Track Only One Object:** If the sequence contains only one object the Track only one object option can be checked to eliminate other objects on the image, which can be within threshold levels, but insignificant for tracking.

**Coherence filtering:** If the sequence represents *coherent motion*, in which spatially close particles are likely to have similar velocity vectors, the **Coherence filtering** option should be activated. The filtration discards those velocity vectors whose orientation is incompatible with the dominant orientation of the surrounding vectors. The dominant orientation is the median in a window centered on the origin of the vector being considered.

**Size:** The window **size** is defined in percents of image size. Incompatible outliers are detected as falling outside the limits specified by the median.

**Angle Range:** **Angle range** is defined in degrees (default is  $\pm 60$  degrees). Tracks that include one or more incompatible vectors



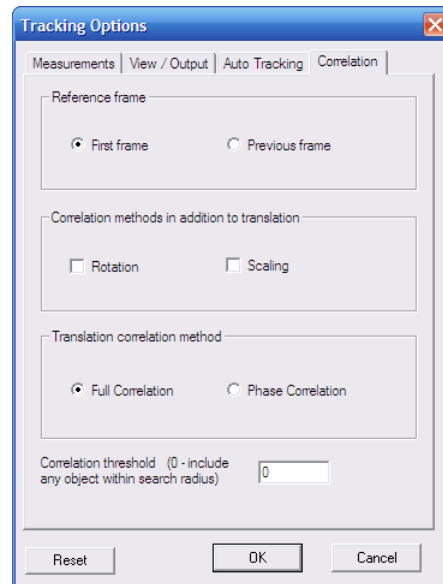
are removed. The **Allow shared objects** option should be *on* with this option

**Show outlines during auto-tracking:** You may choose to display the outlines of the objects in all frames, or only in the active frame, by clicking the appropriate radio button.

**Show objects' outlines with semi-automatic tracking:** When the image has too many frames and many objects, the outlines of the objects can hide the image itself, making object picking in semi-automatic tracking difficult. The overlays can be switched off using the **Show objects' outlines with semi-automatic tracking** option.

### Correlation Page

This page contains the options used with **Correlation** tracking:



**Reference frame:** The **Reference frame** defines which frame will be used for cross-correlation for the current frame.

**First frame:** The **First frame** can be used when object does not change shape from frame to frame.

**Previous frame:** The **Previous frame** option can be activated when the moving object changes slightly.

## Measure:Track Objects

**Correlation methods in addition to translation:** **Rotation** and **Scaling** are additional correlation methods.

**Rotation :** If the object changes orientation during tracking the **Rotation** option should be activated.

**Scaling:** If the tracking object changes size the **Scaling** option can be switched on.

Note that activating these options will slow down the tracking process.

If the tracking object moves without changing orientation and/or scale, the **Rotation** and **Scaling** options should be switched *off*.

**Translation correction method:** The best-match position between the reference and current frame can be calculated using **Full Correlation** or **Phase Correlation**. Full correlation works better for most images. Phase correlation ignores image intensity, and may be preferable if the overall image intensities vary while the patterns remain similar. Click the appropriate radio button to select the correction method.

## Manual tracking

**Add track manually:** When you click the **Add track manually** button, following dialog is displayed:



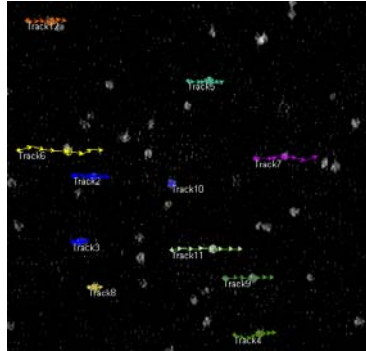
You will need to click on the object's position in every frame to create the track.

**Continue:** To cancel the track, click the **Continue** button .

**Close Track:** The track is started from the **Start** frame of the sequence and closes on the **End** frame. You can use the **Close track** button to close the track before the **End**.

Note that the tracks can be created only on sequences where the number of frames is greater than 1.

A sample image with tracks is displayed here:

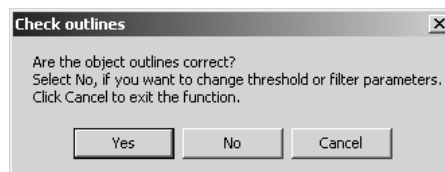


This is an example of the tracks on the image. A crossed circle on each track shows the position of the object on the current frame.

## Semi-Automatic Tracking

**Add track automatically** : You can add tracks in semi-automatic mode by clicking on the **Add track automatically** button.

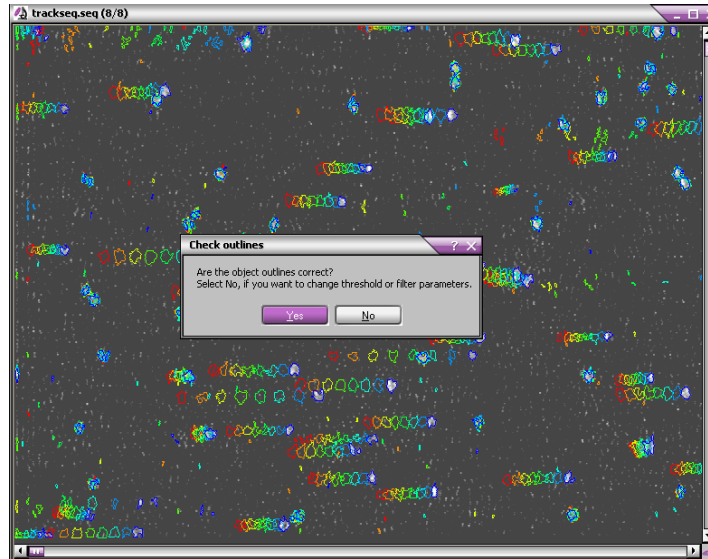
The first time you click this button, the following message is shown:



Click **Yes** to activate the **Count/Size** dialog . At this stage, you should define all the parameters in the **Count/Size** dialog to identify the tracking objects. You may adjust threshold level, and set measurement filters to eliminate unwanted objects (like dust) from tracking, and perform other tasks. You may click the **Count** button (on the **Count/Size** dialog) to check the correctness of the outlines on the first frame. When the parameters are set, click the **Continue** button.

In the following step the program finds object outlines on all frames and shows them to you for confirmation.

## Measure:Track Objects

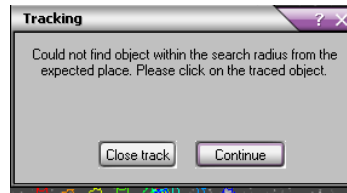


If the outlines are not correct, click the **No** button to reset the **Count/Size** parameters. If the outlines are correct, clicking the **Yes** button starts semi-automatic tracking.

For every track, you will be prompted to click on the position of an object in the first frame.



When you click on the object position on the first frame, the program tries to create a track starting from that point. On the next frames, all objects within the search radius are considered as track point candidates. The point, which can create a track with the lowest cost is selected from the available candidates. Objects that move in erratic patterns are considered to be higher-cost candidates. The program uses 2-frame forward tracking for every tracking point candidate. If there are no points found within the search radius, you will be prompted to locate the point manually.



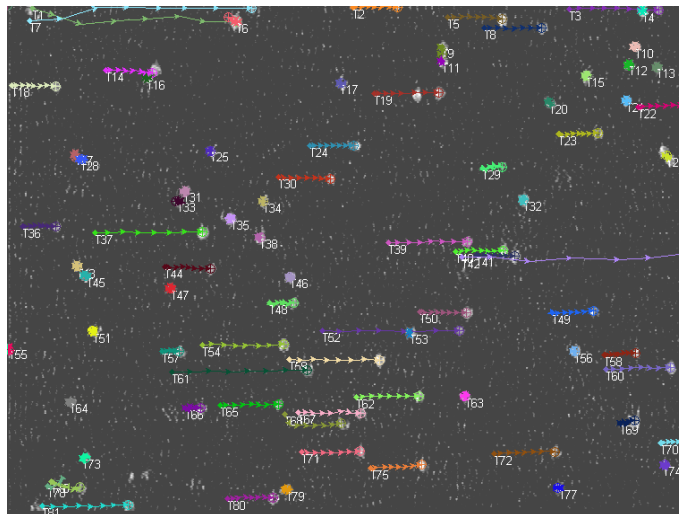
**Continue:** To cancel the track, click **Continue**.

**Close track:** The track can be closed by clicking on the **Close track** button; in this case a partial track will be saved in the list of tracks.

## Automatic tracking

**Find all tracks automatically:** You can find all the tracks on the image automatically by clicking on the **Find all tracks automatically** button.

In the first step, you will be prompted to set the correct **Count/Size** parameters (*see semi-automatic tracking*). The program then finds tracks for all objects located in the first frame. If a track cannot be completed in automatic mode, it will be canceled.



## Intensity Tracking

Intensity tracks can be created to control changing of intensity parameters over time within an AOI. The intensity tracks can be **static** or **dynamic**.


To create a **static** intensity track, follow the steps below:

1. draw an AOI around the object of interest .

2. Then click the **Add Intensity track**  button.

A new track with 0 length will be created and the intensity (and other parameters in the measurements list) in every frame will be measured. If there are no *Intensity* measurements in the list, a warning message will be displayed prompting you to select one of intensity measurements: Density (mean), Density (red), Density (green), Density (blue), IOD, Density (std.dev.), Density (min.), Density (max) or Density (sum).


A **dynamic** intensity track traces the intensity parameters of moving objects. To create a dynamic intensity track, follow these steps:

1. First, you need to create a *base track*, either manually or automatically. The track has to follow the moving object of interest..
2. Then, create an AOI around the object of interest on the first frame of the active sequence portion .
3. Next, select the base track and click the **Add Intensity track**  button. A new track that follows the *base track* with density measurements within the moving AOI will be created.

## **Correlation Tracking**

Correlation tracking can be used to track objects when image segmentation is difficult or not possible. Correlation tracking uses Fourier cross-correlation to find the position of the object outlined by an AOI on the next frame of the sequence.

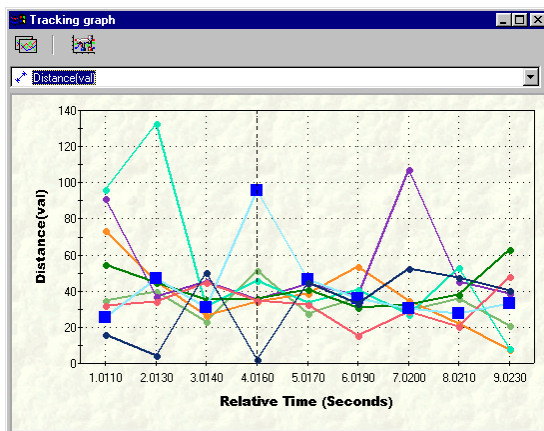
Use the following steps to perform correlation tracking:

1. Draw a rectangular AOI around the object
2. Click the **Add correlation track**  button. The area around the current AOI position expanded by the **Search radius** on the following frame will be analyzed to find the best-match position of the object. This procedure will be repeated for all active frames of the sequence. Then the tracking module will create a new track, using the central positions of the AOIs.

## Tracking Graph

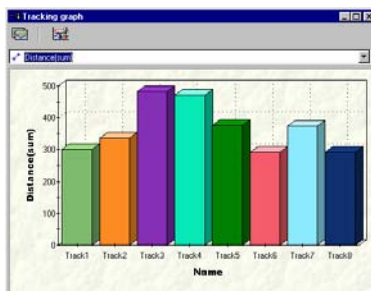
The **Tracking Graph** window can be opened by clicking on the **Show**

**Graph** icon  in the **Tracking Data Table's** toolbar.



The window shows the track values for all **Visible** tracks according to the **selected measurement** parameters (from the **Measurements** combo-box). The marker (vertical dashed line) shows the current frame position on the active image.

The window has tool tips showing the name of the track under the cursor. Clicking on a point on the graph selects the track and moves the active frame of the sequence to the selected position. The selected tracks are shown with large points. Selections on the image, the data table and the graph are synchronized. For statistical parameters, a bar graph is used:





**Tracking Graph Toolbar buttons:**



**Copy graph to clipboard:** Clicking this button copies the graph to the Windows Clipboard in Enhanced Metafile format.



**Graph options:** Clicking this button opens the **Graph Options** window, as shown here:



**Vertical range:** The **Vertical range** controls define the minimum and maximum data ranges if the **Auto-range** flag is off. If the flag is on, the ranges are defined from the data values of the corresponding measurement.

**X labels :** The **X labels** control defines labels for X axis. The choices are : *Frame Number*, *Relative Time* and *Absolute Time*. When **Relative time** is selected, measurement points along the X axis are distributed according to its relative time. In other cases, the measurement points are spaced equidistantly.

**Reset:** Clicking the **Reset** button resets all graph parameters to the original state.

*Measure:Snap Measurements*

## **Snap Measurements**

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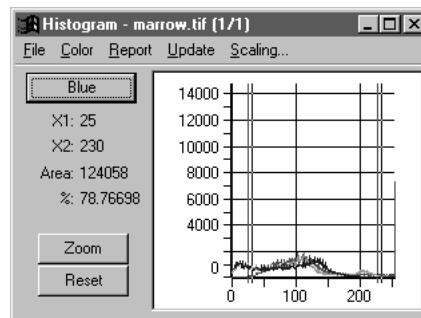
*Snap Measurements* allows you to capture a picture of your image with measurement overlays. This feature will not store the measurements as overlay objects. The result will be a new 24-bit color image showing the image as displayed in the image workspace, with all measurement, AOI and annotation overlays. This feature has no user interface or options.

## Histogram

---

Use the *Histogram* command to open the histogram window and display the intensity histogram and statistics for the active image or AOI. A histogram shows you a frequency distribution of the intensities in your image. It describes, in graphic form, the contrast and dynamic-range characteristics of your image. If you are not familiar with histograms, you may want to review the *Histogram* and *Intensity Analysis* discussions in *Section 1* of the Start-Up Guide before you begin using the *Histogram* command.

When you select the *Histogram* command, a **Histogram** window is opened.



The Histogram window is not a “common access” window like the *Count/Size* or *Measurement* windows. A Histogram window is associated with, and accessed by, only one image (the name of the owning image is displayed in its title bar). The **Histogram** window remains open until you explicitly close it, or until you close the image to which it is attached.

By default, the histogram’s X-axis will be calibrated to your *intensity* unit-of-measure, and the Y-axis to your *spatial* unit-of-measure. However, histogram data can be displayed in uncalibrated form by disabling the Intensity/Spatial calibration options in the histogram *Report* menu.

**Zoom:** Clicking on the **Zoom** button enables you to enlarge and focus on a particular area of the histogram.

**Reset:** Clicking on the **Reset** button returns the Histogram to its original size.

## Measure:Histogram

**Color:** For a color image, clicking on the color list box changes the color channel displayed on the Histogram.

***Important** - the histogram measures pixel values as they exist in the image bitmap. It does not reflect values as they are interpreted through the LUT. This allows you to make visual adjustments to your image for viewing purposes without affecting the data used for analysis. In the event that you want to have LUT intensity adjustments reflected in your histogram, you must apply the LUT to your bitmap using the **Apply LUT** command.*

The appearance of the histogram can be modified, and its data saved to a file, using the Histogram menu commands. These commands are listed in the following sections.

---

***Note:** In the command and option descriptions below, the general terms “intensity” and “pixels” are used, but keep in mind that on your system these values will actually be reported in the terms you have specified with the **Calibration** command (optical density and millimeters, for example).*

---

## Histogram File Menu

The options on the *Histogram* window's *File* menu are used to store histogram data. The current set of histogram data may be stored to a new file, or appended to an existing histogram file. The *File* menu also contains an option that lets you close the *Histogram* window.

---

◆ **Data to File:** Use the *Histogram* window's *Data to File* command to store the current histogram data to a file. Histogram data are saved in an ASCII file containing the intensity intervals and the spatial frequency reported for each. The saved data can be imported into a spreadsheet application or used as input to an external program.

When the *Data to File* command is selected, the **Save Histogram** dialog box is presented.

Enter the name of the file to which you want the histogram data stored, then click **OK**. Your histogram data will be written to the file.

If you have sub-divided the X-axis into "bins" using the Histogram **Bins** command, the data will be expressed and recorded in the intervals you have specified. Each bin along the X axis will be labeled with its midpoint value.

---

◆ **Append Data to File..:** Use the *Histogram* window's *Append* command (on the *File* menu) to add the current histogram data to an existing histogram file.

When the *Append* command is selected, the **Save Histogram** dialog box is presented.

Select the file to which you want the current data appended, then click **OK**. The current histogram data will be written to the end of the specified file.

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◆ **Statistics to File:** Use the *Statistics to File* command to store the current Statistic or Range/Area data to a file.

---

**Note:** The **Statistics to File** command can also be used to save the current range information. If the "Range/Area" option is enabled in the "Report" menu when the "Statistics to File" command is performed, the current range information will be saved to the file instead of statistics.

---

## Measure:Histogram

Data are saved to an *Image-Pro* histogram file (.HST) in ASCII format. The saved data can be imported into a spreadsheet application or used as input to an external program.

When the *Statistics to File* command is selected, the **Statistics to File** dialog box is presented. Within it, specify the name of the file to which you want the statistic or range data saved. Then click **OK**.

- 
- ◆ **Append Statistics to File:** Use the **Histogram** window's *Append Statistics* command to add the current Statistic or Range/Area data to the end of an existing histogram file.

---

**Note:** The **Append Statistics** command can also be used to append the current range information to a histogram file. If the **Range/Area** option is enabled in the **Report** menu when the **Append Statistics** command is performed, the current range information will be saved to the file instead of statistics.

---

When the *Append Statistics* command is selected, the **Statistics to File** dialog box is presented. Within it, specify the name of the file to which you want the statistic or range data appended. Then click **OK**.

- 
- ◆ **Data To Clipboard:** Use the **Histogram** window's *Data To Clipboard* command to store the current Statistic or Range/Area data to the Windows Clipboard. Once on the Clipboard, the histogram data can be pasted into any Windows application that accepts text from the Clipboard.

- 
- ◆ **Data to Printer:** Select the *Data To Printer* command to print out the current histogram data.

- 
- ◆ **Graph To Clipboard:** Select the *Graph To Clipboard* command to copy the current histogram graph to the Windows Clipboard. From there, the data can be pasted into any application that accepts text from the Clipboard.

- 
- ◆ **Data Export:** Select the *Data Export* command to transfer data to Microsoft's Excel spreadsheet. Before selecting *Data Export*, you should use *Export Options* to determine the correct placement (within Excel) for the data. When you select *Data Export*, the transfer occurs with little visible evidence that it has happened. By switching to the Excel application, you can confirm that the transfer has occurred.

## Measure:Histogram

For additional information about DDE, see the *Dynamic Data Exchange* discussion.

---

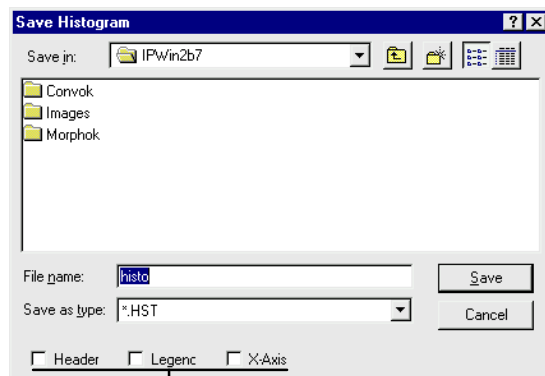
- ◆ **Export Options:** Select the *Export Options* command to tell *Image-Pro* how and where to export the current data via DDE. For additional information about DDE, see the *Dynamic Data Exchange* discussion.
- 

- ◆ **Close** Use the *Histogram* window's *Close* command (on the *File* menu) to close the histogram window.

If you close the image with which the *Histogram* window is associated, the *Histogram* window will automatically close, too.

### Saving Header, Legend and Axis Information

The Histogram **Data to File**, **Append Data to File**, **Statistics to File** and **Append Statistics to File** commands provide the ability to include or suppress the following kinds of information: the header, the legend and the X-axis values.



Enable the option to include the information; disable the option to suppress it.

**Header:** When enabled, this option will include descriptive header information at the beginning of the data set, as shown in the example below.

```
# Image-Pro Plus Histogram Data
# Time: Mon Mar 07 16:06:31 1998
# Image: dna.tif
# Spatial Unit: Pixels
# Intensity Unit: Gray Level
# Number Of Channels: 1
# Number Of Samples: 256
    30
    30
    32
    .
    .
```

descriptive "header" information.

histogram data

**Legend:** When enabled, this option will include column headings with the data (see example below). Columns will be labeled with the unit names specified by the **Calibration** command, or with the default labels of *Intensity* and *Pixels*, if unit names have not been specified.



#	<u>Density</u>	<u>Pixels</u>
		.
0		30
1		30
2		32
⋮		⋮

— data legends

histogram data

**X-axis:** When enabled, this option will store the X-axis values (the intensity values for the histogram), along with the pixel counts for each intensity. See examples below.

0		30
1		30
2		32
⋮		⋮

histogram data stored with X-axis data (leftmost column).

30		
30		
32		
⋮		

histogram data stored without X-axis data.

## Histogram Color Menu

The options on the *Histogram* window's *Color* menu are used to specify which color model you want your image's color information measured in, and which color channels you are interested in seeing. If you are not familiar with color models, you may want to review the *Intensity Analysis* discussion in *Section 1* before working with this command.

Each command on the histogram *Color* menu is described below.

---

**Note:** The **Color** command will be dim if the active image is not a True Color image. If your image is a Palette class image, you must convert it to True Color if you want to extract histogram data for each color channel. See the *Convert To* command.

---

- ◆ **RGB:** Check this command on the *Histogram* window's **Color** menu if you want your color data expressed in terms of its Red, Green and Blue components.

When **RGB** is checked, you can select the channels you want to measure, Red, Green or Blue, in the middle segment of the *Color* menu. If there is a check by the channel name, it will be measured. Click on the channel name to toggle it off and on.

- 
- ◆ **HSI:** Check this command on the *Histogram* window's **Color** menu if you want your color data expressed in terms of its Hue, Saturation and Intensity components.

When **HSI** is checked, you can select the channels you want to measure, Hue, Saturation or Intensity, in the middle segment of the *Color* menu. If there is a check by the channel name, it will be measured. Click on the channel name to toggle it off and on.

- 
- ◆ **HSV:** Check this command on the *Histogram* window's **Color** menu if you want your image data expressed in terms of its Hue, Saturation and Value components.

When **HSV** is checked, you can select the channels you want to measure, Hue, Saturation or Value, in the middle segment of the *Color* menu. If there is a check by the channel name, it will be measured. Click on the channel name to toggle it off and on.

- ◆ **YIQ:** Check this command on the *Histogram* window's *Color* menu if you want your image data expressed in terms of its Y-axis (luminance), In-phase and Quadrature components.

When **YIQ** is checked, you can select the channels you want to measure, Y-axis, In-phase or Quadrature, in the middle segment of the *Color* menu. If there is a check by the channel name, it will be measured. Click on the channel name to toggle it off and on.


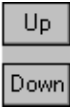
- ◆ **Order :** Use this command on the *Histogram* window's **Color** menu to arrange the way in which the individual channel plots are layered in the graph. This might be used when you have graphed more than one channel simultaneously, and the plot for one channel is obscured by another.

When the histogram *Order* command is selected, the **Histogram Order** dialog box is presented:



The channels listed inside the box show you the order in which the plots are currently layered, from front to back. In the **Line Profile Order** box above, the Blue channel is in front, and the Red channel is in back.

To rearrange the layers, click on the channel you want to reposition, then click the Up or Down button to exchange the two channels.

	<p>To move the Green channel in front of the Blue channel, click the <b>Up</b> button.</p> <p>To move the Green channel in back of the Red channel, click the <b>Down</b> button.</p>	
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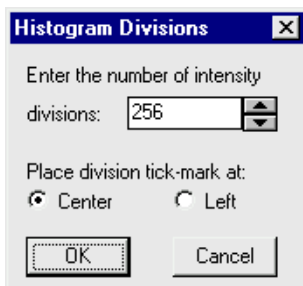
**Note:** If you have selected a different color model (e.g., HSI or YIQ), then your choices will be expressed in terms of the color model chosen (e.g., “hue, saturation, intensity”).

---

## Histogram Report Menu

The options on the *Histogram* window’s *Report* menu are used to determine the format in which data are presented in the histogram graph. Choices relating to interval width and X/Y-axis calibration are available within this menu. You can also choose to display the data in bar/line/table form, or summarize the data into a set of statistics.

- ◆ **Bins:** Select this command on the *Histogram* window’s *Report* menu to specify the number of intervals you want the X-axis segmented into. When you select the *Bins* command, the following dialog box is presented.



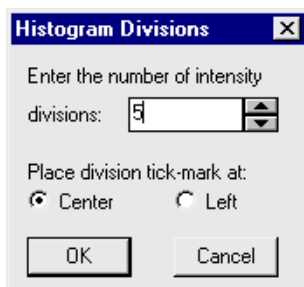
Type the number of intervals (bins) you want your scale divided into. By default, *Image-Pro* will divide the scale into the following number of segments:

<i>Gray Scale</i>	256
<i>True Color</i>	256
<i>Gray Scale 12</i>	4096
<i>Gray Scale 16</i>	65536
<i>Floating Point 25</i>	6

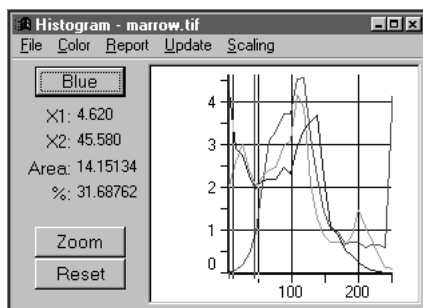
You may change the number of intervals to any whole number that does not exceed the intensity range defined by the image bit depth; i.e., no more than 256 for an 8-BPP image, no more than 4096 for a 12-BPP image. *Floating Point* scales can be segmented into a maximum of 256 bins.

*Measure:Histogram*

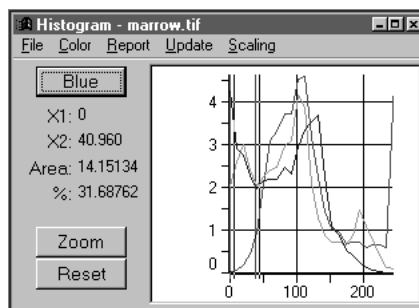
The **Bins** command on the *Histogram* window's *Report* menu now offers the option of locating the tick mark between adjacent bins to the Left of each bin or in the Center of each bin.



The calculation is the same in both instances. Only the placement of the value on the report changes. In the following histograms, five bins are selected.



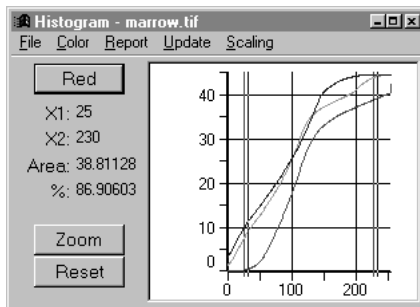
*Division marks at Left*



*Division marks at Center*

**Important** - Changing the bin value affects your histogram in a couple of important ways. First, it affects the statistics produced by the **Report** menu's **Statistics** command. Some precision will be lost due to averaging that occurs within the consolidated bins. Second, if histogram data are saved to a file, the data will reflect the number of bins selected here (i.e., if 10 bins were specified, 10 data points would be recorded in the file).

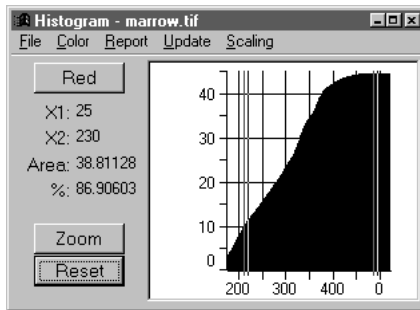
- 
- ◆ **Accumulated:** Select this command on the *Histogram* window's *Report* menu to display pixels values cumulatively. An accumulated histogram indicates the number of pixels having the measured intensity value, *or a lesser value*, as shown in the example below.



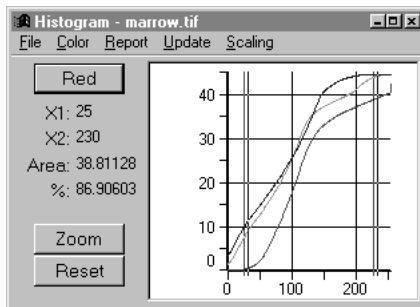
When you select *Accumulated*, a check will appear next to it indicating that it is enabled.

- 
- ◆ **Spatial Cal:** Toggle this option on the *Histogram* window's *Report* menu *ON* to measure Y-axis values in their calibrated form. Toggle this option "off" to measure spatial values (Y-axis data) in uncalibrated form. In uncalibrated form, the Y-axis reflects the number of pixels for a given intensity value. By default this option is enabled.
  - ◆ **Intensity Cal:** Toggle this option on the *Histogram* window's *Report* menu *ON* to measure X-axis values in their calibrated form. Toggle this option "off" to measure intensity values (X-axis data) in uncalibrated form. In uncalibrated form, the X-axis measures the pixel's actual intensity value. By default this option is enabled.

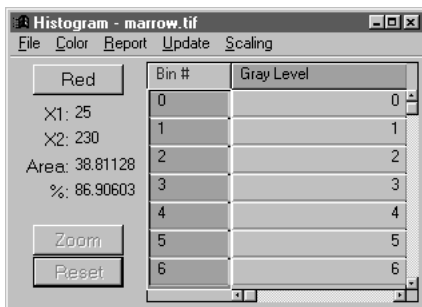
- ◆ **Bar:** Select this option on the *Histogram* window's *Report* menu to display your histogram in bar graph form. In bar form, *Image-Pro* indicates the measurement points with a bar, as shown in the example below:



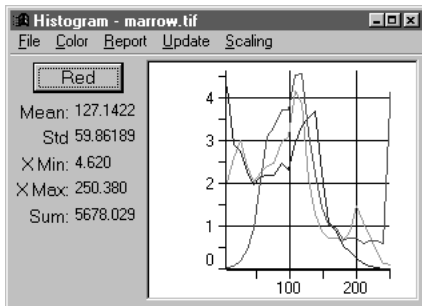
- ◆ **Line:** Select this option on the *Histogram* window's *Report* menu to display your histogram in line form. In line form, *Image-Pro* connects the measurement points with a line, as shown in the example below:



- ◆ **Table:** Select this option on the *Histogram* window's *Report* menu to display the histogram data in tabular form, as shown in the example below:



- ◆ **Statistics:** Select this option on the *Histogram* window's *Report* menu to display statistics concurrently with the histogram, as shown in the example below. If the active image contains *True Color* data, the statistics in this box will relate to the color channel that is the backmost layer in the graph (you can relayer the channels using the *Order* command on the *Color* menu).

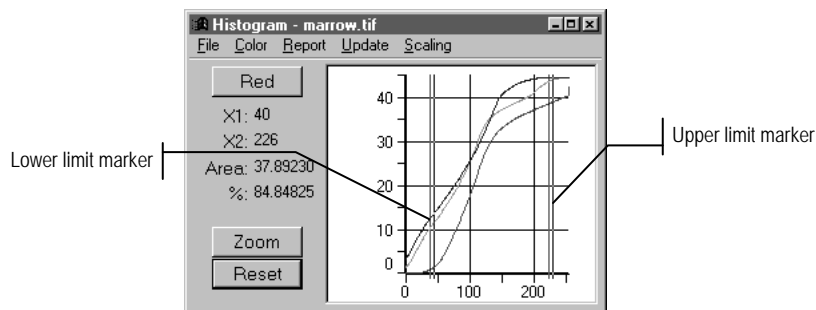


The following statistics will be included:

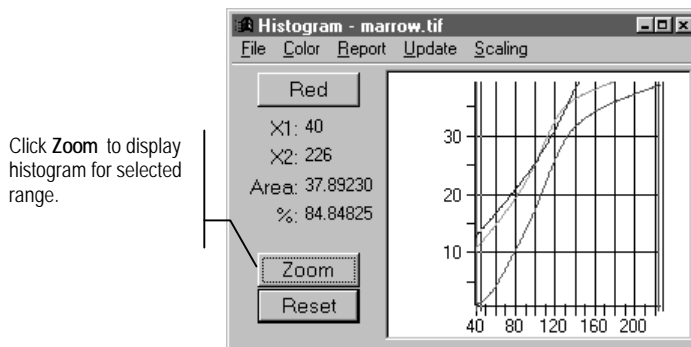
- ◆ **Mean:** Reports the average (mean) intensity value for your the active image or AOI.
- ◆ **Std Dev:** Reports the standard deviation of the intensities contained in the active image or AOI.
- ◆ **X Min:** Reports the smallest intensity value contained within the active image or AOI.
- ◆ **X Max:** Reports the largest intensity value contained within the active image or AOI.
- ◆ **Sum:** Reports the sum of all the intensity values contained within the active image or AOI.



- ◆ **Range / Area:** Select this option to isolate a segment (range) of the X-axis, and display information relating to this range. When this option is selected, two movable boundary markers are placed in the histogram.



The boundary markers can be positioned to select just the range you want to study (see also the *Histogram* window's *Scaling* menu, which can be used to accomplish the same thing). Information about the selected range is displayed along the right-hand side of the *Histogram* window. The window also contains a *Zoom* button to re-scale the graph to the selected range (i.e., display the histogram for just the selected range, as shown below).



---

**Note:** The **Range/Area** and **Statistics** options are mutually exclusive, i.e., they cannot be enabled simultaneously. Image-Pro will automatically disable the **Statistics** option when **Range/Area** is selected.

---

## Measure:Histogram

The following information is reported for the selected range.

- ◆ **X1:** Reports the intensity level that marks the beginning of the current range (it identifies the position of the left boundary-marker).
- ◆ **X2:** Reports the intensity level that marks the end of the current range (it identifies the position of the right boundary-marker).
- ◆ **Area:** Reports the number of pixels between the left and right boundary-markers (“the area under the curve”).
- ◆ **%:** Reports the number of pixels within the current range (under the curve) expressed as a percentage of the number of pixels in the entire histogram.

**Zoom:** Click this button to “zoom-in” on the selected range, and display just that portion of the histogram. Data outside the boundary markers are eliminated from the graph. The Y-axis is re-scaled to accommodate the largest value in the selected range.

---

*Note:* If the *Statistics* command is enabled while the display is “zoomed-in” on a segment of the X-axis, the reported statistics represent the entire histogram, not just the selected segment.

---

**Reset:** Click this button to reset the X and Y axes to their default values after having “zoomed-in” on a histogram range using the **Zoom** button (see above).

## **Histogram Update Menu**

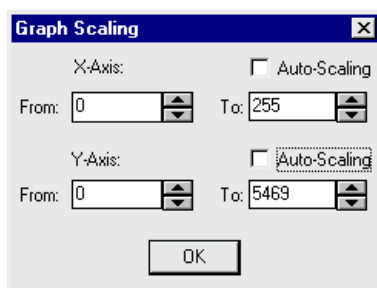
Select the *Histogram* window's *Update* menu to refresh the contents of the histogram window to reflect any changes that have been made to the image data. For example, if you moved the AOI from which the histogram was generated, you would need to select *Update* to plot the data at the AOI's new position. Or, if you filtered an image after creating its histogram, you would need to update the histogram to reflect the intensity changes produced by the filter.

To update the histogram, simply click the *Update* menu. The contents of the histogram will be rewritten based upon the contents of the currently selected image or AOI.

## Histogram Scaling Menu

The *Scaling* menu lets you define how much of the data you want graphed by controlling the length of the X and Y axes. This lets you graph just part of your intensity data.

When you select the *Scaling* menu, the **Graph Scaling** dialog box is presented:

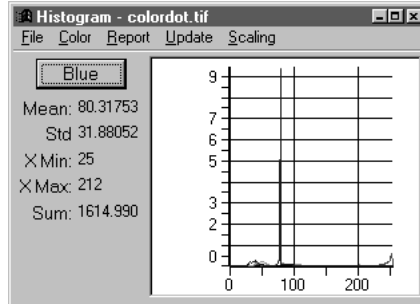


By default, the *Auto-Scaling* option will be selected for both axes. If you want to change the endpoints of an axis, you must disable this option. Doing so will allow you to enter values into the **From** and **To** fields.

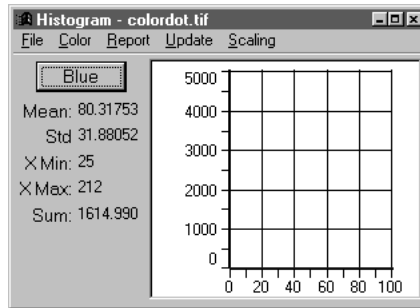
For each axis, the following fields are available:

**Auto-Scaling:** Check this box if you want *Image-Pro* to use the default length of the axis. For the X-axis, the default length is the full intensity range (e.g., 0 - 256 for *Gray Scale* and *True Color* images, 0 - 4095 for *Gray Scale 12*). For the Y-axis, the default range is determined by the largest value measured on the Y-axis for that particular AOI or image. When the **Auto Scaling** box is selected for an axis, its **From** and **To** fields and values are dimmed.

**From/To:** In these fields, enter the values to be used as the axis beginning and end points. When you define a range on the X-axis, just the intensities within the range you specify are graphed. You can also specify the Y-axis limits. This is usually done to reduce its scale to make small values visible. For example, in the histogram below, the huge number of pixels at 79 dwarfed the measurements at either end of the X-axis.



By scaling both the X and Y axes, the intensity measurements at the lower-end of the scale can be visualized:



In this case, the X-axis was changed to range from 0 to 101, and the upper limit of the Y-axis was set to 5000.

In cases where the upper limit of the Y-axis is not large enough to accommodate a value in the graph, the value is simply clipped at the top of the chart.

---

**Note:** The values specified in the **Graph Scaling** dialog box do not affect your actual histogram data in any way. These values merely determine how much of the data will be displayed in the graph, and how it will be scaled vertically. If the histogram data were to be viewed in table form, or stored to a file while these scaling value were in effect, the entire set of histogram data would be displayed or stored.

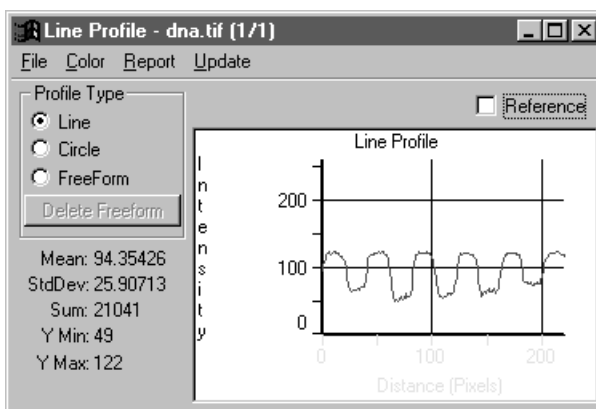
---

## Line Profile

---

Use the *Line Profile* command to obtain a plot of the intensity values of a single line within your image, or the average values of a band of lines. When you select the *Line Profile* command, the *Line Profile* window is opened. The *Line Profile* window is not a “common access” window like the *Color Map* or *Count/Size* windows. A *Line Profile* window is associated with, and accessed by, only one image (the name of its owner is displayed in its title bar).

The *Line Profile* window will remain open until you explicitly close it, or close the image to which it is attached.

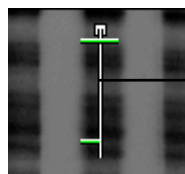


---

**Note:** You can change the size of the dialog box by positioning your cursor near an edge or corner and dragging the two-way arrow to the desired position.

---

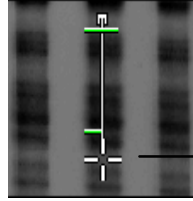
When you select the *Line Profile* command, a **Line Profile** window is opened, and a defining-line is placed within your image. The defining-line specifies the line of pixels to be plotted.



Position the "defining line" over the line of pixels you want to measure.

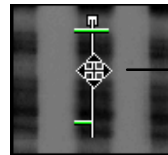
## Measure:Line Profile

Use the mouse to position the defining-line over the line of pixels you want to measure. The line can be drawn to any length or angle. When you move your cursor over the line, special cursor symbols



Position your cursor over an endpoint to change its position. Drag the line when you see the 4-spoked cursor.

will appear, letting you know that you are in a position to move the line or one of its endpoints. To move the entire line, place your cursor over the middle



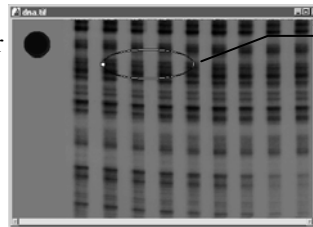
Position your cursor within the line to move the entire line. Drag the line when you see the 4-way cursor.

of the line. Your cursor will change into a 4-way arrow. When you see the 4-way arrow, drag the line to its new position.

To reposition an endpoint, place your cursor over the endpoint you want to move. Your cursor will change into a wheel. When you see the wheel, drag the endpoint to its new position. *Image-Pro* will redraw your **Line Profile** graph to reflect the line's new position.

The **Line**, **Circle**, and **Freeform** options allow you to select the area to be graphed. An example of the **Line** option is shown above.

The **Circle** option places a circle on the image, and you can use your cursor to position it.



Use your cursor to define the shape and placement of the circle.

The **Freeform** option allows you to draw an irregular shape on the image. Clicking the **Del Freeform** button removes the drawing from the image.

The graph in the **Line Profile** window displays the type of plot you have selected in the *Line Profile : Report* menu (i.e., **Normal**, **Thick Horizontal**, or **Thick Vertical**). By default, a **Normal** line plot is created. It plots intensity values along a single line of pixels. If you have calibrated your intensity and/or spatial scales, the calibrated values will be reflected in the graph (the Y-axis depicts intensity values, and the X-axis, spatial values).

## Measure:Line Profile

**Important** - the line profile measures pixel values as they exist in the image bitmap. It does not reflect values as they are interpreted through the LUT. This allows you to make visual adjustments to your image for viewing purposes without affecting the data used for analysis. In the event that you want to have LUT intensity adjustments reflected in your line profile, you must apply the LUT to your bitmap using the **Apply LUT** command.

If the **Reference** option is checked, the defining line becomes a “baseline” against which a subsequent line is measured (see **Reference** below). You can also **Freeze** a line plot on the graph so that it, and a subsequent line, can be viewed simultaneously (see *Report* menu below).

If *True Color* data are being measured, *Image-Pro* will plot the values of the color channel(s) you have specified in the *Color* menu.

Line profile commands are performed using the commands from its menu bar. These commands are described in the sections that follow.

---

**Note:** The line profile X-axis will be calibrated to your spatial unit-of-measure, and the Y-axis to your intensity unit-of-measure. In the command and option descriptions below, the general terms “intensity” and “pixels” are used, but, on your system, these values will actually be reported in the terms you have specified using the **Calibration** command (which might be optical density and millimeters, for example)

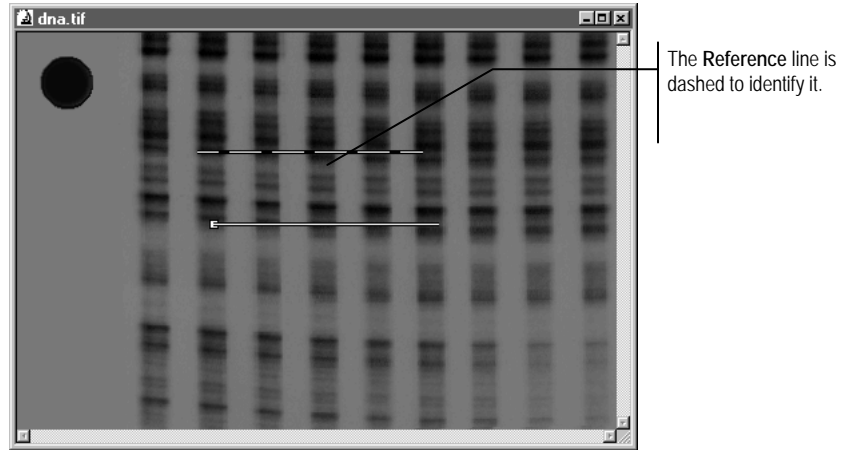
---

**Reference:** Check this box if you want the current line to become the baseline against which all further measurements will be plotted. This option lets you visualize the differences between two lines of pixels. To create such a plot you must:

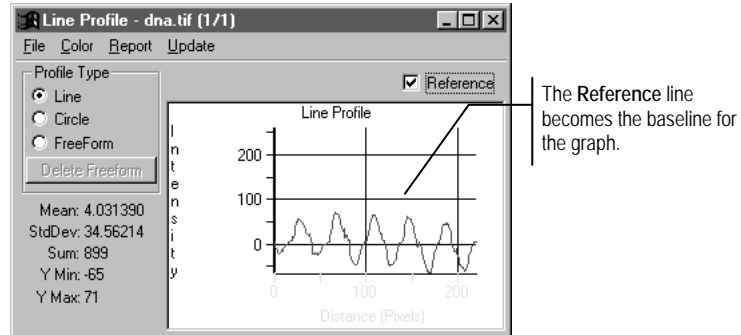
1. **Position the defining line** in the image to represent the first line in the pair.
2. **Check the Reference option.** This will establish the first line as the baseline. The defining line in your image will appear in dashed form to indicate this. You will also see the profile for that line plotted as the baseline (0) in your graph.



### Measure:Line Profile



3. Place your cursor over the middle of the reference line in your image. When the 4-way cursor appears, drag the line to its new position. When the line is in its new position, the differences between it and the reference line are plotted in the graph as shown below.



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**Note:** Once a reference line has been established, its position can be moved, but its length should not be changed. Changing its length will cause it to lose its reference attribute and will change it back into an ordinary defining line.

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## Line Profile File Menu

Use the *Line Profile* window's *File* menu to store your line profile data. This menu lets you store the current data to a new file or append it to an

## Measure:Line Profile

existing histogram file. The *File* menu also contains an option that lets you close the *Line Profile* window.

The commands on the *Line Profile* window's *File* menu are described below.

- 
- ◆ **Data to File:** Use the *Data to File* command to store the current line profile data to a file. Line profile data are saved in an *Image-Pro* histogram file (.HST), in ASCII format.

---

The file will contain the pixel positions along the line and the intensity associated with each. If the reference option has been selected, *Image-Pro* will save the offsets (positive and negative) from the reference line. The saved data can be imported to a spreadsheet application or used as input to an external program.

When the *Data to File* command is selected, the **Save Profile** dialog box is presented.

Enter the name of the file to which you want the line profile data stored, then click **OK**. Your line profile data will be written to the histogram file.

- 
- ◆ **Append Data to File:** Use the *Append* command on the *Line Profile : File* menu to add the current line profile data to an existing histogram (.HST) file.

When the *Append* command is selected, the **Save Profile** dialog box is presented.

Select the file to which you want the current data appended, then click **OK**. The current line profile data will be written to the end of the specified file.

- 
- ◆ **Statistics to File:** Use the *Statistics to File* command to store current Line Profile statistics to a file.

---

**Note:** The **Statistics to File** command can also be used to save the current range information. If the **Range/Area** option is enabled in the **Report** menu when the **Statistics to File** command is performed, the current range information will be saved to the file instead of statistics.

---

Data are saved to an *Image-Pro* histogram file (.HST) in ASCII format. The saved data can be imported into a spreadsheet application or used as input to an external program.

When the *Statistics to File* command is selected, the **Statistics to File** dialog box is presented. Within it, specify the name of the file to which you want the statistic or range data saved. Then click **OK**.

---

- ◆ **Append Statistics to File:** Use the *Line Profile* window's *Append Statistics* command to add the current Line Profile data to the end of an existing file.
- 

**Note:** The **Append Statistics** command can also be used to append the current range information to a file. If the **Range/Area** option is enabled in the **Report** menu when the **Append Statistics** command is performed, the current range information will be saved to the file instead of statistics.

---

When the *Append Statistics* command is selected, the *Statistics to File* dialog box is presented. Within it, specify the name of the file to which you want the statistic or range data appended. Then click **OK**.

---

- ◆ **Data To Clipboard:** Use the *Line Profile* window's **Data To Clipboard** command to copy the current Line Profile data to the Windows Clipboard. Once on the Clipboard, the histogram data can be pasted into any Windows application that accepts text from the Clipboard.
- 

- ◆ **Data to Printer:** Select the *Data To Printer* command to print out the current line profile data.
- 

- ◆ **Graph To Clipboard:** Use the *Line Profile* window's **Graph To Clipboard** command to copy the current Line Profile graph to the Windows Clipboard. Once on the Clipboard, the histogram data can be pasted into any Windows application that accepts text from the Clipboard.

- ◆ **Data Export:** Select the *Data Export* command to transfer data to Microsoft's Excel spreadsheet. Before selecting *Data Export*, you should use *Export Options* to determine the correct placement (within

### *Measure:Line Profile*

Excel) for the data. When you select *Data Export*, the transfer occurs with little visible evidence that it has happened. By switching to the Excel application, you can confirm that the transfer has occurred. For additional information about DDE, see the *Dynamic Data Exchange* discussion.

---

◆ **Export Options :** Select the *Export Options* command to tell *Image-Pro* how and where to export the current data via DDE. For additional information about DDE, see the *Dynamic Data Exchange* discussion

---

◆ **Close:** Use the *Close* command on the *Line Profile : File* menu to close the **Line Profile** window.

## Line Profile Color Menu

The options on the *Line Profile* window's *Color* menu are used to specify which color model you want your image's color information measured in, and which color channels you are interested in seeing. If you are not familiar with color models, you may want to review the *Color Models* discussion in *Section 1* before working with this command.

Each command on the line profile *Color* menu is described below.

---

**Note:** The **Color** command will be dim if the active image is not a True Color image. If your image is a Palette class image, you must convert it to True Color if you want to extract histogram data for each color channel. See the **Convert To** command.

---

- ◆ **RGB B:** Check this command on the *Line Profile : Color* menu if you want your color data expressed in terms of its Red, Green and Blue components.

When **RGB** is checked, you can select the channels you want to measure, Red, Green or Blue, in the middle segment of the *Color* menu. If there is a check by the channel name, it will be measured. Click on the channel name to toggle it off and on.

- 
- ◆ **HSI :** Check this command on the *Line Profile : Color* menu if you want your color data expressed in terms of its Hue, Saturation and Intensity components.

When **HSI** is checked, you can select the channels you want to measure, Hue, Saturation or Intensity, in the middle segment of the *Color* menu. If there is a check by the channel name, it will be measured. Click on the channel name to toggle it off and on.

- 
- ◆ **HSV :** Check this command on the *Line Profile : Color* menu if you want your image data expressed in terms of its Hue, Saturation and Value components.

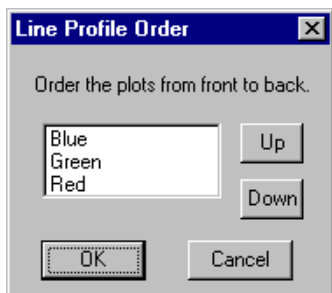
When **HSV** is checked, you can select the channels you want to measure, Hue, Saturation or Value, in the middle segment of the *Color* menu. If there is a check by the channel name, it will be measured. Click on the channel name to toggle it off and on.

- 
- ◆ **YIQ** : Check this command on the *Line Profile : Color* menu if you want your image data expressed in terms of its Y-axis (luminance), In-phase and Quadrature components.

When **YIQ** is checked, you can select the channels you want to measure, Y-axis, In-phase or Quadrature, in the middle segment of the *Color* menu. If there is a check by the channel name, it will be measured. Click on the channel name to toggle it off and on.

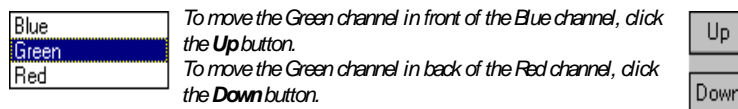
- 
- ◆ **Order** : Use this command on the *Line Profile : Color* menu to rearrange the way in which the individual channel plots are layered in the graph. This might be done so you can view the statistics of a particular channel (statistics represent the values of the backmost layer in the graph).

When the *Order* command is selected, the **Line Profile Order** dialog box is displayed:



The channels listed inside the box show you the order in which the plots are currently layered, from front to back. In the **Line Profile Order** box above, the Blue channel is in front, and the Red channel is in back.

To rearrange the layers, click on the channel you want to reposition, then click the **Up** or **Down** button to exchange the two channels.



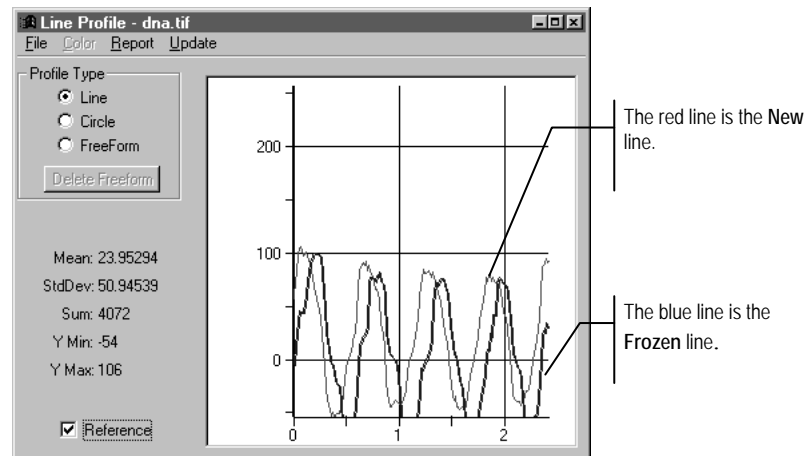
When the cross hair appears at the position of your choice, click your mouse. The highlighted channel will be inserted at that point. Press **OK** when the channels are arranged as you like.

## Line Profile Report Menu

Use the options on the *Line Profile : Report* menu to format the way in which data are presented in the line plot. Choices relating to the X and Y axis calibration and multiple line plotting are available within this menu. It also allows you to display the data in tabular form, or summarize it into a set of statistics.

Each command on the line profile *Report* menu is described below.

- ◆ **Freeze:** Select this command if you want to maintain a copy of the current plot within the graph when you plot another line or band. **Freeze** allows you to view the plots of two lines (or bands) simultaneously .



When you have a line or band that you want to freeze, select the *Freeze* command. The defining line within your image will be displayed in blue to indicate that it is frozen.

To plot the intensities of a companion line, place your cursor over the middle of the frozen line. When the 4-way cursor appears, drag the line to its new position. The frozen line will remain in place, but an identical defining line will be created at the position you specify. The same procedure can be applied to a band that you have frozen.

Once a companion line or band has been created, it can be moved about the image freely. Each time you drag it to a new position, *Image-Pro* will plot the intensities for that location.

## Measure:Line Profile

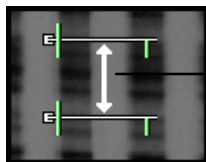
Although you can change the companion's position, you must not change its endpoints (modify its length or orientation). Doing so will release your freeze line.

If you save data while both a frozen plot and a companion plot are active, the data from the companion line are written to the file.

A frozen line or band will remain active until you either toggle off the *Freeze* command, modify its endpoints or close the *Line Profile* window. A check mark will appear next to this command when it is enabled.

- 
- ◆ **Normal:** Select the *Normal* command on the *Line Profile : Report* menu to measure the intensity of a *single* line of pixels. When this mode is enabled, a check mark will appear next to it. By default, this command is enabled when you select the *Line Profile* command.

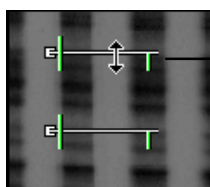
- 
- ◆ **Thick Horz:** Select the *Thick Horz* command on the *Line Profile : Report* menu to plot the specified measurement (mean or standard deviation) for a band of pixel columns. Values are measured from left to right in the band. To obtain the measurements for a *Thick Horz* plot, *Image-Pro* calculates and plots the requested statistic (average or standard deviation) for each column in the band.



A Thick Horz averages the pixels in each column of the band.

When you select the *Thick Horz* command, *Image-Pro* will position two defining lines within your image. You must position these two lines such that they encompass the band of pixels you want to measure.

To define the height of your band, place the cursor over the *middle* of one of the defining lines. When the cursor changes to a 2-way arrow, drag the line up or down to its new position.



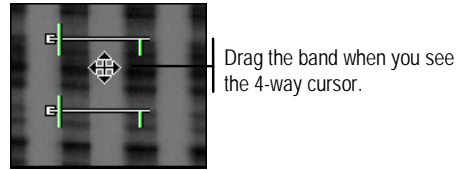
Drag the line when you see the 2-way cursor.

Note that a single line cannot be moved in a horizontal direction.

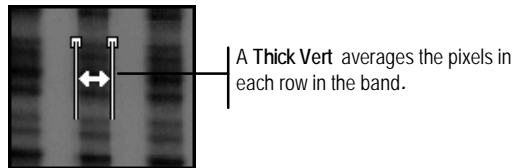


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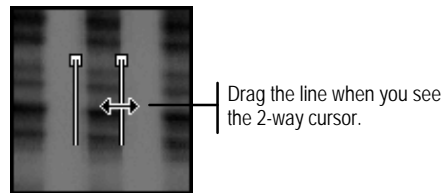
To change the position of your band, place your cursor between the pair of lines. When you see a 4-way arrow, drag the pair to their new position.



- ◆ **Thick Vert:** Select the *Thick Vert* command on the *Line Profile : Report* menu to plot the specified statistic (mean or standard deviation) for the specified band of pixel rows. Values are measured from top to bottom in the band. To obtain the measurements for a *Thick Vert* plot, *Image-Pro* calculates and plots the requested statistic (average or standard deviation) for each *row* in the band.

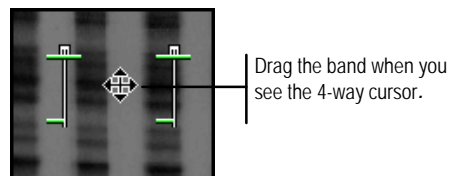


To define the width of your band, place your cursor over the middle of one of the defining lines. When the cursor changes to a 2-way arrow, drag the line left or right to a new position.



Note that a single line cannot be moved in a vertical direction.

To change the position of your band, place your cursor between the pair of lines. When you see the 4-way arrow, drag the pair to their new position.



- ◆ **Thick Options:** Select this command on the *Line Profile : Report* menu to specify the statistic (mean or standard deviation) you want plotted.
  - ◆ **Average:** Select this option if you want *Image-Pro* to calculate the mean value for each strip of pixels in the band. When you select **Average**, a check mark will appear next to it. The **Average** option is the measurement *Image-Pro* sets as the default when *Line Profile* command is selected.

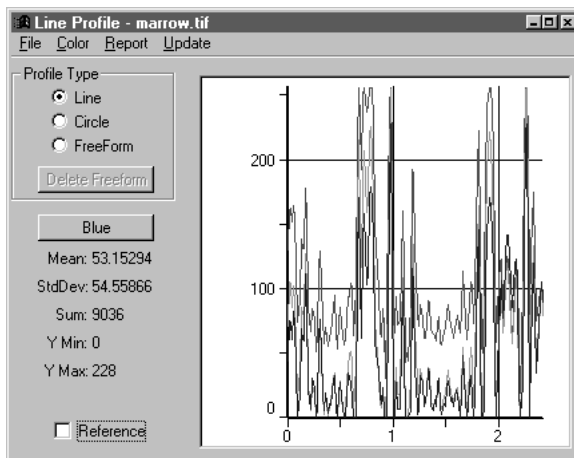
Measure:Line Profile

- ◆ **Std Dev:** Select this option if you want *Image-Pro* to calculate the standard deviation for each strip of pixels in the band. When you select **Std Dev**, a check mark will appear next to it.

- 
- ◆ **Spatial Cal:** Toggle this option “on” to measure X-axis values in their calibrated form. Toggle this option “off” to measure spatial values (X-axis data) in uncalibrated form. In uncalibrated form, the X-axis reflects the pixel position along the line. By default this option is enabled.

- 
- ◆ **Intensity Cal:** Toggle this option “on” to measure Y-axis values in their calibrated form. Toggle this option “off” to measure intensity values (Y-axis data) in uncalibrated form. In uncalibrated form, the Y-axis measures the pixel’s actual intensity value. By default this option is enabled.

- 
- ◆ **Statistics:** Select this option on the *Line Profile : Report* menu to display statistics concurrent with your line profile, as shown in the example below. If the active image contains *True Color* data, the statistics in this box will relate to the color channel that is the backmost layer in the graph (you can relayer the channels using the *Order* command on the *Color* menu).

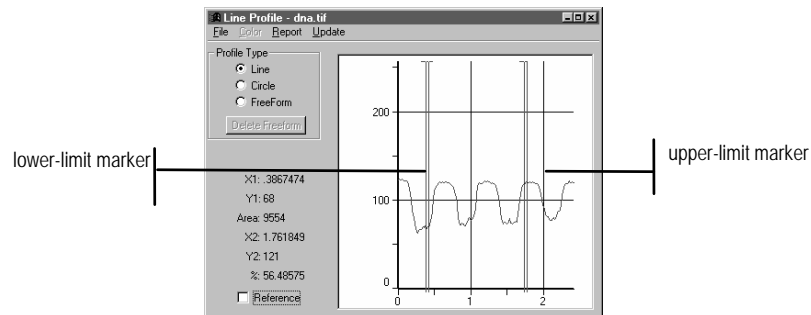


The following statistics will be included:

- ◆ **Mean:** Reports the average (mean) intensity value along the line.

## Measure:Line Profile

- ◆ **Std Dev:** Reports the standard deviation of the intensities along the line.
  - ◆ **Y Min:** Reports the smallest intensity value along the line (if you are plotting a band, this will be the minimum of the averaged values, not the actual minimum intensity value contained in the band).
  - ◆ **Y Max:** Reports the largest intensity value along the line (if you are plotting a band, this will be the maximum of the averaged values, not the actual maximum intensity value contained in the band).
  - ◆ **Sum:** Reports the sum of all the intensity values along the line.
- 
- ◆ **Range / Area:** Select this option to isolate a segment (range) of the X-axis, and to display information relating to this range. When this option is selected, two movable boundary markers are placed in the line profile.



The boundary markers can be positioned to select just the range you want to study. Information about the selected range is displayed along the right-hand side of the **Line Profile** window.

---

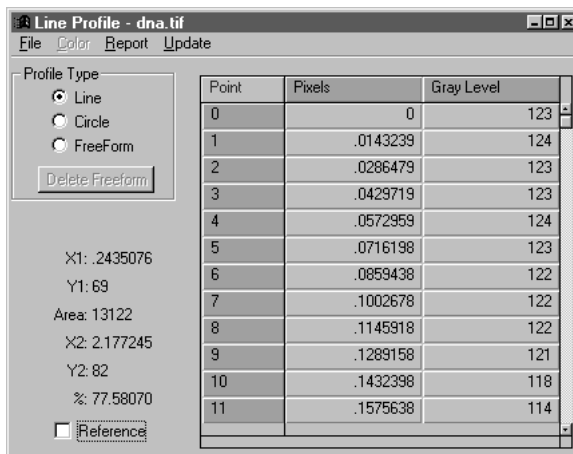
**Note:** The **Range/Area** and **Statistics** options are mutually exclusive, i.e., they cannot be enabled simultaneously. *Image-Pro* will automatically disable the **Statistics** option when **Range/Area** is selected.

---

## Measure:Line Profile

The following information is reported for the selected range.

- ◆ **X1:** Reports the spatial position that marks the beginning of the current range (it identifies the position of the left boundary-marker).
  - ◆ **Y1:** Reports the spatial position that marks the beginning of the current range (it identifies the position of the top boundary-marker).
  - ◆ **X2:** Reports the spatial position that marks the end of the current range (it identifies the position of the right boundary-marker).
  - ◆ **Y2:** Reports the spatial position that marks the beginning of the current range (it identifies the position of the bottom boundary-marker).
  - ◆ **Area:** Reports the sum of all intensity values in the selected range (“the area under the curve”).
  - ◆ **%:** Reports the area of the selected range as a percentage of the entire profile’s area.
- 
- ◆ **Table:** Select this option on the *Line Profile : Report* menu to display the line profile data in tabular form, as shown in the example below:



The screenshot shows a dialog box titled "Line Profile - dna.tif" with a menu bar (File, Color, Report, Update). On the left, there are radio buttons for "Line" (selected), "Circle", and "FreeForm", along with a "Delete Freeform" button. Below these are fields for X1: .2435076, Y1: 69, Area: 13122, X2: 2.177245, Y2: 82, and %: 77.58070. A "Reference" checkbox is at the bottom left. On the right is a table with three columns: Point, Pixels, and Gray Level.

Point	Pixels	Gray Level
0	0	123
1	.0143239	124
2	.0286479	123
3	.0429719	123
4	.0572959	124
5	.0716198	123
6	.0859438	122
7	.1002678	122
8	.1145918	122
9	.1289158	121
10	.1432398	118
11	.1575638	114

- ◆ **Full Scale:** Select this command to force a Line Profile plot to scale the Y-axis to the length of the entire intensity range (e.g., 0 - 255 in a *Gray Scale* image). If this option is not set, the Y-axis is scaled to the minimum and maximum intensity values produced by the profile.

## **Line Profile Update Menu**

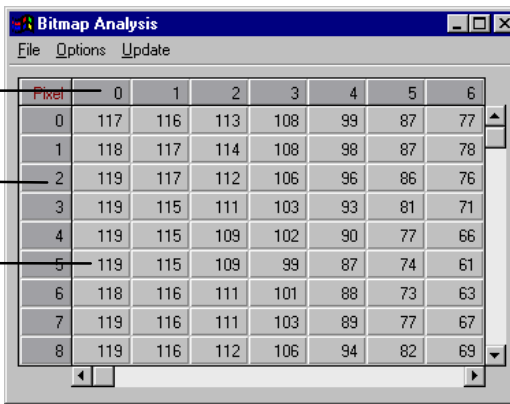
Select the *Line Profile* window's *Update* menu to refresh the line profile to reflect changes that have been made to the image data. For example, if you filtered an image after creating its profile, you would need to update the profile to reflect the intensity changes produced by the filter.

To update the line profile, simply click the *Update* menu. The contents of the *Line Profile* window will be rewritten based upon the contents of the currently selected image or AOI.

## Bitmap Analysis

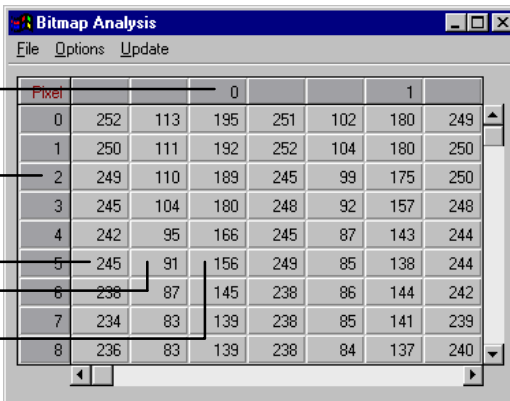
The **Bitmap Analysis** command on the *Measure* menu is used to view the pixel values of the active window (or AOI) in numeric format. These values can be saved to an ASCII file for later use with an external program, or copied to the Clipboard and pasted into another application (a 3D plotting package, for example).

When the **Bitmap Analysis** command is selected, the **Bitmap Analysis** window is opened and the pixel values associated with the active image or AOI are displayed.



Pixel	0	1	2	3	4	5	6
0	117	116	113	108	99	87	77
1	118	117	114	108	98	87	78
2	119	117	112	106	96	86	76
3	119	115	111	103	93	81	71
4	119	115	109	102	90	77	66
5	119	115	109	99	87	74	61
6	118	116	111	101	88	73	63
7	119	116	111	103	89	77	67
8	119	116	112	106	94	82	69

Bitmap analysis of a Gray Scale image



Pixel	0	1	2	3	4	5	6
0	252	113	195	251	102	180	249
1	250	111	192	252	104	180	250
2	249	110	189	245	99	175	250
3	245	104	180	248	92	157	248
4	242	95	166	245	87	143	244
5	245	91	156	249	85	138	244
6	238	87	145	238	86	144	242
7	234	83	139	238	85	141	239
8	236	83	139	238	84	137	240

Bitmap analysis of a True Color image

### *Measure:Bitmap Analysis*

Pixel values can be displayed as they actually exist in the image or in their calibrated form (see the *Bitmap Analysis Options Menu*, below). They are not interpreted through the display LUT however.

The **Bitmap Analysis** window imposes no limit on the number of pixels that can be processed. There is, however, a maximum of 30,000 pixels that can be saved, copied to the Clipboard or transferred to Excel. If you need to save more than this number of pixels, you can define an AOI that is less than or equal to 30,000 pixels, and process the image in chunks (i.e., move the AOI systematically across the image and save the data at each interval). Or, you can use the **Sampling** option to read the pixel values at larger intervals (see the *Bitmap Analysis Options Menu*, below).

---

**Note:** Consider carefully how much data you actually need to save in ASCII form; a 100 x 100 segment of a Gray Scale image (10K in binary image form) will consume over 60K, and a True Color segment will take three times that amount.

---

## Bitmap Analysis File Menu

Use the commands on the **Bitmap Analysis** window's *File* menu to save the displayed bitmap values to disk or to the Clipboard. This menu also contains the command to close the **Bitmap Analysis Measure** window.

- 
- ◆ **Data to File:** Select the **Data to File** command to store the current bitmap values to a file *in ASCII format*. The bitmap values in the ASCII file are padded with leading spaces and are delimited with a tab character.

---

*Note:* A maximum of 30,000 pixels can be saved at one time. You may save more by successively defining AOIs and saving the data.

---

If the **Pixel Values Only** option has been checked, *Image-Pro* will save the data without the pixel coordinates, which are contained in the first row and column of the **Bitmap Analysis** window. See *Pixel Values Only*, below, for more information about this option.

When you select the **Data to File** command, the **Save Bitmap** dialog box is displayed.

Enter the name of the file to which you want the ASCII data saved, and click **OK**. If you do not specify a file extension, *Image-Pro* will automatically attach the “.BIT” extension to your file name.

- 
- ◆ **Append Data to File:** Select the **Append Data** command on the **Bitmap Analysis** window's *File* menu to add the current bitmap values to an existing ASCII file. The ASCII values are padded with leading spaces and are delimited with a tab character.

---

*Note:* A maximum of 30,000 pixels can be saved or appended when the box is displayed.

---

Enter the name of the file to which you want the ASCII data appended, and click **OK**. If you specify a file that does not already exist, a new file will be created.

- 
- ◆ **Data to Clipboard:** Select the *Data To Clipboard* command on the **Bitmap Analysis** window's *File* menu to copy the current bitmap values to the Windows Clipboard. The values are placed on the Clipboard in ASCII (text) format (the values are padded with leading



spaces and are delimited with a tab character). The text can be pasted into any application that accepts text from the Clipboard.

---

*Note:* A maximum of 30,000 pixels can be copied to the Clipboard at one time. You may copy more by successively defining AOIs and copying the data.

---

◆ **Data to Printer:** Select the *Data To Printer* command to print out the current bitmap analysis data.

◆ **Data Export:** Select the *Data Export* command to transfer data to Microsoft's Excel spreadsheet. Before selecting *Data Export*, you should use *Export Options* to determine the correct placement (within Excel) for the data. When you select *Data Export*, the transfer occurs with little visible evidence that it has happened. By switching to the Excel application, you can confirm that the transfer has occurred. For additional information about DDE, see the *Dynamic Data Exchange* discussion.

---

*Note:* A maximum of 30,000 pixels can be transferred to Excel at one time. You may transfer more by successively defining AOIs and transferring the data.

---

◆ **Export Options :** Select the *Export Options* command to tell *Image-Pro* how and where to export the current data via DDE.

◆ **Pixel Values Only:** The **Pixel Values Only** command on the **Bitmap Analysis** window's *File* menu determines whether the coordinate-identifying row and column (i.e., the first row and column in the table) are written along with the bitmap analysis when it is saved using the **Data to File**, **Append Data** or **Copy To Clipboard** commands. A check mark appears next to this command when it is enabled. When **Pixel Values Only** is checked, the first row and column of the data sheet are not saved with the data. Clicking this command toggles the option off and on.

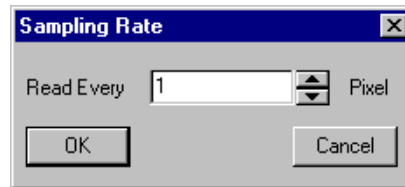
◆ **Close:** Use this command on the **Bitmap Analysis** window's *File* menu to close the **Bitmap Analysis** window.

## Bitmap Analysis Options Menu

Use the commands on the **Bitmap Analysis** window's *Options* menu to set sampling rate and intensity calibration options.

- ◆ **Sampling** : Use this command to specify whether the value of every pixel is to be displayed, or whether sampling should be performed at some other regular interval (e.g., you might choose to display the value of every other pixel, or every 10<sup>th</sup> pixel).

When the **Sampling** command is selected, the **Sampling Rate** dialog box is presented.



Enter a value from 1 to 100 in the **Read Every** field. This value specifies the frequency at which pixel values are presented in the **Bitmap Analysis** data sheet. This value applies to both the X and Y dimension.

For example (assuming data were being viewed from the upper-left segment of an image): a sample rate of 1 would display pixels 0,0 0,1 0,2 0,3 0,4 on the first line; a sample rate of 2 would display pixels 0,0 0,2 0,4 0,6 ; a sample rate of 10 would display pixels 0,0 0,10 0,20 0,30 , and so forth.

- ◆ **Intensity Cal**: The **Intensity Cal** command on the **Bitmap Analysis** window's *Options* menu determines whether the pixel values displayed in the **Bitmap Analysis** menu are calibrated values or actual image values. A check mark appears next to the command when this option is enabled. When **Intensity Cal** is checked, the values are displayed in calibrated form. Clicking this command toggles the option off and on.

## **Bitmap Analysis Update Menu**

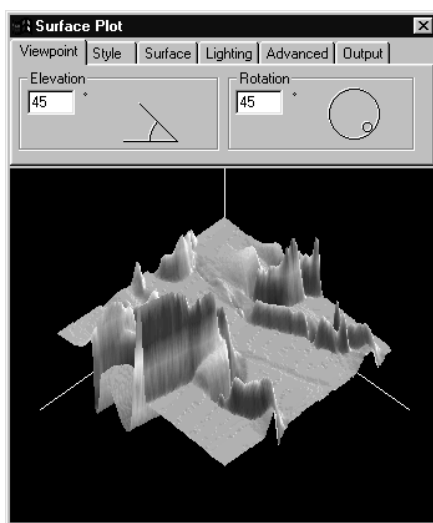
Select the **Bitmap Analysis** window's *Update* menu to refresh the values in the data sheet following a change to the image data. For example, if you filtered the image while its bitmap values were displayed, you would need to update the **Bitmap Analysis** window to retrieve the filtered image values. To update the **Bitmap Analysis** data sheet, simply click the *Update* menu. The values in the data sheet will be rewritten based upon the contents of the active image or AOI.

## Surface Plot

---

The *Surface Plot* (or 3-D Plot) tool is a new feature in *Image-Pro Plus* version 7.0. It creates a three-dimensional representation of the intensity of an image. When using the *Surface Plot* tool, keep in mind that **X** = length; **Y** = width; and **Z** = height.

When you select the *Surface Plot* command, you will see the following tabbed dialog:



### Viewpoint

The **Viewpoint** page describes the positioning of the viewer toward the object.

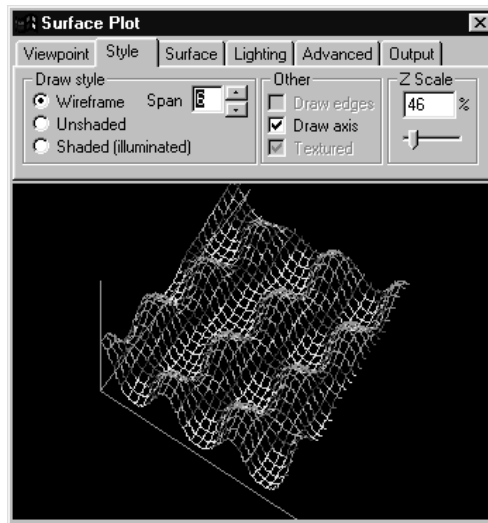
**Elevation:** Indicates the height of the display. You can adjust the elevation by moving the arms of the “angle” indicator in the **Elevation** group box, or typing new numbers in the spin box. For example, elevating the image 90 degrees provides a view looking directly down on the object; zero degrees of elevation would be looking straight at the object. In the picture above, the elevation is 45 degrees.

**Rotation:** Indicates the position of the viewer around the object. You can adjust the rotation by moving the smaller circle around the large

one in the **Rotation** group box, or by typing in new numbers. In the picture above, the rotation is set to 45 degrees.

## Style

The options on the **Style** page govern the display of the surface plot:



**Wireframe:** This option speeds up the surface plot display by not drawing every pixel. To do this, set the **Span** to the required pixel interval. For example, if **Span** is set to 10, the display will sample every 10<sup>th</sup> pixel. Intervening pixels will be transparent, as in the picture above.

**Unshaded:** This option displays the surface plot without reflections or shading; the whole surface of the plot is colored.

**Shaded/Illuminated:** This option displays the surface plot with any shadows resulting from the position of the light source (see the Lighting page). This setting also displays the horizon to help in maintaining perspective.

**Draw Edges:** Checking this box displays the edges or “curtain” of the surface plot.

**Draw Axis:** Checking this box displays lines representing the X, Y, and Z axes of the image.

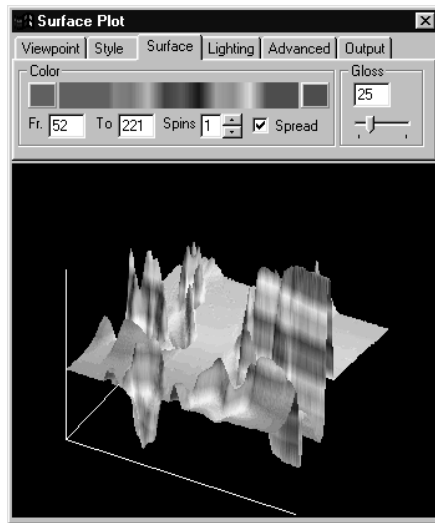
## Measure:Surface Plot

**Textured:** This option activates the textured mode. The texture image can be selected in the Surface tab of the dialog. If this switch is off a color spread applies to the image.

**Z Scale:** Use the slider to adjust the relative height of the display of the Z scale. The possible range is 0 - 400%.

## Surface

The **Surface** page options control the color and glossiness of the surface plot.



**Color:** The **Color** group box lets you select the colors to map to the gray values found in the surface plot. The buttons at each end of the color strip bring up the **Colors** dialog, which allows you to select the starting and ending colors of the range. (Please refer to the *Pseudo-Color* discussion earlier in this manual for more information.)

**From:** Enter a number to indicate the starting value for the color range. The default is 0.

**To:** Enter a number to indicate the ending value for the color range. The default is 255.

**Spins:** Enter a number to indicate the number of iterations of the color wheel to use for coloring the surface plot. Each “spin” is

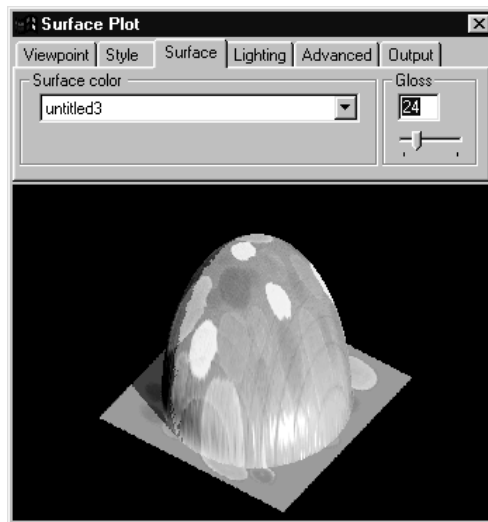
*Measure:Surface Plot*

equivalent to one cycle of the color wheel. The range of possible values is 0 -5.

**Spread** : Check this box to create a color spread. If the box is not checked, and the *From* has a value greater than 0, or *To* has a value lower than 255, the pixels with values below (From) or above (To) will be displayed in grayscale.

**Gloss:** Use the slider to adjust the “reflectivity” of the surface plot image. Matte surfaces have lower reflectivity, shiny or glossy surfaces have higher reflectivity. The range of possible values is 0 -100.

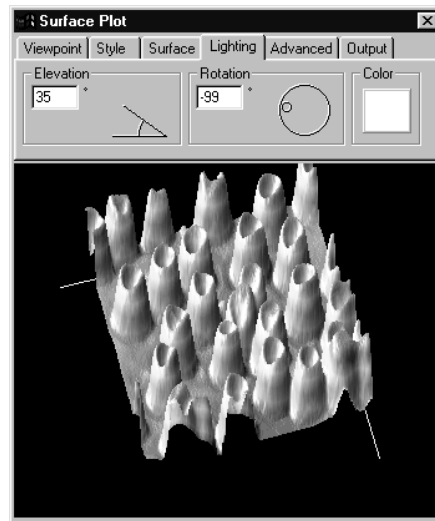
In **Textured** mode the page has another appearance.



The **Surface color** control shows the texture image superimposed on the active image.

## Lighting

**Lighting** options controls the position and color of the light source.



**Elevation:** Indicates the angle of the light striking the image. 90 degrees would be as if the sun were overhead at noon, with very few shadows. Lower angles make more shadows visible.

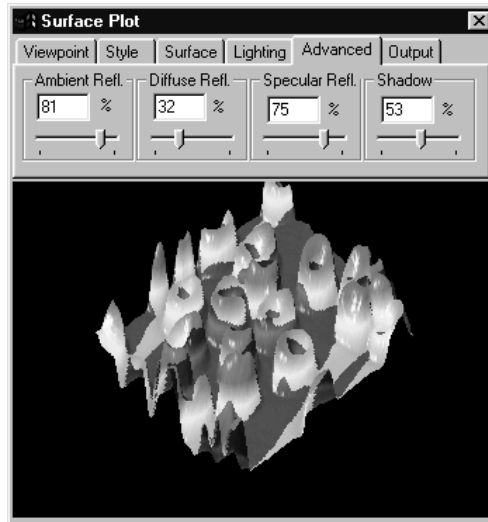
**Rotation:** Indicates the location of the light source around the image. You can adjust the rotation by moving the smaller circle around the large one in the Rotation group box, or by typing in new numbers.

**Color:** Clicking this box allows you to change the color of the light striking the image.



## Advanced

The **Advanced** options determine the characteristics of the surface when it reflects light. Note that these settings are independent and **do not** combine to total 100%.



**Ambient Reflection:** Ambient Reflection uses the available light normally present to light the entire surface plot image. Ambient light is of uniform brightness caused by multiple reflections of light from the many surfaces present in most environments. This component presents the surface color, which is set on the **Surface** tab. When **Ambient Reflection** is set to 100, Diffuse to 0 and Specular to 0, the image appears as it does in the *unshaded* mode.

**Diffuse Reflection:** Diffuse reflection uses diffused light to soften and smooth the surface of the surface plot image. Dull matte surfaces exhibit diffuse reflection, scattering light equally in all directions so that all the surfaces appear to have the same brightness from all viewing angles. When **Diffuse Reflection** is set to 100, Ambient to 0 and Specular to 0, the image appears as it would in moonlight. Objects do not show their own surface color in this case.

**Specular Reflection:** Specular reflection uses a “spotlight” effect to focus bright light on the surface plot image. Specular reflection is best observed on a shiny or glossy surface; the highlighted effect is

## Measure:Surface Plot

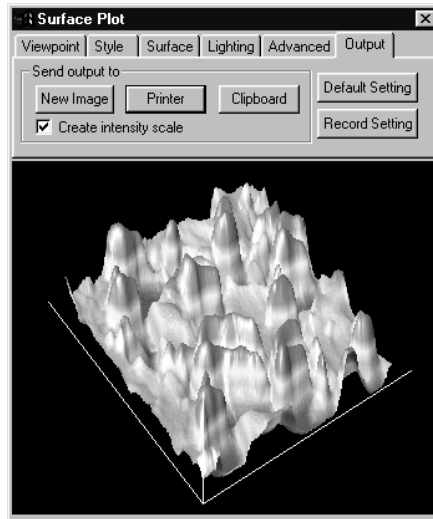
caused by specular reflection. When Ambient Reflection is set to 100, Diffuse to 0 and **Specular** to 100, the image appears in a glass or candy-like material, because glass has very low diffuse reflection.

**Shadow:** sets depth of the shadow. When the **Shadow** is 0 the shadow mode is turned off.

When all the color components are added together, this creates to color of the image pixels in *shaded* mode.

## Output

The **Output** options determine where your surface plot settings and information will be directed.



**New Image:** Clicking this button creates the surface plot in a new *Image-Pro* workspace. This function creates the surface plot in the same size as the original image. It is a good idea to create a new image when you want to save or print the surface plot, because in this case the surface plot has maximal resolution.

**Printer:** Clicking this button sends a copy of the surface plot image to your printer.

*Measure:Surface Plot*

**Clipboard:** Clicking this button sends your surface plot information to the Windows clipboard, where it can be pasted into other applications that accept data from the clipboard.

**Create Intensity Scale:** Check this box to create an additional image or “key” with the intensity scale displayed in it. This is available for a **new image** (above) only.

**Default Settings:** Click this button to return all the surface plot settings to the original, default settings. Your modifications will not be saved.

**Record Settings:** Click this button to record your settings in an *Auto-Pro* macro.

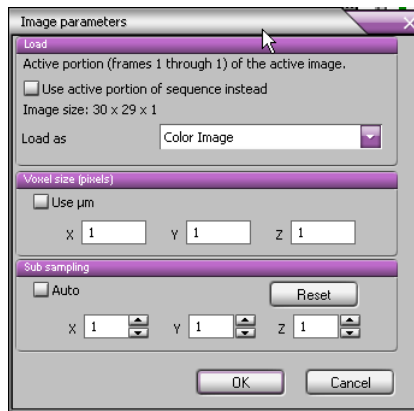
## 3D Viewer

---

The *3D Viewer* window allows you to examine an image stack in a variety of different ways: by volume, in thick or thin slices, and as an animated display. In the *3D Viewer*, all geometric objects are shown in 3D space. It can be regarded as a virtual camera which can be moved to an arbitrary position within or around the 3D scene.

The first time that you select the **3D Viewer** from the *Measure* menu, you will see the **Image parameters** dialog, which loads the active image into the *Viewer*:

The **Image parameters** dialog determines the parameters of the active stack.



**Load:** The **Load** group box determines which images to load:

- **Use active portion of sequence instead:** The default behavior loads the current Z stack of the current set. This checkbox allows you to use only a portion of the active sequence, instead of the current stack. The **image size** is given as width x height (in pixels) x depth (in number of frames). (Please see the note under **Time** if you want to view a single time point.)
- **Load As:** If you are loading a color image, a drop-down list box appears, giving you a choice about how to display your color image: Color images can be displayed as:
  - **Color Image:** All channels are loaded into an indexed color volume (The sequence is converted to a palette class using a palette containing the most popular colors in the sequence).

- **Red Channel:** The red channel of the image is displayed and a red palette is created.
- **Green Channel:** The green channel of the image is displayed and a green palette is created.
- **Blue Channel:** The blue channel of the image is displayed and a blue palette is created.

If you are loading a black and white sequence, it is displayed unmodified and a gray palette is created.

---

*Note:* 256-color palette mode supports interactive brightness/contrast/gamma adjustments throughout the palette settings.

---

**Voxel Size:** **Voxel size:** This group box defines the dimensions of one voxel (a pixel in 3 dimensions). The values displayed are the last ones used. If the image does not have a spatial calibration, and no system calibration has been set, the values are 1, 1, 1. If the image is spatially calibrated, the X and Y values and the units are taken from the calibration. If the active sequence or stack includes information about the Z frame position, the Z size is set from the distance between first and second frame in the sequence. If Z information is absent, Z size is set to the average value of X and Y sizes. These values can be modified to achieve the desired volume ratio.

- **Use  $\mu\text{m}$  (microns):** If this box is checked, the values are presented in microns. If the box is not checked, the units of the image's spatial calibration are used. (For more information about spatial calibration, please refer to your *Image-Pro Plus Reference Guide*.)

**Sub-sampling:** This group box defines the sub-sampling values for loading images. Default values are 1,1,1. The values can be set to higher numbers for larger images, to decrease loading time and increase performance. However, the image quality in this case will be lower.

- **Auto:** Selecting the **Auto** checkbox activates automatic sub-sampling mode. In this mode, the sub-sampling is calculated based on the size of the image. This provides faster loading and rendering of Volume images, but the maximum size of a loaded volume in any direction is limited to 64 voxels. By default this option is *on*. Advanced users can switch this option Off and set sub-sampling manually.

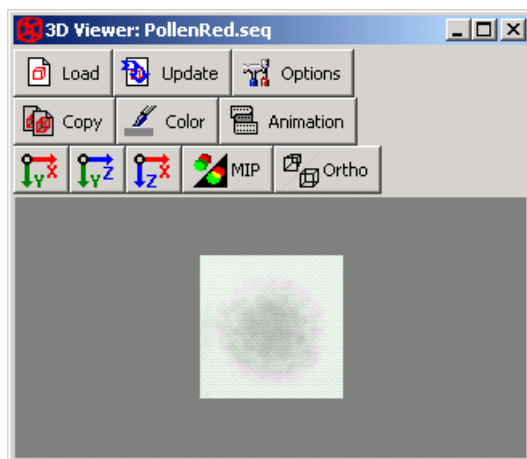
### Measure:3D Viewer

- **Reset:** Clicking the **Reset** button clears all your volume settings and sets 1:1:1 sub-sampling. The **Reset** button is disabled when **Auto** is on.

## 3D Viewer Toolbar and Buttons

There are a number of tools, buttons, and icons to help you examine the data in the **3DViewer**.

Once you have entered the required information in the **Image Parameters** dialog, and clicked **OK**, you will see the following dialog:



The **3D Viewer** dialog contains controls to zoom, rotate, and modify the image. You have the option of viewing both icons and text on the toolbar buttons, or just the icons. To turn the button text on or off, place your cursor over one of the buttons and click the right mouse button. Then you can choose **Show icons only** (above) or **Show icons and text**, as shown above.

**Show icons and text** is the default for the **3D Viewer** toolbar.

---

**Note:** The **3D Viewer** documentation in this section sometimes refers to a "camera". This does not mean a physical camera, but a virtual camera, as if the **Viewer** were actually the view-finder in a typical camera. Changes in the **Viewer's** perspective or point of view can be considered as changes in the position of the camera.

---



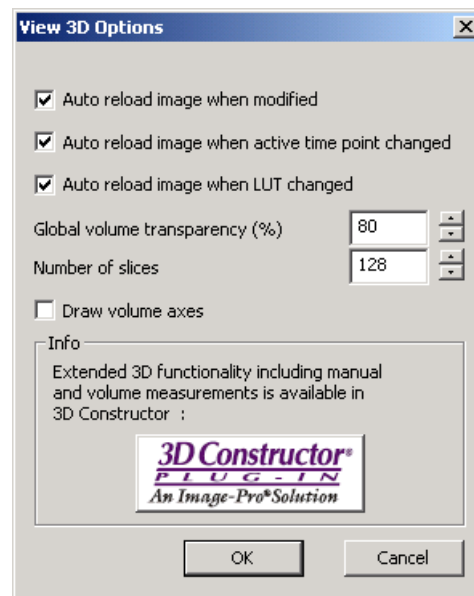
**Load new image from the active workspace.** Clicking this button opens the **Image Parameters** dialog (above).



**Update image.** Click this button to reload the image manually after modifying some of the options. (If **Auto-reload** options are on, the image will be reloaded automatically).



**3D Viewer Options:** Clicking this button opens a dialog with active 3D elements and controls to adjust these elements, as shown here:



**Auto Reload:** **Auto reload** check-boxes define when the image is automatically reloaded:

The image in the 3D Viewer is updated when:

- **Auto reload image when modified:** The original image is modified (filtered). When the option is checked, the *3D Viewer* window will be closed when the original image is closed.
- **Auto reload image when active time point changed:** The time point of the time sequence has changed, so the 3D viewer will be updated .

- **Auto reload image when LUT changed:** Adjusting the brightness, contrast, or display range on the original image will be immediately reflected in the 3D Viewer.

These options can be switched off in the options dialog. The image can be updated manually clicking the **Reload image** button.

- **Global volume transparency:** Defines the volume transparency in percents (affects volumes only in blending mode).
- **Number of slices:** Defines the number of slices drawn for 2D multi-texture map rendering. Rendering fewer slices will generally improve performance (with reduced image quality).
- **Draw volume axis:** This option turns the display of the X, Y, and/or Z axes of the volume *on* or *off*.

**Info:** The **Info** group provides information about an advanced 3D visualization module, *3D Constructor*. Clicking the **3D Constructor** button opens the *3D Constructor* web page.



**Copy to Clipboard:** Clicking this button copies the active view to the clipboard as a picture.



**Background options:** Background options allow you to select the background color of the 3D viewer. **Background color** defines the background color when the **Gradient background** is off. (default)

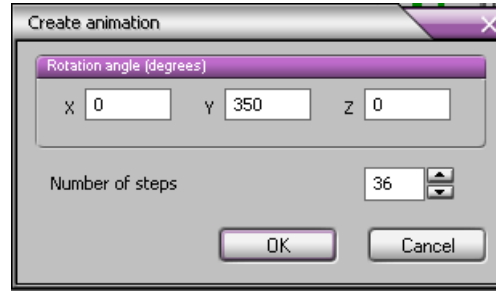
When the **Gradient background** control is *on*, (box is checked) the color gradient is drawn as a background for a scene. The **Background color** appears at the top of the window and the second color appears at the bottom of the window.

When the **Image** option is *on* (box is checked), an image will be displayed as the background. The following image formats are supported: BMP (Windows only), GIF, JPEG 2000, JPEG, PGX, PNG, PNM, SGI RGB and TIFF.



**Create animation sequence:** Clicking this button opens the **Create Animation** dialog, shown here:





- **Rotation angle:** These controls define the rotation angle of the total animation around X, Y and Z axes in degrees. (X = horizontal, Y = vertical, Z= perpendicular to the screen).
- **Number of steps:** These spin buttons define the number of frames in the output animation.

**OK:** Clicking the **Ok** button creates an animation in the *Image-Pro Plus* workspace. The created sequence then can be saved as multi-frame \*.TIF or \*.AVI file.

Use these buttons to move the camera to the X, Y, or Z position.



Sets the **XY** projection of the camera.



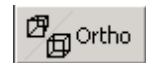
Sets the **ZY** projection of the camera.



Sets the **XZ** projection of the camera.



Toggles between **Blend** (*Off*) and **Maximum Intensity Projections** (*On*).



Toggles between **Perspective** (*Off*) and **Orthogonal** (*On*) views.

An image can be **zoomed** pressing the <Ctrl> button, then clicking the left mouse button on the image and dragging left (zoom out) or right (zoom in). You may also use the mouse wheel, or <Ctrl> +<L>.

*Measure:Report*

## **Report**

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Use the *Report* command to work with the *Image-Pro Plus Report Generator*. This feature lets you compile your *Measure* menu information into a report with graphs, tables, and images. For information about using the *Report Generator*, please refer to the *Report Generator* online help.

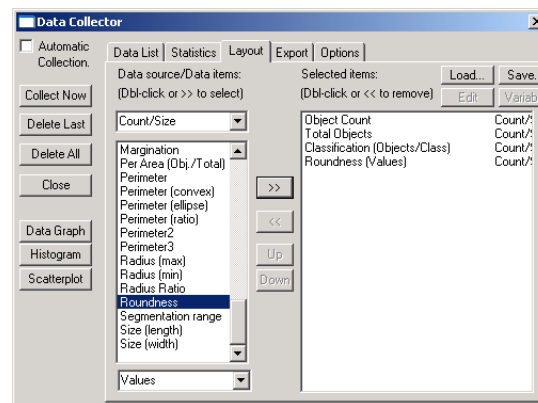
## Data Collector

*Data Collector* provides a place where you can accumulate, summarize, and store information from the analysis of multiple images.

Unlike most tools in *Image-Pro* which show data one image at a time, *Data Collector* provides a place where you can collect analysis data from multiple images. Tools such as *Count/Size*, *Histogram*, and *Manual Measurements* are set up to respond to collection requests from *Data Collector*. More tools will have that capability in the future. To check whether a given tool supports *Data Collector*, simply invoke the tool and look for its name in the **Layout** page of *Data Collector*, under **Sources**. Tools that supply data to *Data Collector* are called **Data Sources**.

Data can be collected in two ways: *Automatically*, every time new data become available from one of the data sources, *or at specific times* the user specifies by pressing the **Collect Now** button. Each new collection updates the statistical summary available on the **Statistics** page.

When you first select *Data Collector*, you will be shown the **Layout** page. This page may be empty if no data source has been invoked yet. One way for *Data Collector* to be aware of the various data items that can be collected is to first invoke the tools that supply them. It is also possible to specify a list of collected items via macro before or after any data source is invoked. In this case though, data will not be collected until the tools are invoked and supply the data.



On the left-hand side of the dialog, you will find a number of buttons that are always present, regardless of which page is show:

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**Automatic Collection:** This check box turns automatic collection on or off. With **Automatic Collection** on, the collection process is triggered each time one of the tools generates new data. When **Automatic Collection** is off, no new data is collected until the **Collect Now** button is pressed.

**Collect Now:** Pressing this button will trigger the collection of a new data block. Each selected data source will be asked to supply its selected data in turn (See Layout page to learn more about selecting data sources and data items).

**Delete Last:** Press this button to delete the last collected data block.

**Delete All:** Press this button to delete the whole collection. Only data stored in *Data Collector* will be deleted. This does not affect any of the data stored by the data sources or data collected prior to that and saved to disk.

**Close:** To close the *Data Collector* dialog. This does not delete any of the data collected so far. Nor does it affect ongoing automatic data collection. In the case the dialog is closed while the **Automatic Collection** option is on, a message will come up reminding the user of that fact.

**Data Graph:** This button opens a new **Data Graph** window.

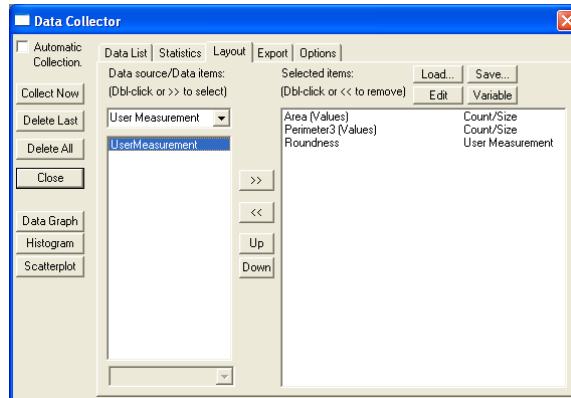
**Histogram:** This button generates a new Histogram window.

**Scatterplot:** This button opens a new **Data Graph** window.

The new data graph, histogram, and scatterplot windows will be discussed in subsequent sections.

## Layout

The **Layout** page is the first page that you see when you start *Data Collector* for the first time.



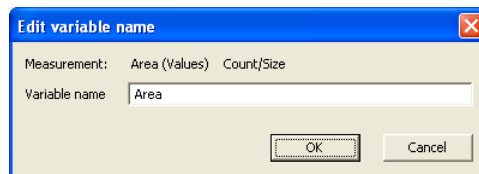
This page may be empty if no data source was invoked first and no selection macros were called. Tools such as *Count/Size* which support *Data Collector* are called **Data Sources**. Each data source supplies one or several **Data Items**. Some of the data items can have several representations. Data source and data items are found on the left hand side of the **Layout** page. Items selected for collection are found in the list box on the right hand side of the page.

**Load:** Clicking this button lets you load a saved measurement from a \* .DCL file.

**Save:** Clicking the save button allows you to save your list of selected measurements and variables to a \* .DCL file.

**Edit** Clicking this button allows you to make changes to a selected measurement.

**Variable:** Click this button to edit the name of a selected variable:



Click **OK** to save your changes, or **Cancel** to start over.

**Data Source Lists:** The top-most drop-down list box contains the list of all the data sources registered thus far. Remember that for a data source to appear in this list, its dialog must have been invoked first. Selecting a data source causes its related data items to be listed in the list box below it. Certain data items may have different representations. These representations are listed in the bottom drop-down list. This list is grayed out for data items that have one representation only.

To select a given data item for collection, double-click on its name with the left mouse button. Alternatively, you can click once on the item to highlight it and press the >> button. In either case, make sure that you picked the right representation for that item before selecting it.

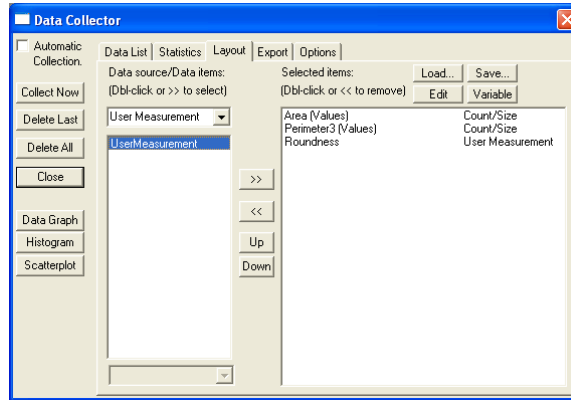
In version 7.0 of *Image-Pro Plus*, the *Caliper* feature can export information to the *Data Collector*. When at least one sampler exists with at least one detector, *Caliper* will add a **Detector** group to the *Data Collector*. This group supports **Name** and **Count**. Together, these can be used to see how many intercepts are detected, e.g. checking the number of gear teeth found. The name is the name of the sampler (e.g. "C1") followed by the detector label (e.g. "A") for a result like "C1 A."

**Selected Lists:** Once an item is selected, it will appear on the right hand side list box (**Selected box**), with its name, representation (in parentheses), and data source name. To deselect an item, double-click on its name with the left mouse button. Alternatively, you can highlight one or more items and press the << button.

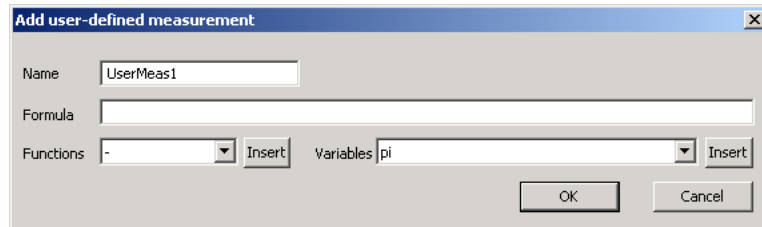
Collection can begin as soon as one or more items have been selected.

## User-Defined Measurements

The last item in the **data source list** is **User Measurement**. It allows you to define a formula not already included in *Image-Pro*.



To define a measurement, highlight the **User Measurement** list item and click the >> button. You will see the **Add User Measurement** dialog:



**Name:** Enter the name of your new measurement here.

**Formula:** The formula that you are creating appears here.

**Functions:** You may select a function from the drop-down list in this box. Click the **Insert** button to include this function in your formula. (See the list of supported functions and operators at the end of this chapter.)

**Variables:** You may select a variable from the drop-down list in this box. Click the **Insert** button to include this variable in your formula.

## Measure:Data Collector

**OK:** Click the **OK** button when you are satisfied with your formula. You will return to the data collector Layout page with your formula added to list of selected items.

**Cancel:** Click the **Cancel** button to erase your formula without saving it.

**Edit:** Clicking this button allows you to make changes to a selected measurement.

**Variable:** Click this button to edit the name of a selected variable.

## Data List

This page shows the list of data points collected thus far. Each data item selected in the **Layout** page will appear as one or several columns. Each row represents a data point for one of several of the data items. Each time the **Collect Now** button is pressed, a new data block will be appended to the list. Similarly, under **Automatic Collection** mode, new blocks will be appended every time that new data become available.

Row:Block	Object Count	Total Objects	Classification (Objects/Class)	Roundness (Values)
1: 1	190	315	0	1
2: 1				1
3: 1				1
4: 1				1
5: 1				1
6: 1				1
7: 1				1
8: 1				1
9: 1				1
10: 1				1
11: 1				1
12: 1				1
13: 1				1
14: 1				1
15: 1				1

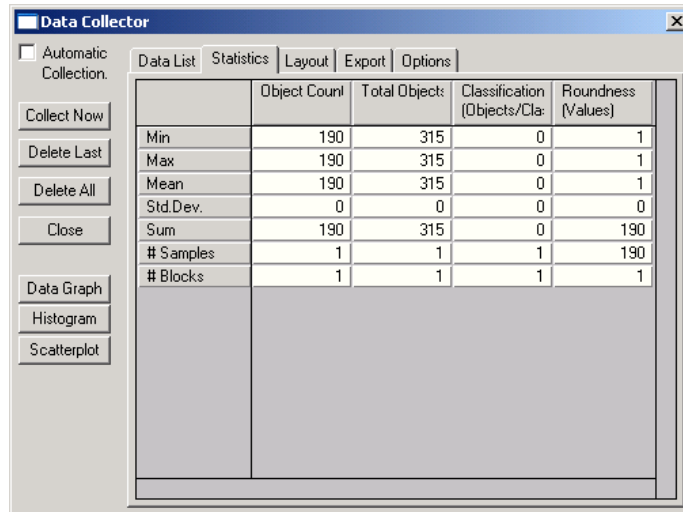
## Statistics

This page shows a statistical summary of the data collected thus far. The column layout of this page is identical to that of the **Data List** page. The rows show the minimum (**Min.**), maximum (**Max.**), average (**Mean**), standard deviation (**Std.Dev.**), and accumulated (**Sum**) values



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of each column. The number of samples (# **Samples**) and data blocks (# **Blocks**) collected is also shown for each column. Note that the number of blocks collected is always the same for each column.

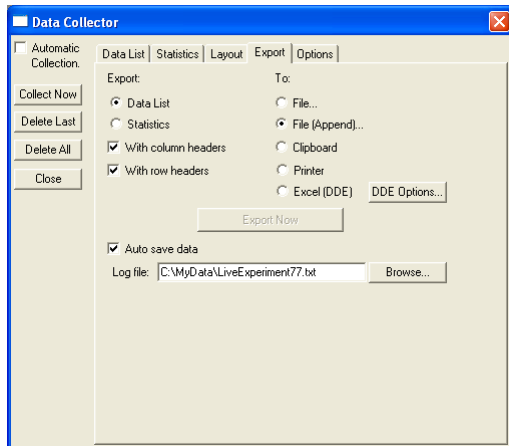


The screenshot shows the 'Data Collector' window with a 'Statistics' tab selected. The window includes a sidebar with buttons for 'Collect Now', 'Delete Last', 'Delete All', 'Close', 'Data Graph', 'Histogram', and 'Scatterplot'. The main area contains a table with the following data:

	Object Count	Total Object	Classification (Objects/Clas)	Roundness (Values)
Min	190	315	0	1
Max	190	315	0	1
Mean	190	315	0	1
Std.Dev.	0	0	0	0
Sum	190	315	0	190
# Samples	1	1	1	190
# Blocks	1	1	1	1

## Export

This page provides different ways in which the collected data can be saved or exported.



**Export:** The left hand side options identify what data is saved or exported.

**Data List:** Click here if you want to save or export the data listed in the Data List page.

**Statistics:** Click here if you want to save or export the results listed in the Statistics page.

**With column headers:** Click this option to include column headers.

**With row headers:** Click this option to include row headers.

**To:** The right hand side options indicate the destination for the exported data.

**File:** To save the data into a file on disk.

**File (Append):** To append the data to an existing file.

**Clipboard** : To copy the data in text form to the clipboard.

**Printer** : To print the data.

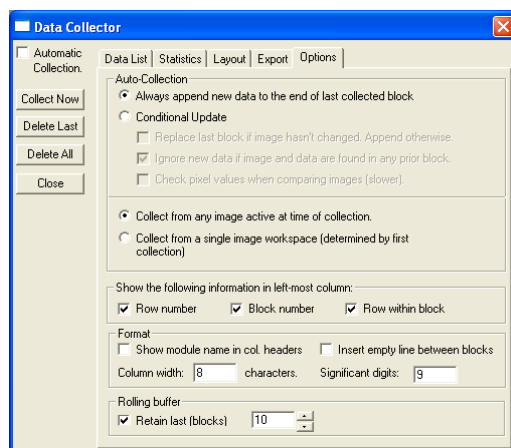
**Export Target:** To send the data to Excel or Origin via DDE. Either program will be started automatically if it is installed on your system.

**Export Options:** Press this button to bring up the **Export Options** dialog. For additional information about these options, see the **Dynamic Data Exchange** discussion in the first part of this manual.

**Auto-Save Data:** Checking this box allows you to save the data in tab-delimited text format every time it's collected (only last block is saved). The data is appended to text file defined in the **Log file** window.

## Options

The **Options** page contains selections that affect the way **Automatic Collection** works. It also contains options for controlling the way the data gets presented in the **Data List** and **Statistics** pages.



**Auto-Collection:** This set of options controls the way **Automatic Collection** is done. These options have no effect until **Auto-Collection** is turned on via the **Automatic Collection** check box found on the main page. They have no effect on manual collection (see **Collect Now** button). **Automatic Collection** is triggered by the data sources when they generate new data.

**Always append new data to the end of the last collected block:** Select this option if all new data must be collected regardless of its contents or regardless of which image it comes from.

**Conditional Update:** Select this option to restrict automatic data collection according to the following criteria.

**Replace last block if image hasn't changed.** Append otherwise: New data will be appended to the current collection, or will replace the last collected block. It will be appended if the data comes from a different image than that of the previous block. It will replace it if the new data comes from the same image. This can be useful when collecting from a data source (tool) that triggers Auto Collection several times before the final data has stabilized. For instance, when working with Count/Size, Auto Collection is triggered after the Count button is pressed. It is also triggered each time individual objects are deleted or split. By replacing the previous block with the new data instead of appending it, one can refine the Count/Size data without collecting temporary and unnecessary data.

**Ignore new data if image and data are found in any prior block:** New data will be rejected if it is found in any prior block, and if that block came from the same image. Playing a sequence for example may cause the Histogram tool to trigger Automatic Collection for each frame in the sequence. Auto Collection can be restricted to one loop through the sequence by selecting this option.

**Check pixel values when comparing images (slower):** The two previous options (above) will check to see if the image the data is collected from has changed since prior blocks were collected. Image identity can be checked in two ways: By checking whether the image workspace has changed, or by checking whether the image pixel values have changed. The second option is slightly slower and should be used only when needed. To illustrate when it is needed, let's assume that each new image is captured into the same workspace (see Capture module). In this case, the only way to check whether the image has changed is to physically check whether pixel values are different. In the case where each new image is captured into a new workspace, then comparing pixel values is unnecessary.

**Collect from any image active at time of collection:** Select this option if you want to collect from any image workspace.

**Collect from a single image workspace (determined by first collection):** Select this option if you want to collect from a single image workspace only. Which image workspace is used is determined by which workspace is active during the first collection. Following the first collection, any data collected from any other image workspace will be ignored.

**Show the following information in left-most column:** This gives you the choice of what to display in the left most column of the **Data List** and **Statistics** pages: Row number and/or Block number and/or the Row number within the block.

**Show module name in col. headers:** Check this option to display the data source (module) name in each column in addition to the data item name.

**Insert empty lines between blocks:** Insert empty lines in the **Data List** table to separate (in the display only) the different blocks collected. This does not affect any of the statistical results.

**Column width:** To set the width (in characters) of the columns in the **Data List** and **Statistics** tables.

**Significant digits:** To set the number of significant digits used to display number values in the **Data List** and **Statistics** pages.

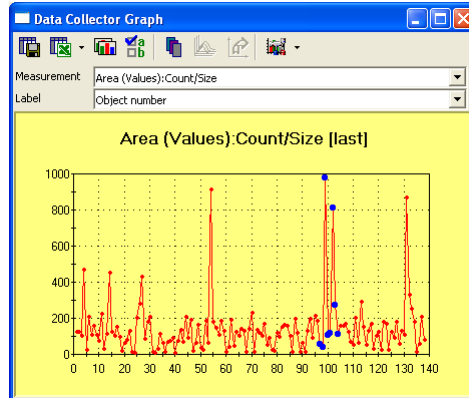
**Rolling Buffer:** This group contains the options that control live data collection. When the **Retain last** checkbox is *off* (default), there is no limit on the amount of data collected (limited only by RAM). If the control is *on*, only last N blocks are retained in the **Data Collector**. When the number of collected blocks exceeds the limit the first data block in the buffer will be removed before acquiring a new data block. Every new data block is appended to the end.

## Data Collector Charts

The **Data Graph**, **Histogram**, and **Scatterplot** have a common toolbar and similar display options. Data selection can be synchronized between the Data Collector, data charts, and the source modules that generate the data. In this way, changing or updating one of these features updates or changes the others.

Clicking the **Data Graph** button opens a window similar to this one:

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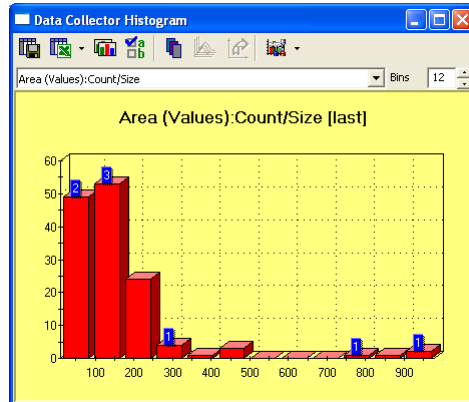


The **Data Charts** all have combo-boxes which include a list of measurements and labels available in the **Data Collector**.

**Measurements:** This box displays only numeric measurements from the data collector.

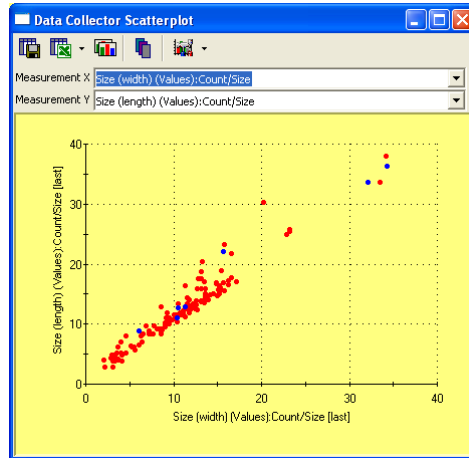
**Label:** This combo-box includes all text measurements, such as **Image Name** and **Object number**.

The **Histogram** looks something like this:



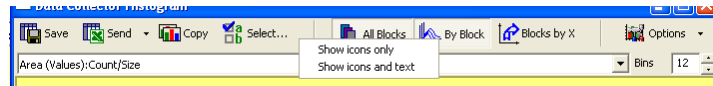
**Bins:** The **Bins** control of the **Histogram** defines the number of bins.

The **Scatterplot** appears like this:



**Measurements:** The **X** and **Y** combo-boxes display only numeric measurements from the **Data Collector**.

The chart window toolbar has two modes: icons only, or icons and text. Clicking the right mouse button over the toolbar switches the modes.



The following buttons are on the **Data Collector Chart** toolbar:



**Save:** Clicking this button saves the chart data into a file. The data can be saved in tab-delimited text format (TXT), comma-delimited text format (CSV) or as an html page (HTML). The number of **Significant digits** in the data is defined on the **Options** page of the **Data Collector** dialog.



**Send:** Clicking this button sends the chart data to a target program selected in the **Data Exchange Options** dialog.

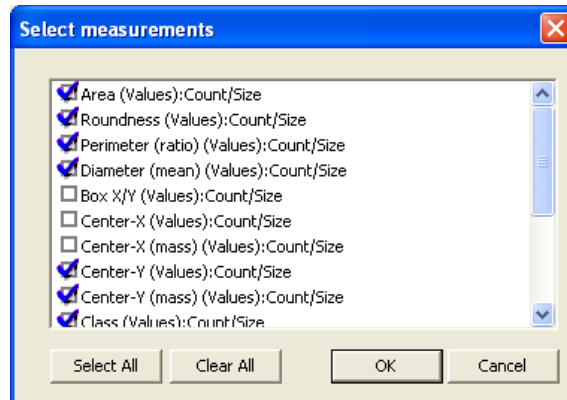


**Copy:** Click this button to copy a chart to the clipboard in enhanced metafile format.



**Select...** Clicking this button opens the **Select Measurements** dialog, where multiple measurements can be selected for display, as shown here:

## Measure:Data Collector



**Select All:** Clicking the **Select All** button selects all measurements,

**Clear All:** clicking the **Clear All** button resets the selection.

The next three buttons allow you to display groups of data collected in multiple blocks in the **Data Collector**:



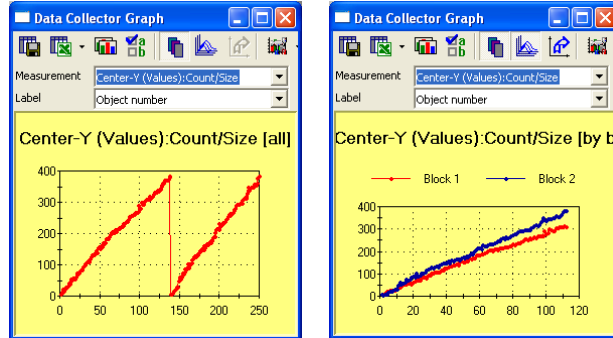
**All Blocks** : Clicking this button toggles between **All blocks** and **Last block** of data. When the button is *off*, the last data block is displayed in the chart. When the button is *on*, all data blocks are displayed with one graph per measurement.



**By Block:** When this button is *on*, different data blocks are shown in separate graphs. Only one measurement per block can be displayed. When this button is *off*, all blocks of one type of measurement are shown in one graph, so that multiple measurements may be displayed in one chart.

An example is shown here:





By blocks *on* B

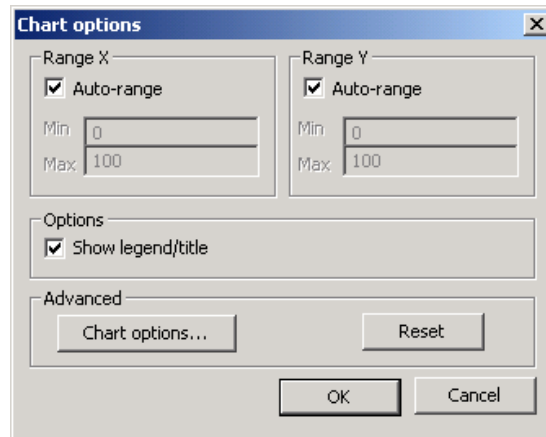
y blocks *off*



**Blocks by X** : When this option is *on*, one graph per value is displayed, and the block numbers are on the X axis. This mode can be used for displaying intensity measurements over time, or frames collected from a fixed number of AOIs.



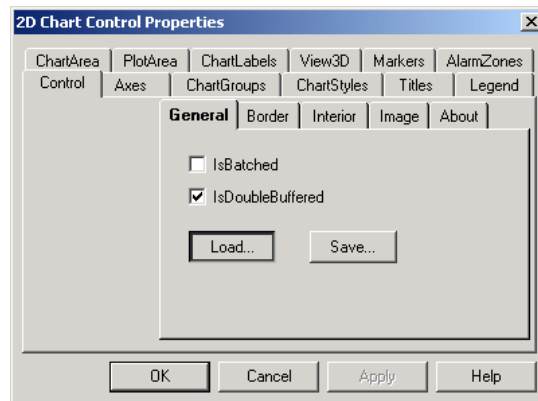
**Options**: Clicking this button opens the **Chart Options** dialog, as shown here:



**Range X** and **Range Y** groups define data ranges on the chart in the horizontal (X) and vertical (Y) directions. When the **Auto-range** is *on*, the ranges are defined by the actual data. When the option is *off*, the ranges are defined by the values in **Min** and **Max** controls.

**Options**: Checking the **Show legend/title** box turns the chart title and legend on or off.

**Advanced:** The **Chart options...** button opens an advanced chart control dialog where various chart options, such as colors, fonts, positions, chart types or new template can be set, as shown here:



If the **Options** dialog is closed using the **Ok** button, the changed chart options are saved as part of the chart template, and will be used the next time that a new chart window is created. The **Data Graph**, **Histogram** and **Scatterplot** windows have different templates located in the *Template* folder of the *Image-Pro Plus* directory.

**Reset:** Clicking the **Reset** button sets the default template to the chart.

## Data Selection in Data Collector Charts

Data selection is synchronized among the Data Collector, data charts, and the source modules that generate the data. In this way, changing or updating one of these features updates or changes the others.

Selection for the Scatterplot is available in two modes: *Rectangular* and *Irregular*. For *rectangular* selection, click-and-drag with the left mouse button. *Irregular* selection mode is activated by **Shift and clicking the Left mouse button**, and closed by clicking the **Right button** mouse button.

Hold the **<Ctrl>** key down to add or toggle a selection.

## Supported Functions and Operators

Symbol	Equivalent	Description	Example
()		Prioritizes an expression	$5*(1+1) = 10$
!	FACT	Factorial	$5! = 120$ $\text{fact}(5) = 120$
%		Percentage	$35\% = 0.35$
^	**	Raised to the power of	$4^5 = 1024$
*		Multiply by	$3 * 6 = 18$
/		Divide by	$9 / 2 = 4.5$
\	DIV	Integer divide by	$9 \setminus 2 = 4$
MOD		Modulo (remainder)	$7 \text{ mod } 4 = 3$
+		Add	$1 + 1 = 2$
-		Subtract	$9 - 5 = 4$
>		Greater than	$9 > 2 = 1$ * see note
<		Less than	$7 < 4 = 0$
==	=	Equal test	$5 == 4 = 0$
>=	=>	Greater or equal	$3 >= 3 = 1$
<=	=<	Less or equal	$\#h3E <= 9 = 0$
<>		Not equal	$\#b10101 <> 20 = 1$
NOT		Bitwise NOT	$\text{NOT}(15) = -16$
AND	&	Bitwise AND	$\#b101 \text{ AND } \#h1E = 4$
OR		Bitwise OR	$13 \text{ OR } 6 = 15$
XOR		Bitwise Exclusive OR	$9 \text{ XOR } 3 = 10$
EQV		Bitwise Equivalence	$6 \text{ EQV } 9 = -16$
IMP		Bitwise Implication	$1 \text{ IMP } 5 = -1$
IIF		If condition	$\text{Iif}(1+1=2,4,5) = 4$
MIN		Minimum value	$\text{min}(10,3,27,15) = 3$
MAX		Maximum value	$\text{max}(1,9)=9$ *see note
SIN		Sine	$\text{sin}(\pi) = 0$ *see note
COS		Cosine	$\text{cos}(\pi) = -1$
TAN		Tangent	$\text{tan}(\pi) = 0$
ASIN		Arc sine	$\text{asin}(1) = 1.570$
ACOS		Arc cosine	$\text{acos}(-1) = 3.141$
ATAN	ATN	Arc tangent	$\text{atan}(0) = 0$
SEC		Secant	$\text{sec}(0) = 1$
CSC		Cosecant	$\text{csc}(1) = 1.18$
COT		Cotangent	$\text{cot}(1) = 0.642$
SINH		Hyperbolic sine	$\text{sinh}(3) = 10.01$

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<b>Symbol</b>	<b>Equivalent</b>	<b>Description</b>	<b>Example</b>
COSH		Hyperbolic cosine	$\cosh(2) = 3.76$
TANH		Hyperbolic tangent	$\tanh(1) = 0.76$
COTH		Hyperbolic cotangent	$\coth(1) = 1.31$
SECH		Hyperbolic secant	$\operatorname{sech}(0) = 1$
CSCH		Hyperbolic cosecant	$\operatorname{csch}(1) = 0.85$
ASINH		Hyperbolic arc sine	$\operatorname{asinh}(2) = 1.44$
ACOSH		Hyperbolic arc cosine	$\operatorname{acosh}(9) = 2.89$
ATANH		Hyperbolic arc tangent	$\operatorname{atanh}(.1) = 0.10$
ACOTH		Hyperbolic arc cotangent	$\operatorname{acoth}(7) = 0.14$
ASECH		Hyperbolic arc secant	$\operatorname{asech}(.3) = 1.87$
ACSCH		Hyperbolic arc cosecant	$\operatorname{acsch}(2) = 0.48$
ABS		Absolute value	$\operatorname{abs}(-8) = 8$
EXP		e to the power of	$\operatorname{exp}(3) = 20.08$
EXP2		2 to the power of	$\operatorname{exp2}(3) = 8$
EXP10		10 to the power of	$\operatorname{exp10}(3) = 1000$
LOG	LN	Natural log	$\log(16) = 2.77$
LOG2		Log base 2	$\log_2(8) = 3$
LOG10		Log base 10	$\log_{10}(100) = 2$
CEIL		Round up	$\operatorname{ceil}(7.0) = 7$
RND		Random number	$\operatorname{rnd}(1) = .969$
INT		Truncate to an integer in	$\operatorname{t}(6.8) = 6$
SGN	SIGN	Sign of expression (-1, 0, or 1)	$\operatorname{sgn}(-9) = -1$
SQR	SQRT	Square root	$\operatorname{sqr}(64) = 8$

## The Macro Menu

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Use the *Macro* command to work with the contents of the selected script (.IPM) file. A script file (.IPM) contains one or more macros that have been created using the *Record Macro* command, or have been manually created using *Image-Pro's* macro language, IPBasic. A list of macros can be stored in a Macro Player List (\*.MPL) file. By default all macros are stored in a default file, but using the *Macro* command you can direct *Image-Pro* to store macros, or play them back, from a file of your choice.

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**Important:** In *IPP 7.0*, the preferred new extension for script files is .IPM. If you want to use older files with the extension .SCR, you must manually type the name of the file (\*.SCR to see a list of all files with that extension) into the file name field.

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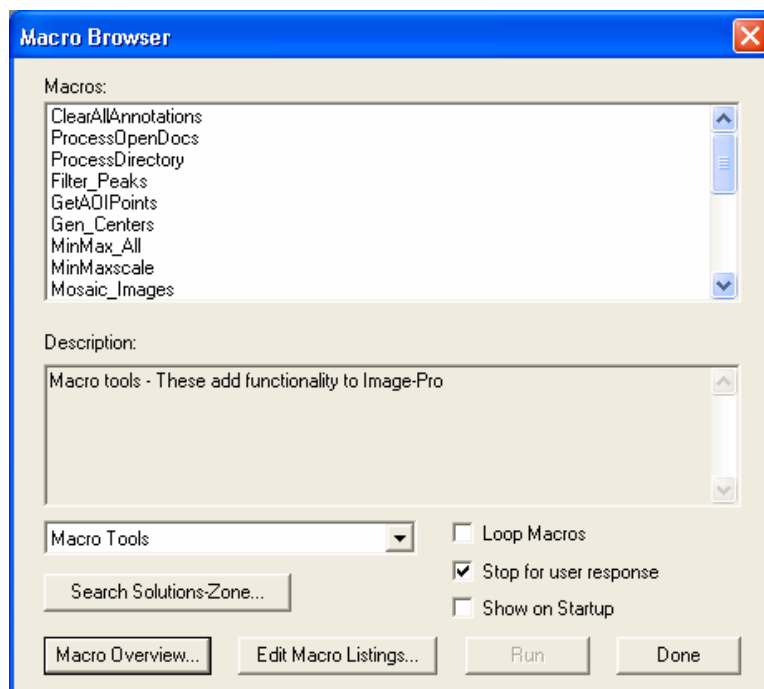
**Note :** You can also incorporate *Image-Pro* macros into a Visual Basic™ or Visual C++™ program. See the **Using Auto-Pro with Visual Basic** and **Using Auto-Pro with Visual C++** sections in your *Auto-Pro* manual.

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## Macro Browser

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Use the *Macro Browser* command to run the Image-Pro Plus demos and script files. When you select the *Macro* command, the **Macro Browser** dialog box is presented.

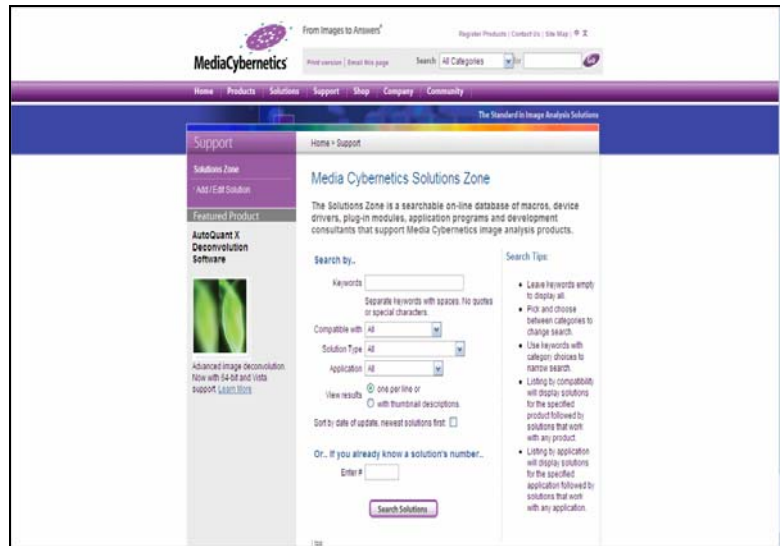


**Macros:** This is a list of all available *Image-Pro Plus* macros.

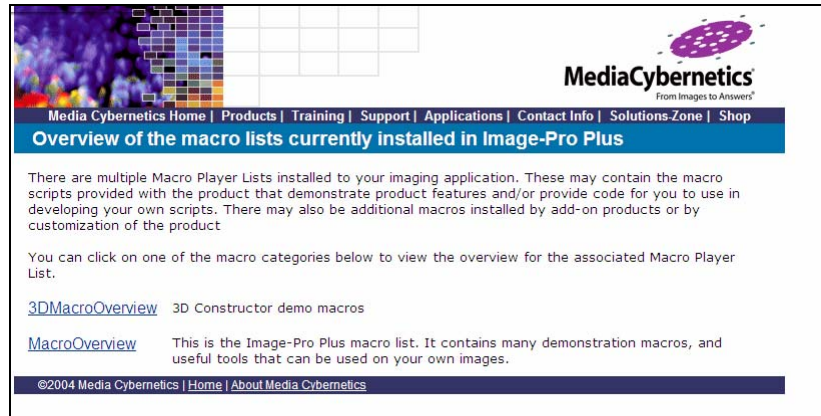
**Description:** This “view-only” field will display the selected macro's description, if one was assigned to it when it was created.

**Macro Tools:** In this list box, select the macro with which you want to work. A macro is selected by clicking its name in the list box.

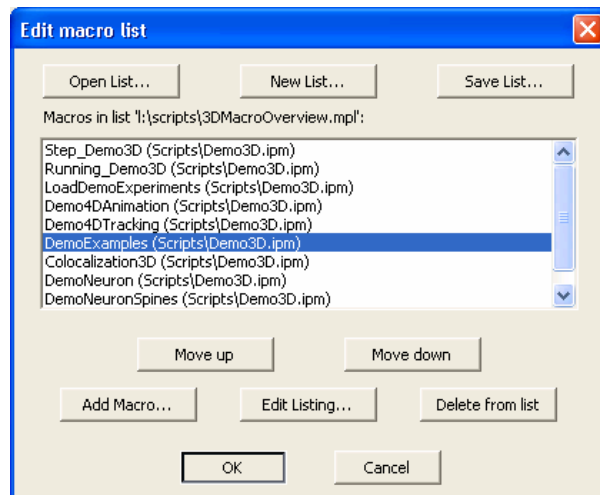
**Search Solutions Zone:** Click this button to go to the *Solutions Zone* portion of the *Media Cybernetics* website to look for new macros, as shown here:



**Macro Overview:** Click this button to see an overview of the macros currently installed with *Image-Pro Plus*:



**Edit Macro Listings:** Click this button to edit the list of available macros. You will see the following dialog:



The first set of buttons is used to manage which macro list is being edited in the rest of the dialog.

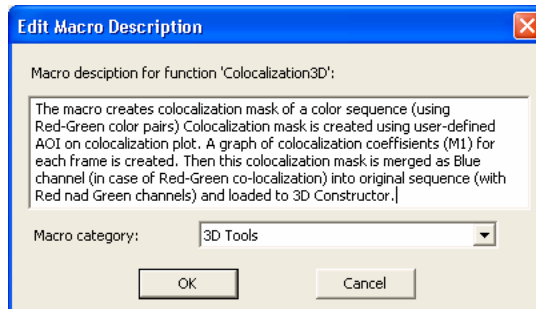
- **Open List:** Click this button to see the “Open Macro List” dialog that allows you to select the MPL macro list file to edit.
- **New List:** Click this button to open a small dialog where you can enter the list description and other list attributes, and then create a new, empty list.
- **Macros in list:** This window will be empty until the **Add Macro...** button is used to add macros to the list.
- **Save List...** This button opens a **Save Macro List** dialog which lets you save the list to a new .MPL file, or to save over the existing file if one was opened earlier.

The bottom set of buttons are used for editing the current list that is displayed in the **Macros in list...** control. They will all enable or disable appropriately as described for each button.

- The **Move Up** and **Move Down** buttons are used to shift the selected macro up or down in the list, and are disabled when there is no macro selected, or the selected macro is already at the top or bottom of the list (respectively).

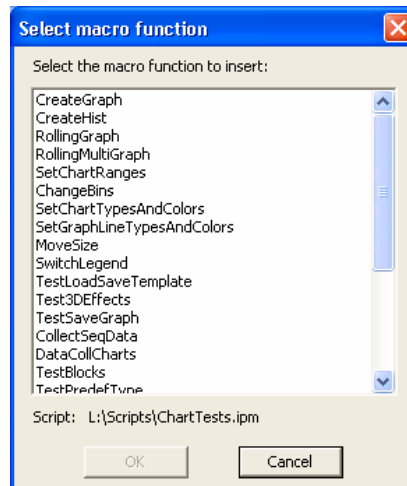


- **Delete from list:** This button will only be enabled when a macro listing is selected, and will delete the selected macro from the list (which does not affect the macro's availability from the Macro menu or from the script file).
- **Edit Listing:** This button will only be enabled when a macro listing is selected, and will invoke the “**Edit Macro Description**” dialog, which will allow the user to edit the description or change the macro category:



- **Add Macro...** Clicking this button invokes a series of dialogs that allow you to select a macro to add to the list, and to provide the macro's description and category. When the **Add Macro...** button is clicked, theyou will see the “**Open Macro Script**” dialog in order to determine the macro script from which the macro will be selected. If there is more than one suitable function in the macro script, then the “**Select macro function**” dialog will be invoked to allowyou to specify the macro function to be added to the list:

## Macro:Macro Browser



- The **OK** button will be enabled when you select the macro function to add to the list. After double-clicking the macro function or selecting the function and clicking **OK**, the **Edit Macro Description** appears to allow you to specify the macro description and category. At any point in this process, you can press **Cancel** to stop the addition of the macro to the list.
- Clicking **OK** on the **Edit Macro Description** dialog will add the macro to the list at the current insertion point (after the currently selected macro listing, or at the top of the list if no macro is selected).

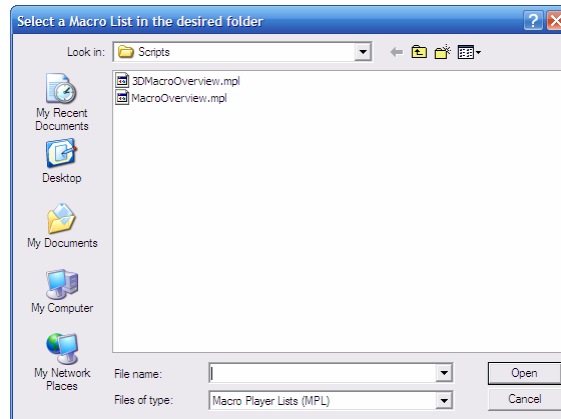
**Loop Macros:** Check this box to run the macros in a continuous loop.

**Stop for User Response:** Check this box to run interactive macros and demos where your input is required.

**Show on Start-Up:** Check this box to display the **Macro Browser** dialog every time you start *Image-Pro Plus*.

*Macro:Macro Browser*

**Insert Macro:** Click this button to load a new macro, as shown here:



**Run:** Click this button to play your selected macro.

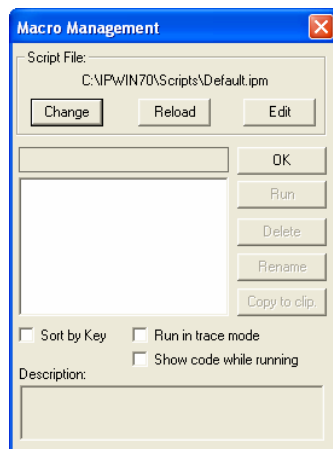
**Done:** Click this button to close the *Macro Browser*.

## Macro Management

---

Use the **Macro Management** dialog to run, rename, delete, or copy the macros that have been recorded into the selected script file. You can also use the *Macro Management* command to edit a script file.

When you select the *Macro Management* command, the **Macro Management** dialog box is presented.



**Script File:** In this group box, select the script file with which you want to work. A script file contains zero, one, or more macros. By default, the script file named “default.ipm” is selected when you start *Image-Pro*, unless you specify a new default using the “-s” command line parameter (see the *Start-Up Macro* topic) .

**Change:** Click this button to select a different script file. When you click **Change**, the **Select Script File** dialog box is presented. Within it, select the script file you want to use and click **OK**. The name of the selected script file will appear in the **Script File** group box, and the macros it contains will be listed in the **Macro Name** box (see below).

**Reload:** Click this button to reload the selected script file. This action would be required if you had manually edited the selected script file using an editor other than *Image-Pro's* built-in Macro Editor, while *Image-Pro* was active. The edited macros would not be recognized within *Image-Pro* until the script file was reloaded.

A **Reload** is required under these circumstances because macros are maintained in, and executed from, memory. When you edit the selected script file, you are modifying the disk copy of the file, but the macros in memory are unchanged. The **Reload** button instructs *Image-Pro* to refresh the macros in memory based upon the current contents of the disk file.

---

**Note:** Besides clicking the **Reload** button, there are three other actions that will cause the contents of the selected script file to be written to memory: 1) starting *Image-Pro* (the macros in the default script are loaded into memory), 2) selecting a new script file using the **Change** button (see above), and 3) editing and saving the script file with *Image-Pro's Macro Editor* (see below) .

---

**Edit:** Click this button to edit the selected script file. This action will invoke *Image-Pro's* built-in Macro Editor, and will automatically load the current script file into it. You may modify the macros as needed (refer to your *Auto-Pro* manual for macro-language syntax). When you have finished your edits, remember to save the file using the *Save* command in the *Macro Editor : File* menu. Then close the editor to return to *Image-Pro*. The newly revised copy of the script file will automatically be loaded into memory.

When you click the **Edit** button, the **Macro Editor** window appears. For a description of the menus and usage of this window, see the *Edit Macro* command.

---

**TIP** *You can have Image-Pro open the **Macro Editor** window and position the cursor at the macro of your choice. This is especially helpful if you have many macros in the script file. In the **Macro** dialog box, select the script file of your choice. Names of macros within that file will be listed in the large central list box. Select the macro name you want, then click the **Edit** button. Image-Pro positions the cursor at the first line of that macro.*

---

**Sort by key:** Enable this option if you want the macros contained in the selected script file to be listed by their assigned shortcut keys.

When disabled, the macros will be listed in alphabetical order, by name.

## Macro:Macro Management

**Run in trace mode:** Enable this option if you want to run your macros in step-by-step fashion. In “trace mode,” *Image-Pro* presents each macro statement before it is executed. The presented command is not executed until you click the **Step Into** button in the **Edit Macro** panel. Trace mode can be very useful for pinpointing a statement that is causing a macro to fail. For more about trace mode, see *Running a Macro in Trace Mode* later in this section.

**Show code while running:** Enable this option if you want to display the code during execution. In this mode, *Image-Pro* presents each macro statement before it is executed, but the macro operates continuously, rather than in step-wise fashion.

**Run:** Click this button to play back the selected macro (this is just one of several way to invoke a macro — see also *Running A Macro* later in this section). This button will be dimmed if a macro has not been selected.

**Delete:** Click this button to delete the selected macro from the script file. Be careful with this button; once a macro has been deleted there is no way to retrieve it.

**Rename:** Click this button to rename the selected macro. When you click *Rename*, the **Rename Macro** dialog box is presented. Enter the macro's new name and click **OK**. You may enter up to 15 characters. The name must conform to the naming conventions outlined under *Record Macro/Stop Recording* below.

The name you enter must also be unique to the selected script file — if you enter a name already assigned to another macro, you will be prompted to enter a new one.

**Copy to Clip.:** Click this button to copy the contents of the selected macro to the Windows Clipboard. You might use this feature to copy a macro into a Visual Basic or Visual C++ program.

## Record Macro/Stop Recording

---

Use the *Record Macro* command to record a series of *Image-Pro* commands as you perform them. When *Record Macro* is enabled, *Image-Pro* begins saving your commands to a script file using the *Auto-Pro* scripting facility. It continues to record your actions until you instruct it to stop by clicking the **Stop Recording** button in the **Recording** dialog box, or selecting the *Stop Recording* command in the *Macro* Menu.

While a macro is being recorded, the message “Image-Pro - Recording” will flash in the Title bar and the **Recording** dialog box will be displayed. The **Template Mode** option in this dialog box can be enabled at any point while the macro is being recorded

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**Note:** If you are recording a macro, and you call an image from the File menu that is no longer accessible, the steps requiring that image will not be recorded.

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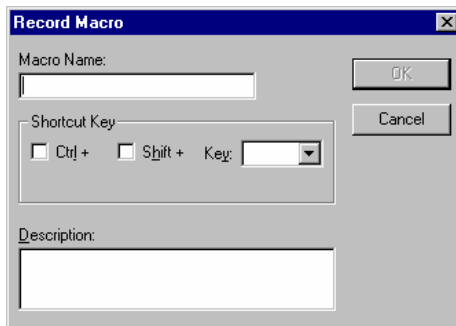
Enabling this option causes an `IpTemplateMode(1)` statement to be written to the script file. This statement affects the behavior of the macro when it is played back — instead of using the actual data values written into the macro, the user will be prompted to provide input during playback.

The **Template Mode** option can be enabled and disabled at any time (even multiple times) in a macro. When you disable the option, an `IpTemplateMode(0)` statement is written to the script file. When played back, the commands following this statement are executed in “literal” mode. For more about **Template Mode** see your *Auto-Pro* manual.

The **Show Recording** option, which can also be enabled at any time, lets you view the macro statements as they are being recorded (see the *Output Window* command for more information about this).

To begin recording a macro, select the *Record Macro* command. The **Record Macro** dialog box is presented. Within this box, enter a name that will uniquely identify the macro you are about to record and (optionally) assign it a shortcut key that can be used to play it back. You may also enter optional comment information.

## Macro:Record Macro/Stop Recording



**Macro Name:** Enter a name for your macro. You may assign a name of up to 30 characters. This name must conform to the following rules (nonconforming names will cause an error during playback):

- ◆ the name must begin with a letter.
- ◆ the name must be made up of only letters, numbers, spaces or the "\_" (underscore) character (note - *Image-Pro* will convert all spaces to the underscore character in the final name).
- ◆ the name must not duplicate one of *Auto-Pro's* reserved words. Reserved words are words with special meaning to *Image-Pro*, such as command names (e.g., `IpDocClose`, `IpFftForward`, `IpLutApply`) and BASIC-like keywords (e.g., `If`, `Or`, `Open`, `loop`). See *Appendix B* in your *Auto-Pro* manual for a list of reserved words.

---

**TIP** *If you want to ensure that your macro name does not conflict with a reserved word, you can make it distinct by appending the underscore character to it, e.g., `Open_`, `Close_`, `LOOP_`.*

---

The name you enter must also be unique to the selected script file. If you enter a name that is already assigned to another macro, you will be prompted to enter a new one.

**Shortcut Key:** Enter a key combination that can be used to play back the macro you are about to record.



**Note:** You are not required to assign a shortcut key. A shortcut key's purpose is simply to let you play back your macro quickly and easily — basically, with the touch of a key. If you do not assign a shortcut key, you can play back your macro using the Run Macro or Macro command.

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A macro can be assigned to a function key (activate the **Key** list box, to find the available function keys), or to a <Ctrl>+<key>, <Shift>+<key> or <Ctrl>+<Shift>+<key> combination.

**Ctrl:** Select the **Ctrl** box to use the <Ctrl> key plus another key to activate your macro. The <Ctrl> key can be used in combination with an available Function or Alphanumeric keys (activate the **Key** list box, to find the available keys). It can also be combined with the <Shift> key to create <Ctrl>+<Shift>+<key> combinations.

**Shift:** Select the **Shift** box to use the <Shift> key plus another key to activate your macro. The <Shift> key can be used in combination with an available Function key. It can also be used with an Alphanumeric keys (activate the **Key** list box, to find the available keys), as long as it is combined with the <Ctrl> key. If the <Ctrl> key is not checked, the alphanumeric choices will be dimmed.

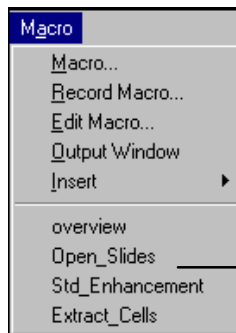
**Description:** This field can be used to include an optional comment or description with your macro. The description is displayed when you use the *Macro* command to browse the contents of a script file.

An unlimited number of characters can be entered. Use <Ctrl>+<Enter> to enter a line ending.

## Running a Macro

There are five ways of invoking a macro that you have created:

1. **Press its shortcut key**, if one has been assigned to the macro.
2. **Click the macro's name** listed at the bottom of the *Macro* menu.



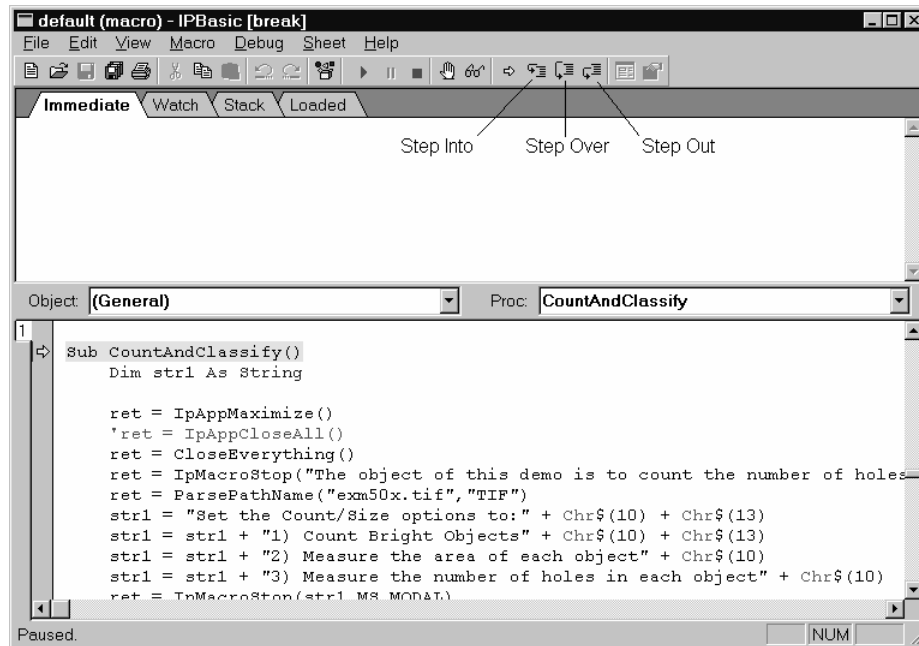
Macros are listed at the bottom of the Macro menu.

3. **Select the macro name in the *Macro Management* command's dialog box** and click the **Run** button.
4. **Select it from a menu (or Toolbar button) to which it has been assigned** (see *Adding a Macro to a Menu* under *Customizing the Command Menus and Buttons*).
5. **Call it from another macro or a Visual Basic program** (see your *Auto-Pro Reference* for more about calling a macro from Visual Basic).

## Running a Macro in Trace Mode

For debugging purposes, there are occasions when it is useful to run your macro in “trace mode.” Trace mode lets you execute the macro in step-by-step fashion, allowing you to see the effect of each command. It can help you quickly identify the specific statement that is causing a macro failure.

Enable trace mode by selecting the *Macro* command's **Run in trace mode** option (see the *Macro* command earlier in this section). When you run your macro, *Image-Pro* will display the first macro statement in the **Macro Editor** window. This statement is not executed until you click the **Step Into** button in the **Macro Editor**.



Using the **Macro Editor**, you can step through your entire macro one statement at a time, or step through just a portion of the macro and execute (or terminate) the remainder automatically. Trace mode also lets you view the value of a symbolic construct (i.e., a variable or constant).

*Macro:Record Macro/Stop Recording*

The trace function uses the following buttons:

**Step Into:** Execute the current line. If the current line is a subroutine or function call, stop on the first line of that subroutine or function. (If the macro is not active, start it.)

**Step Over:** Execute to the next line. If the current line is a subroutine or function call, execute that subroutine or function completely.

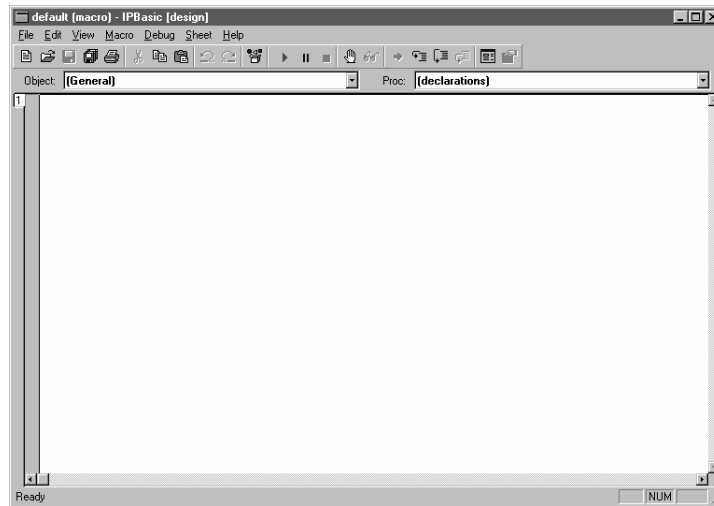
**Step Out:** Step out of the current subroutine or function call.

## Edit Macro

---

Use the *Edit Macro* command to edit an existing macro. This command can be used instead of selecting the macro in the Macro dialog and choosing *Edit*.

When you select the *Edit Macro* command, the **Macro Editor** window appears.



Using the commands on the *Macro Editor* window's menus, the contents of the window can be searched, edited directly, saved to a file or the Clipboard, and printed. You may also open other files, print a file, search for a character sequence, cut, copy, paste, delete text, and query the *Help* files.

---

**Important:** With the release of IPP 7.0, the preferred new extension for script files is `.IPM`. If you want to use older files with the extension `.IPM`, you must manually type the name of the file (or `.IPM` to see a list of all files with that extension) into the file name field.

---

The following information will help you use the IPBasic Macro Language Editor:

## File Menu

The *File* menu provides the following options.

<b>Item Description</b>	
New	Create a new macro.
New Module	Create a new macro module (code, object or class).
Open	Load an existing macro/module from disk.
Close	Close the current macro/module.
Save	Save the current macro/module to disk.
Save As	Save the current macro/module to disk as a particular file.
Save All	Save all the macros/modules to disk.
Print	Print the current macro/module.
Print Setup	Select the default printer.
Exit	Exit IPBasic Editor.

## Edit Menu

The *Edit* menu provides the following options.

<b>Item Description</b>	
Undo	Undo the last edit. <b>Hot Key:</b> <Ctrl+Z>
Redo	Redo the last undo. <b>Hot Key:</b> <Ctrl+Y>
Cut	Move the selected text to the Clipboard. <b>Hot Key:</b> <Ctrl+X>
Copy	Copy the selected text to the Clipboard. <b>Hot Key:</b> <Ctrl+C>
Paste	Paste the Clipboard text over the selected text. <b>Hot Key:</b> <Ctrl+V>
Delete	Delete the selected text. <b>Hot Key:</b> <Del>
Select All	Select all of the text.
Indent	Move selected lines right. <b>Hot Key:</b> <Tab>
Outdent	Move selected lines left. <b>Hot Key:</b> <Shift+Tab>
Tab As Spaces	Toggle the insert tab as spaces mode on/off.
Find	Find a string. <b>Hot Key:</b> <Ctrl+F>
Replace	Replace a string with another. <b>Hot Key:</b> <Ctrl+R>
Again	Repeat last find or replace. <b>Hot Key:</b> <F3>
UserDialog	Edit a User Dialog.
References	Edit the macro/module's references
Properties	Edit the module's properties.

## View Menu

The *View* menu provides the following options.

<b>Item Description</b>	
Macro	Activate the macro editing window. <b>Hot Key:</b> <Ctrl+A>
Immediate	Show the immediate output window. <b>Hot Key:</b> <Ctrl+E>
Watch	Show the watch expressions window. <b>Hot Key:</b> <Ctrl+W >
Stack	Show the call stack window. <b>Hot Key:</b> <Ctrl+T>
Loaded	Show the loaded macros/modules window. <b>Hot Key:</b> <Ctrl+L>
Toolbar	Toggle the toolbar on/off.
Status Bar	Toggle the status bar on/off.
Edit Buttons	Toggle the edit buttons on/off.
Always Split	Toggle the split on/off.
Font	Set the display font.
Tab Width	Set the tab width.
Object/Proc	Select the Object/Proc list display mode.

### Immediate Window

- ◆ Use this window to Evaluate an expression, assign a variable or call a subroutine.
- ◆ Type "?expr" <Enter> to show the value of "expr".
- ◆ Type "var = expr" <Enter> to change the value of "var".
- ◆ Type "Set var = expr" <Enter> to change the reference of "var".
- ◆ Type "subname args" <Enter> to call a subroutine or built-in instruction.
- ◆ Type "Trace" <Enter> to toggle trace mode. Trace mode prints each statement in the immediate window when a macro/module is running.



### **Watch Window**

List the variables, functions and expressions that are calculated and displayed.

- ◆ Each time execution pauses the value of each line in the window is updated.
- ◆ The expression to the left of "->" may be edited.
- ◆ Pressing Enter updates all the values immediately.
- ◆ Pressing <Ctrl+Y> deletes the line.

### **Stack Window**

List the lines which called the current statement.

- ◆ The first line is the current statement. The second line is the one that called the first. And so on.

Clicking on a line brings that macro/module into a sheet and highlights the line in the edit window.

*Macro:Edit Macro*

## **Macro Menu**

The *Macro* menu provides options for starting macros, stopping macros and extending the Basic language.

<b>Item Description</b>	
Run	Run the macro to completion. (If the macro is not active, start it.) <b>Hot Key:</b> <F5>
Pause	Stop the macro/module. Execution can be continued. <b>Hot Key:</b> <Esc>
End	Terminate the macro/module. Execution cannot be continued.

## Debug Menu

The *Debug* menu provides the options for debugging macros/modules.

<b>Item Description</b>	
Step Into	Execute the current line. If the current line is a subroutine or function call, stop on the first line of that subroutine or function. (If the macro is not active, start it.) <b>Hot Key:</b> <F8>
Step Over	Execute to the next line. If the current line is a subroutine or function call, execute that subroutine or function completely. <b>Hot Key:</b> <Shift+F8>
Step Out	Step out of the current subroutine or function call. <b>Hot Key:</b> <Ctrl+F8>
Step to Cursor	Execute until the line the cursor is on is the current line. (If the macro is not active, start it.) <b>Hot Key:</b> <F7>
Toggle Break	Toggle the break point on the current line. <b>Hot Key:</b> <F9>
Clear All Breaks	Clear all break points. <b>Hot Key:</b> <Shift+Ctrl+F9>
Quick Watch	Show the value of the expression under of the cursor in the immediate window. <b>Hot Key:</b> <Shift+F9>
Add Watch	Add the expression under of the cursor in the watch window. <b>Hot Key:</b> <Ctrl+F9>
Browse	Show the methods of the expression under of the cursor.
Set Next	Set the next statement to be executed. Only statements in the current subroutine/function can be selected.
Show Next	Show the next statement to be executed.

Macro:Edit Macro

## Sheet Menu

The *Sheet* menu provides the sheet options.

<b>Item Description</b>	
Open Uses	Open all the #Uses modules for the current macro/module.
Close All	Close all the macros/modules.
1 9	Show this macro/module. <b>Hot Key:</b> <Ctrl+1> to <Ctrl+9>

## Help Menu

The *Help* menu provides the following options.

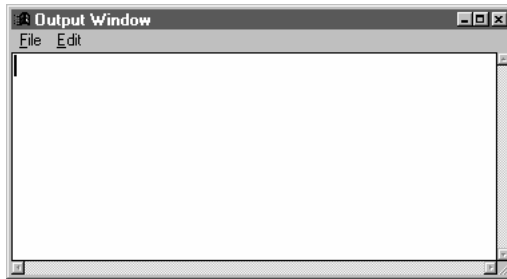
<b>Item Description</b>	
Editor Help	Show the table of contents for IPBasic Editor.
Language Help	Show the table of contents for the Basic language. <b>Hot Key:</b> <Shift+F1>
Topic Search	Show IPBasic language help for the keyword under the cursor. <b>Hot Key:</b> <F1>
About IPBasic	IPBasic Editor's about box.

## Output Window

---

Use the *Output Window* command to display the **Macro Output** window. This window serves a variety of macro-related purposes. It can be used to copy *Auto-Pro* commands to a file or the Clipboard. It can be used as a “scratch pad” on which you can jot notes or annotate an *Auto-Pro* script. It can be used as a window in which to display output from an *Auto-Pro* macro (see the `Print` and `IpOutput` statements in your *Auto-Pro Reference*).

When the **Macro Output** window is opened for the first time in the *Image-Pro* session, it is empty.



From that point on it will begin collecting information that is:

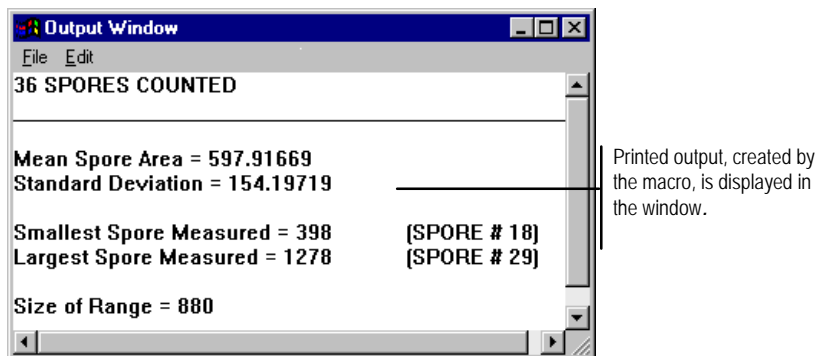
Typed in directly:



Comments and annotations can be typed directly into the window.

### Macro:Output Window

Or, printed by the macro when it is played back (i.e., output produced by the `Print` or `IpOutput` statement).



The **Macro Output** window retains the data it receives until its buffer becomes full (approximately 25,000 characters), *Image-Pro* is closed, or the window is cleared by the user (see the *Edit* menu, below). When the buffer becomes full, the oldest lines are pushed out to accommodate the new text.

Using the commands on the *Macro Output* window's *File* and *Edit* menus, the contents of the window can be edited directly and saved to a file or the Clipboard. See the descriptions of these commands, below.

See also, the `Print` and `IpOutput` statements in your Auto-Pro Reference.

---

**TIP:** To select all the data in the **Output Window**, press `<Ctrl>+<Home>` and then `<Ctrl>+<Shift>+<End>`.

---

## Output Window : File Menu

Use the commands on the *Output Window : File* menu to save the contents of the window to a file or the Clipboard.

- 
- ◆ **Save:** Select the *Save* command on the *Output Window : File* menu to save the contents of the **Macro Output** window to a text file.
- 
- ◆ **Append:** Select the *Append* command on the *Output Window : File* menu to add the contents of the **Macro Output** window to the end of an existing text file.
- 
- ◆ **Copy To Clipboard:** Select the *Copy To Clipboard* command on the *Output Window : File* menu to copy the contents of the **Macro Output** window to the Windows Clipboard. The text can then be pasted into any application that accepts text from the Clipboard.
- 
- ◆ **Data Export:** Use the *Data Export* command on the *Output Window : File* menu to transfer the contents of the Output Window to Microsoft's Excel spreadsheet. Before selecting *Data Export*, you should use *Export Options* to determine the correct placement (within Excel) for the data. When you select *Data Export*, the transfer occurs with little visible evidence that it has happened. By switching to the Excel application, you can confirm that the transfer has occurred.
- For additional information about DDE, see the *Dynamic Data Exchange* discussion.
- 
- ◆ **Export Options:** Use this command on the *Output Window : File* menu to tell *Image-Pro* how and where to export the current output window data via DDE.
- 
- ◆ **Print:** Use this command on the *Output Window : File* menu to print the **Macro Output** window. The window must be open to print the contents.

## Macro:Output Window

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- ◆ **Close:** Use this command on the *Output Window : File* menu to close the **Macro Output** window.

## Output Window : Edit Menu

Use the commands on the *Output Window : Edit* menu to cut, copy and paste text in the **Macro Output** window.

- 
- ◆ **Undo Last:** Select this command on the *Output Window : Edit* menu to undo the last editing operation that was manually performed in the **Macro Output** window (e.g., a typing sequence, a cut operation or a paste operation). This command does not undo data that have been printed by a macro or passed from the macro recorder.
- 
- ◆ **Clear Screen:** Use this command on the *Output Window : Edit* menu to delete the contents of the **Macro Output** window.
- 
- ◆ **Copy:** Use this command on the *Output Window : Edit* menu to copy the *selected* text to the Clipboard (this command will be dimmed if there is no selected text). Text is selected by dragging the mouse across it. When text is selected, it will be highlighted.
- 
- ◆ **Cut:** Use this command on the *Output Window : Edit* menu to copy the *selected* text to the Clipboard and simultaneously remove it from the **Macro Output** window (this command will be dimmed if there is no selected text). Text is selected by dragging the mouse across it. When text is selected, it will be highlighted.
- 
- ◆ **Paste:** Use this command on the *Output Window : Edit* menu to copy the contents of the Clipboard into the **Macro Output** window. Before selecting the *Paste* command, place your insert cursor in the position at which the text is to be inserted. If the Clipboard is empty, or does not contain textual data, this command will be dimmed.



## Insert

---

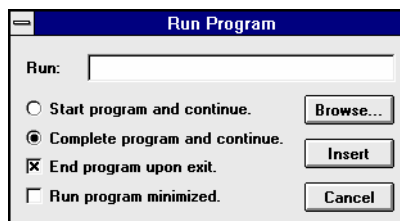
Use the *Insert* command to add messaging and control commands to your macro while it is being recorded. This command can be used instead of manually editing such statements into your macro after it is recorded.

Select the *Insert* command when you are recording your macro and have reached the point at which you want a control statement inserted. You will receive a pop-out menu from which you must choose the kind of statement to insert, or a comment.

*Hint - it is often easier to use the **Insert** command if the "Show Recording" option is enabled so you can see your macro commands as they are being recorded. Seeing a command sequence often makes it easier to identify the points at which a control statement should be inserted.*

Each option on the Insert command's pop-out menu is described below.

- ◆ **Run Program:** Use this command to add an `IpAppRun` statement to your macro. The `IpAppRun` statement is used to execute a DOS or Windows application from your macro. When you select the *Run Program* command, the **Run Program** dialog box is displayed.



**Run:** In this field, type the file name of the program that is to be called by your macro (you can also use the **Browse** button to select a program file using a dialog box; see below). Then, set the options that describe the way in which you want the program called. When you click *Insert*, the `IpAppRun` statement will be written into your program.

A DOS or Windows program can be invoked by this command. A DOS program must be called by its PIF file, which is created with the Windows PIF Editor.

Refer to your *Auto-Pro Reference* for more about the IpAppRun statement and its options.

**Browse:** Click this button to receive the **Select Program Name** dialog, with which you can display the contents of a directory and select a program file. The file name you select will automatically be placed in the **Run** field.

**Start program and continue:** Enable this option if you want your macro to continue processing after the external program has been loaded (i.e., the external application and your macro will be active, simultaneously). The disposition of the external program when *Image-Pro* is subsequently closed, is determined by the **End program upon exit** value (see below).

**Complete program and continue:** Enable this option if you want your macro to suspend its processing until the external application is closed. Setting this option is equivalent to setting IpAppRun's *RunMode* parameter to RUN\_MODAL.

**End program upon exit:** Enable this option if you want the external program to close automatically when you exit *Image-Pro*. Otherwise, the external program will remain active even if *Image-Pro* is no longer running. Setting this option is equivalent to setting IpAppRun's *RunMode* parameter to RUN\_AUTOCLOSE. Setting **Start program and continue** without this option is equivalent to setting IpAppRun's *RunMode* parameter to 0.

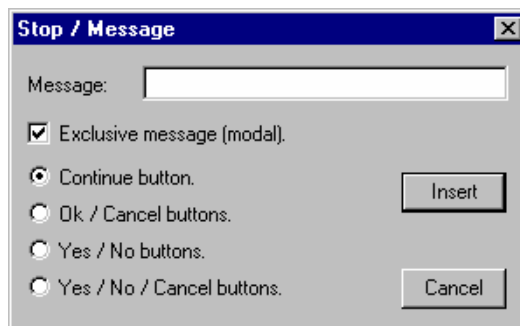
**Run program minimized:** Enable this option if you want the external program's window to be displayed as an icon when it is initially loaded. Setting this option is equivalent to setting IpAppRun's *ShowMode* parameter to RUN\_MINIMIZED. If **Run program minimized** is not enabled, the *ShowMode* parameter is set to RUN\_NORMAL.

---

**Stop/Message:** Use this command to add an IpMacroStop statement to your macro. The IpMacroStop statement suspends macro processing, and displays a message box containing text that you have

specified. The box can be designed to include buttons such as **Yes**, **No**, **Continue**, and **Cancel**. When the user clicks a button, macro processing resumes with the next statement.

When you select the *Stop/Message* command, the following dialog box is displayed:



**Message:** In this field, type the text that is to be displayed when the macro is executed. Set the options that describe the way in which you want the message to be displayed (see definitions, below) and click **Insert**. The `IpMacroStop` statement will be written into your program.

Refer to your *Auto-Pro Reference* for more about the `IpMacroStop` statement and its options.

**Exclusive message (modal):** Enable this option to display a “modal” message box. A modal message box is one that “locks out” *Image-Pro* when it is displayed — i.e., the user is prevented from selecting an image or performing any *Image-Pro* operations while the message is on the screen. Modal messages are useful for reporting events that do not require any user action (e.g., notifying the user that the last image was processed or that an error had occurred). When **Exclusive message (modal)** is selected, the button options are activated, allowing you to include buttons that best suit the purpose of your message box (e.g., *OK*, *Yes/No*, or *Continue/Cancel*). See button options, below.

If **Exclusive message (modal)** is *not* enabled, a “modeless” message box is issued. A modeless message allows the user to

access *Image-Pro* while the message is displayed. This sort of message is generally used to request an action of the user. You might use it to instruct the user to select an AOI, or remind him/her to adjust image brightness to a satisfactory level. The button options (see below) are not active when the **Exclusive message (modal)** is not enabled. A modeless message box can contain only a *Continue* button, which, when clicked, will resume processing of the macro.

**Continue:** Enable this option to include a single button labeled *Continue* in your message box. When the *Continue* button is clicked, macro processing will resume with the next macro statement. This is the only button configuration that can be selected when **Exclusive message (modal)** is disabled (i.e., a modeless message box is requested).

**OK / Cancel buttons:** Enable this option to include two buttons, labeled *OK* and *Cancel*, in your message box. When either button is clicked, macro processing resumes with the next macro statement. This statement should be the first statement of a decision structure that acts upon the value returned by the `IpMacroStop` function (you will need to manually add this structure with the Macro Editor). See the `IpMacroStop` statement in your *Auto-Pro Reference* for the value that is returned by each button, and an example of a decision structure that handles them.

This option is available when **Exclusive message (modal)** is selected. Otherwise, it is dimmed.

**Yes / No buttons:** Enable this option to include two buttons, labeled *Yes* and *No*, in your message box. When either button is clicked, macro processing resumes with the next macro statement. This statement should be the first statement of a decision structure that acts upon the value returned by the `IpMacroStop` function (you will need to manually add this structure with the Macro Editor). See the `IpMacroStop` statement in your *Auto-Pro Reference* for the value returned by each button, and an example of a decision structure that handles them.

This option is available when **Exclusive message (modal)** is selected. Otherwise, it is dimmed.

**Yes / No / Cancel buttons:** Enable this option to include three buttons, labeled *Yes*, *No*, and *Cancel*, in your message box. When any button is clicked, macro processing resumes with the next macro statement. This statement should be the first statement of a decision structure that acts upon the value returned by the `IpMacroStop` function (you will need to manually add this structure with the Macro Editor). See the `IpMacroStop` statement in your *Auto-Pro Reference* for the value returned by each button, and an example of a decision structure that handles them.

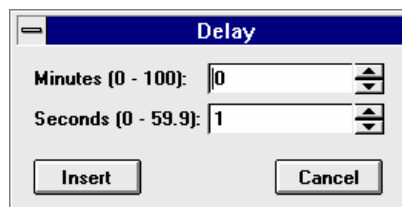
This option is available when **Exclusive message (modal)** is selected. Otherwise, it is dimmed.

- 
- ◆ **Break Point:** Use this command to add a `Stop` statement to your macro. The `Stop` statement halts the macro and activates “trace mode.” It is often included during the debugging stage to stop a macro at a specific point so that the status of certain data values can be examined.

Refer to your *Auto-Pro Reference* for more about the `Stop` statement.

- 
- ◆ **Delay:** Use this command to add an `IpMacroWait` statement to your macro. The `IpMacroWait` statement halts macro processing for a specified duration. You might insert this command to slow down a particular step so that its results can be easily observed on the screen. Or, you might use it to allow sufficient time for an external event to occur; perhaps to await a result from an external application.

When you select the *Delay* command, the following dialog box is displayed:

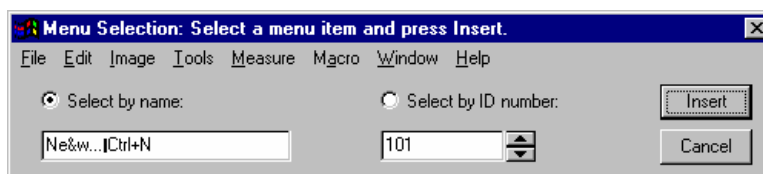


Enter the amount of time that must pass before *Image-Pro* processes the next statement in the macro. When you click *Insert*, the `IpMacroWait` statement will be written into your program (its time will be expressed in 1/10ths of a second).

Refer to your *Auto-Pro Reference* for more about the `IpMacroWait` statement.

- ◆ **Menu Selection:** Use this command to add an `IpAppMenuSelect` statement to your macro. The `IpAppMenuSelect` statement can be used to invoke any item from the *Image-Pro* main menu — it is typically used for user-defined menu commands, which are not recognized by the macro recorder.

When you select the *Menu Selection* command, the following dialog box is displayed:



To insert a menu command, simply select it from the menu listed at the top of the box. (Alternatively, you may enter the command's *Title* or *ID* in the **Select by Name** or **Select by ID number** field, respectively. When you use this method, you must enter the command name and program number *exactly* as they have been defined in the *Title* and *ID* parameters of the MENU.INI file — see `item` and `progitem` under the *MENU.INI Syntax* section in Part 1 of this manual for more about the *Title* and *ID* parameters).

When you click **Insert**, the `IpAppMenuSelect` statement is written into your macro.

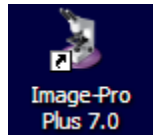
**Select by name:** Enable this option if you want the `IpAppMenuSelect` statement written with its *Mode* parameter set to `MENU_NAME`, which identifies the requested command by its title (as specified in the IPWIN32.MNU file). See the `IpAppMenuSelect` statement in your *Auto-Pro Reference* for more about this parameter.

**Select by ID:** Enable this option if you want the `IpAppMenuSelect` statement written with its *Mode* parameter set to `MENU_ID`, which identifies the requested command by its ID number (as specified in the IPWIN32.MNU file). See the `IpAppMenuSelect` statement in your *Auto-Pro Reference* for more about this parameter.

- 
- ◆ **Comment:** Use this command to add text to your macro. The text statement can be used to describe the actions performed by the macro. Comments do not affect the performance of the macro.
- 
- ◆ **Stop Image Refresh:** Use this command to turn off the image display while the macro is running. This may speed up the time it takes to process the image using macros.
- 
- ◆ **Resume Image Refresh:** Use this command to turn the image display back on while the macro is running. This may slow down the time it takes to process the image using macros.

## A Start-Up Macro

Any macro you create can be made to automatically run whenever *Image-Pro* is loaded. Assigning a start-up macro is accomplished by using the “-m” and “-s” switches on the *ipwin32.exe* command line.

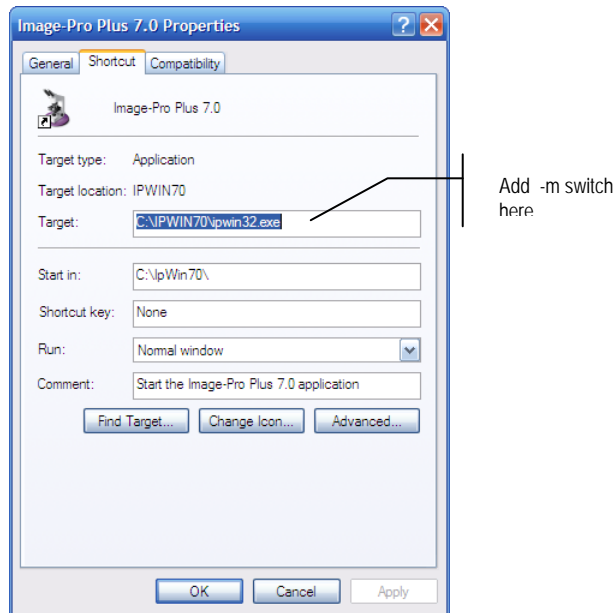


To add these parameters, perform the following steps:

1. Right-click on the Windows **Start** button, then click **Open**.
2. Double-click the **Programs** folder.
3. Double-click the **Image-Pro Plus** group.
4. Right-click the *Image-Pro Plus* icon.
5. Select **Properties**.

Macro:Insert

The **Image-Pro Plus Properties** dialog box will be presented.



Add the “-m” switch to the end of the *ipwin32.exe* statement in the **Command Line** field. Type the name of the start-up macro after this switch. The example below shows how the statement should look to assign a macro called “init” as the start-up macro.

```
C:\IPWIN7\ipwin32.exe -m init
```

Note that a space is placed before and after the -m switch, separating it from both the *ipwin32.exe* file name and the “init” macro name.

If your macro is not contained in the “default.ipm” file, you must also add the “-s” switch to specify the script file in which the macro is located. The example below shows how the command line should look if the “init” macro were located in the “cellwork.ipm” script file.

```
C:\IPWIN7\ipwin32.exe -m init -s cellwork.ipm
```

*Note - The specified script file becomes the "selected" script file once Image-Pro is loaded. Also, be sure to include the extension in the script file's name.*



## Load Demo Macros

---

This selection contains the demonstration script files for *Image-Pro Plus*. A list of available demo macros will appear in the drop-down menu. Click on the name of the demo macro that you wish to run. Some of the available demo macros are:

- ◆ **Separate\_Fluorochromes:** This macro demonstrates the separation of a 24-bit color image into its red, green, and blue channels. After enhancing the blue channel, the individual channels are recombined to create a 24-bit color image.
- ◆ **CountSmall\_Cells:** This macro shows how to count the number of cells in a sample.
- ◆ **MeasureStain:** This macro uses an image of a tissue section to demonstrate the *Count/Size* tools to select objects of interest, based on their color.
- ◆ **MeasureAngles:** This macro uses an x-ray film image to calculate the angle of the leg. First, the image is processed to define area of interest, and second, the angles are measured using the *Count/Size* tools.
- ◆ **CountandClassify:** This macro demonstrates one possible way to count the number of holes (pores) in an object or objects, and then determine the percent area of the holes compared to the objects of interest.
- ◆ **MeasureIronPhases:** This macro demonstrates how to locate and count individual phases of a multiple-phase material. In this macro, individual phases are segmented and then measured using the *Count/Size* tools.
- ◆ **ThoroughfocusSequences:** This macro shows how to open a sequence of images using the *Sequencer* tool, and then apply a pseudocolor to the sequence. For additional detail, the histogram of the image sequence is also opened in order to view the histogram from frame to frame.
- ◆ **MeasureAutoradiograph:** This macro creates an intensity calibration curve and then derives measurements from selected areas of an autoradiograph.

*Macro:Load Demo Macros*

- ◆ **SprocketCountDemo:** This macro demonstrates the creation of a mask image. It then applies various filters, such as Thinning and Branch/EndPoint to locate endpoints, and uses the **Count/Size** tools to count the number of endpoints.

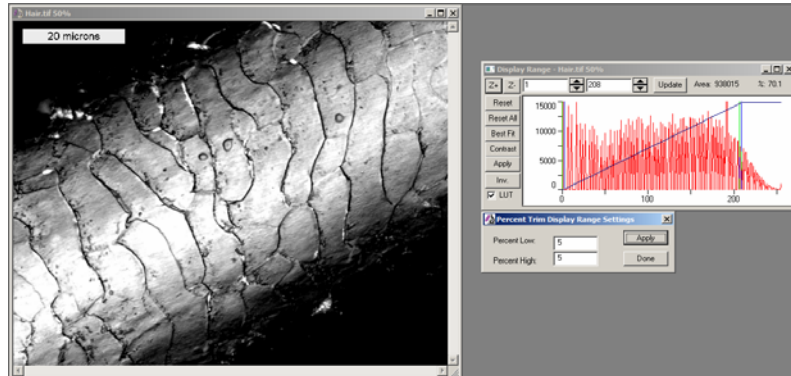
**Note:** Saving an image in lossy format ( \*.jpg, for example) will damage the watermark. If you add a watermark to your image, save the image in a lossless format, such as \*.tif .

The set of demonstration macros available on your *Image-Pro Plus* system may differ.

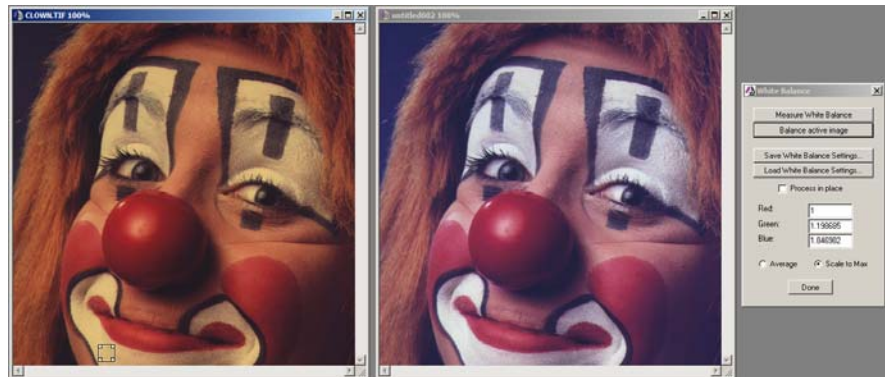
## Extras

The *Extras* are new macros created for *Image-Pro Plus v. 7.0*. A list of available demo macros will appear in the drop-down menu. Click on the name of the demo macro that you wish to run. Some of the available demo macros include:

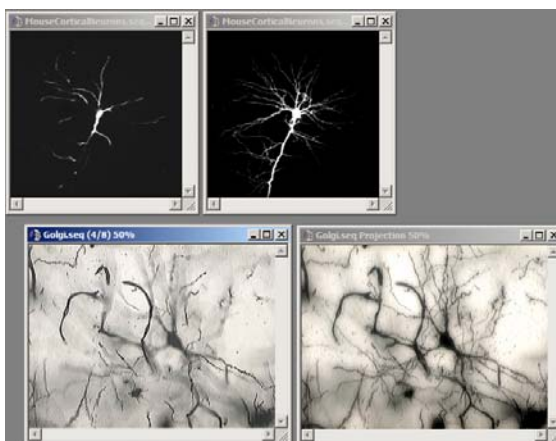
- ◆ **Trim Display Range:** Presents an interface for setting the display range of an image, clipping a set percentage of high and low pixels. For example, clipping the top and bottom 2% from the histogram to better display image intensities.



- ◆ **White Balance:** Measures the RGB values in a known white or gray section of the image, then uses those values to white balance the image.



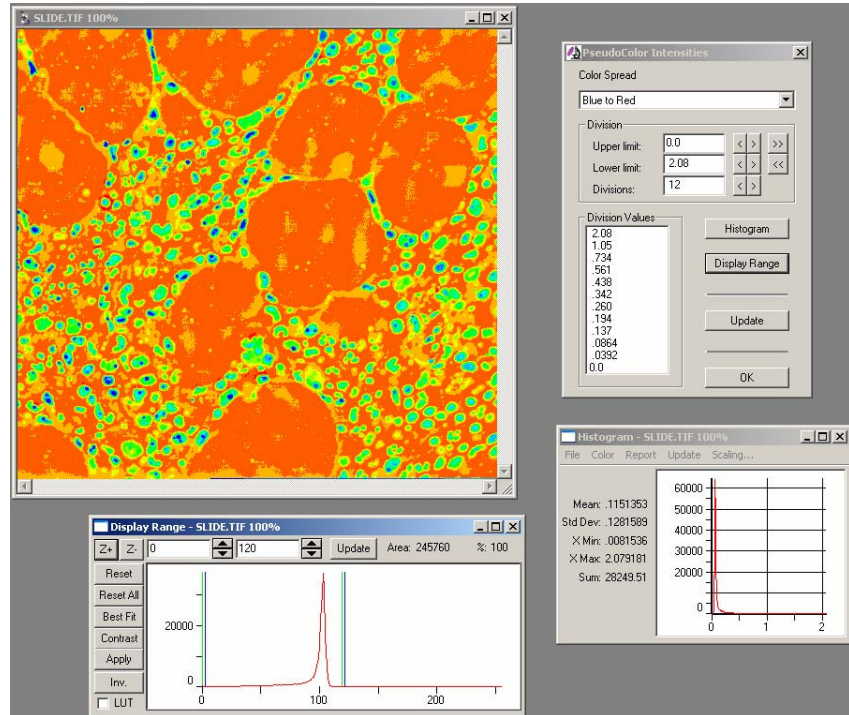
- **Measure White Balance:** Places an AOI on the image, which should be moved over a known white or gray area. When the AOI is selected, the average Red, Green, and Blue values are read, and conversion factors to make them equal are calculated.
  - **Balance Active Image:** Balances the active image by scaling RGB values as computed.
  - **Save/Load White Balance Setting:** Saves or loads the current conversion factors to a text file for later use.
  - **Process in Place:** Converts the current image if checked, or otherwise creates a new image with the applied white balance factors.
  - **Average/Scale to Max:** Either averages the conversion factors to 1.0, or scales up the dimmer components to match the brightest. Averaging retains the image intensity, while scaling to max can better handle cases where one of the channels is saturated.
- ◆ **Quick Contrast:** Scales the current image for visibility. If this is a sequence or set, the central image will be chosen and scaled, as end images often are not very interesting.
- ◆ **Sequence Projection:** Creates a quick projection of the currently active sequence. This is done by averaging all frames in the sequence and then enhancing the contrast of the result.



This brings in data from all planes of a Z-stack or time series at once, although as a qualitative view.

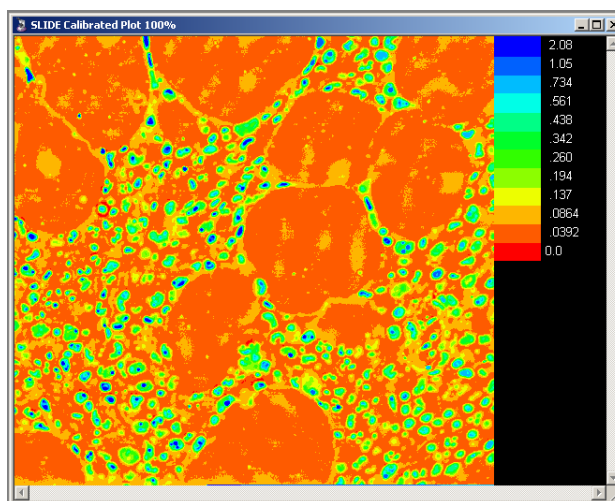
- ◆ **Calibrated Plot:** This macro allows you to apply a pseudo-color with a known number of color steps to an image, and from that to generate a color output with a labeled/calibrated scale bar.

As shown below:



- The **Color Spread** allows you to select from the standard Pseudo-color maps (blue to red, red to blue, various pure colors).
- **Upper and Lower limits**, bounded by the image's display range, show calibrated values for the color mapping. You can map less than the full range, for example, leaving darker items in grayscale and only coloring brighter ones, by adjusting these values.
- **Divisions** set the number of color levels, both in the display and in the eventual scale bar.
- **Division Values** display what the separators are for the various color levels. These are not directly editable.

- **Histogram** brings up the image histogram for information purposes. (Not shown by default!)
- **Display Range** brings up the appropriate dialog, where the image display range can be adjusted. This lets the user select what intensity ranges are valid for the pseudo-color; the dialog will update when you do. (Not shown by default!)
- **Update** forces a refresh of the display. This can be useful if typing values into the settings rather than using the buttons.
- **OK** generates the output image, a color version of the pseudo-colored original with a scale bar appended to the right side of the image, as shown here:



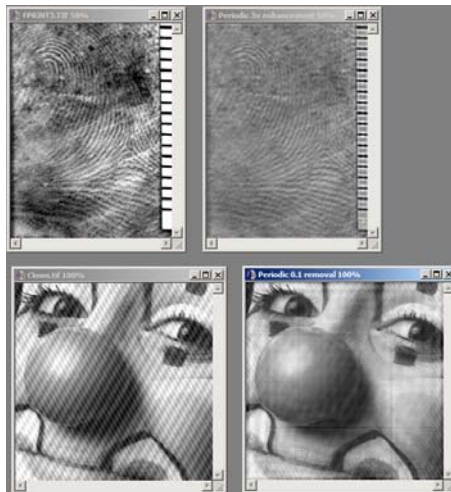
- ◆ **3D Slicer:** This is a very simple 3D volume viewer. It prompts you for a slice thickness, the ratio of the Z distance to the XY pixel distance, and displays an XY, an XZ, and a YZ view of the volume. Clicking in any one view changes the other two to intersecting cut planes.
- ◆ **Sigma Filter:** This is a very specific Median filter. The image is analyzed to identify the local mean and standard deviation, and if individual pixels differ from the mean by more than the stated standard deviations they are replaced by a median filtered pixel. This is an excellent removal of salt/pepper noise in images, while limiting modifications to a few percent of the original pixels. The

*Macro:Extras*

dialog allows choosing the limit of Standard Deviation, the base filter size for variance, mean, and median filters, and displays the percentage of pixels modified.



- ◆ **Remove Periodic Noise:** Using FFT techniques, peaks in the frequency domain are identified, indicating periodic patterns in the image. A scaling factor is requested: a value of 1.0 does nothing, a value  $<1.0$  suppresses the periodic pattern, while a value  $>1.0$  enhances it. This can be used to enhance periodic patterns such as fingerprints, or to remove periodic noise.

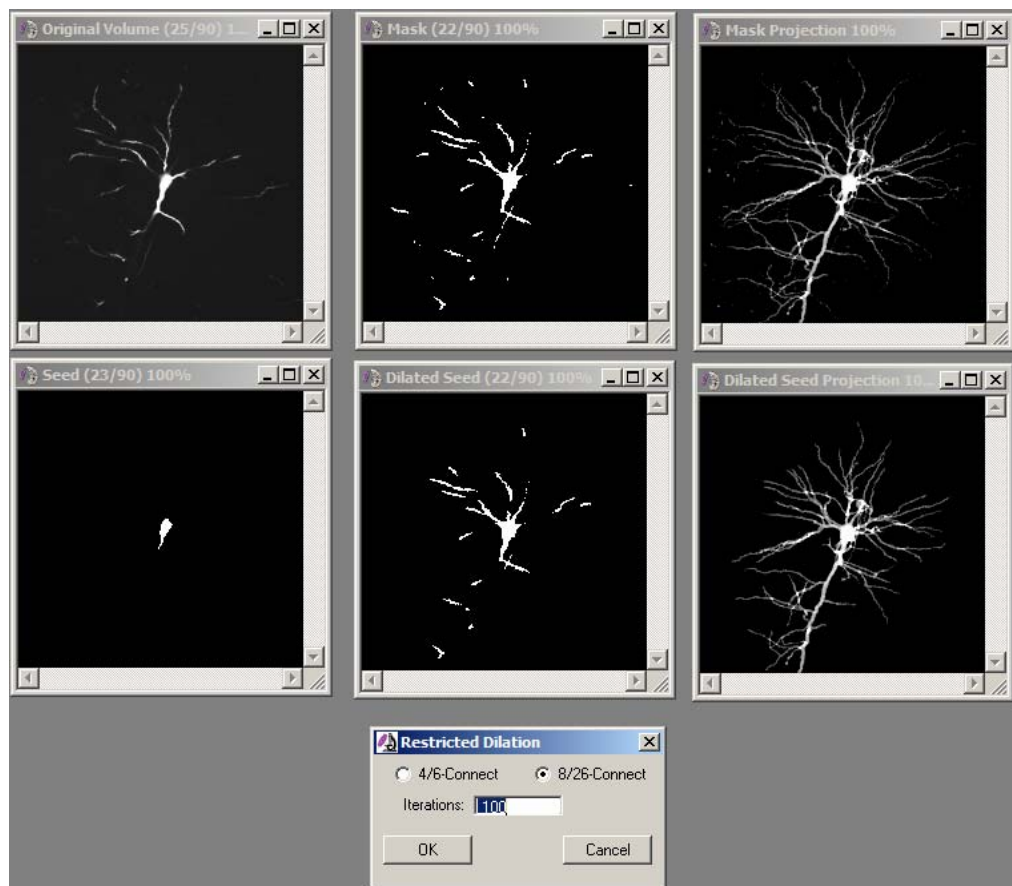


Macro:Extras

- ◆ **3D Restricted Dilation:** Given a mask volume (0-255, or otherwise 'binary' images) and a seed volume, dilate the seed into the mask. This may be used, for example, to seed on a nerve body and dilate along the nerve processes, avoiding external volumes that might otherwise be shown if just using a straight threshold.

This is done by iterating a dilation on the seed followed by ANDing with the mask volume.

Choices are 4/6 connectivity or 8/26 connectivity, reflecting whether the dilation should occur with or without diagonal pixels.





- ◆ **3D Colocalization:** Applies the 2D colocalization functions to a 3D volume and accumulate the results.
- ◆ **Color Deconvolution:** Applies deconvolution to an RGB volume, with estimated emission wavelengths for the three components.
- ◆ **Min/Max Circles:** Calculates and draws the minimum enclosed circle and maximum enclosing circle for counted objects.
- ◆ **Sequence Histogram:** Calculate the accumulated histogram of an entire sequence, as opposed to the single frame histogram tool.
- ◆ **Irregular Duplicate, Irregular Copy/Paste:** This macro provides Copy/Paste and Duplicate functions using AOI's. This allows you to copy an elliptical or irregular AOI from one image to another, or from one image to a new workspace, without carrying along the bounding box of the AOI. The *Image-Pro* default is to copy the rectangular bounding box of an AOI to a new image.
- ◆ **Read/Write ASCII:** Writes an image to disk as a text file with comma, tab, or space delimiters, and read such images from disk. The images are saved as XY arrays of delimited numbers, one row of numbers per row in the image. Note that this is limited to grayscale images. Read images are of type Float, allowing any range of values to be read.

*Macro:Extras*

## **The Window Menu**

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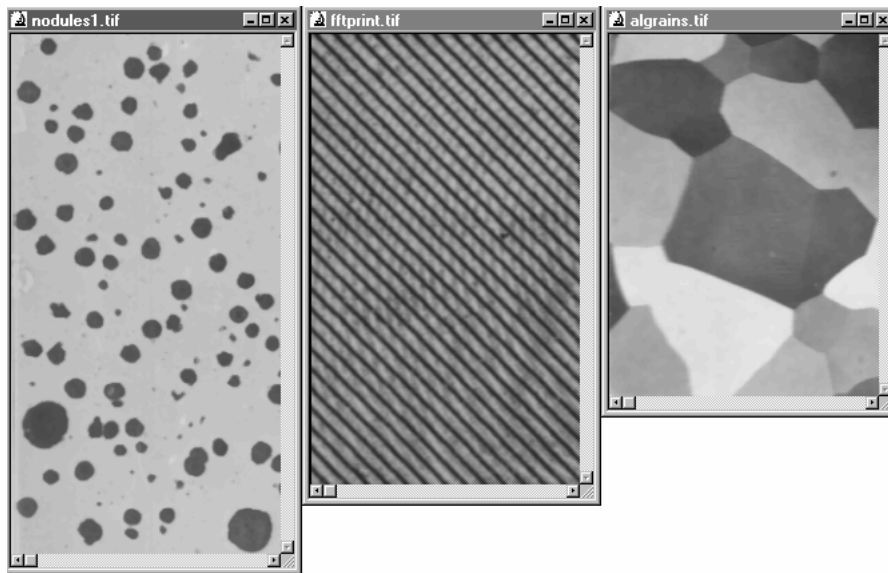
*Window* menu commands control the display of images in *Image-Pro Plus*. You can arrange the display of multiple images in the *Image-Pro Plus* application area, move back and forth from image to image, and hide or show the *Image-Pro Plus* toolbars.

## Tile Images

---

Use the *Tile Images* command to arrange your open image windows so that all windows are visible and roughly equally sized across the screen. *Tile Images* rearranges only the open image windows; command windows (such as *Count/Size*) and data windows (such as histogram windows) retain their positions.

An example of tiled image windows is shown below.

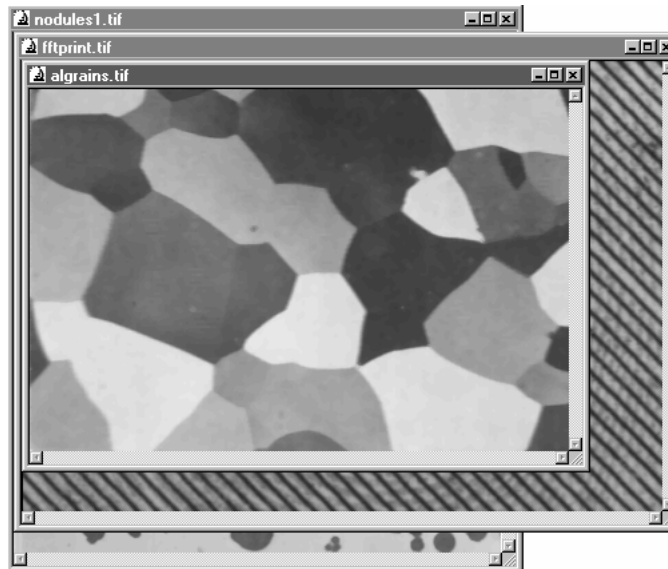


## Cascade Images

---

Use the *Cascade Images* command to arrange your open image windows so that they are roughly equally-sized and layered, leaving only their title bar and left border visible. *Cascade Images* rearranges only the open image windows; command windows (such as *Count/Size*) and data windows (such as histogram windows) will stay in their positions.

An example of cascading image windows is shown below.

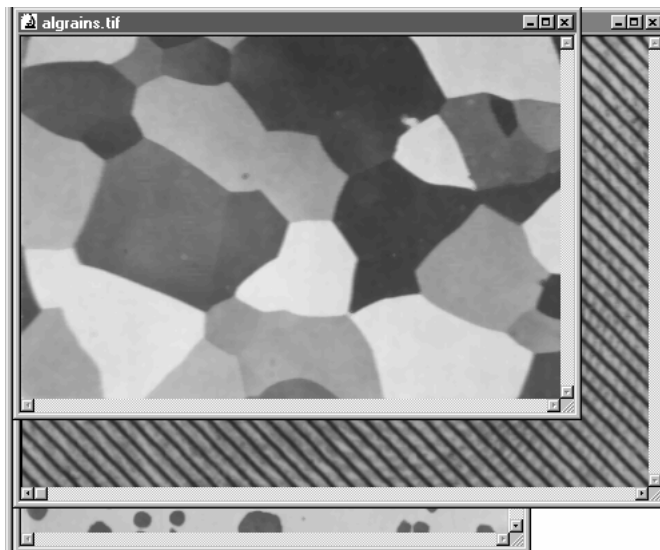


## Overlap Images

---

Use the *Overlap Images* command to arrange your open image windows so that they are stacked one above the other and the active window is on top. *Overlap Images* rearranges only the open image windows; command windows (such as *Count/Size*) and data windows (such as histogram windows) retain their positions.

An example of overlapping image windows is shown below.



## **Next Image**

---

Use the *Next Image* command (<F2>) to select (activate) the next open image window — i.e., the one with the next higher window ID. This is a useful way of specifying an image window in a macro. Because window ID's vary greatly from session to session, it is difficult to ensure that a window ID at playback matches the ID it had when a macro was initially recorded. Specifying an image window relatively using the *Next Image* and *Prev. Image* commands often alleviates this problem.

## **Prev. Image**

---

Use the *Prev. Image* command (<F3>) to select (activate) the previous open image window — i.e., the one with the next lower window ID. This is a useful way of specifying an image window in a macro. Because window ID's vary greatly from session to session, it is difficult to ensure that a window ID at playback matches the ID it had when a macro was initially recorded. Specifying an image window relatively using the *Next Image* and *Prev. Image* commands often alleviates this problem.



## **Close All**

---

Use the *Close All* command to close all open image windows at once.

When an image is closed, its associated histogram and profile windows are closed with it. Many features will also close automatically when the last open image is closed.

Certain windows, such as those containing measurement data sheets and object outlines, will not be closed with the *Close* command from the *File* menu. To close these windows, you must use the *Close* option on their **Control** icon menu.

*Window:Close Tools*

## **Close Tools**

---

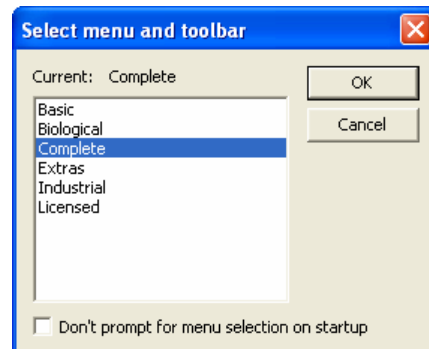
Use the *Close Tools* command to close the *Image-Pro* tools and dialogs. Any open workspaces will remain open.

## Select Menu

---

*Image-Pro Plus* is used by a wide variety of different types of users for a wide variety of purposes. As such, it is a general-purpose “imaging toolbox” with a large number of features, not all of which are useful to any one user. The *Select Menu* feature allows you to select a menu and toolbar configuration that contains a more focused set of features for your particular image processing needs.

When you first start *Image-Pro Plus*, you will see the following dialog:



Use your cursor to highlight one of the choices and then click **OK**.

**Basic:** Displays a minimal set of menus.

**Biological:** Displays a menu set designed for life science and biological applications.

**Complete:** Displays the full set of *Image-Pro* features and functions on all menus.

**Extras:**

**Industrial:** Displays a menu set designed for industrial applications.

**Licensed:** Displays only those items which are currently licensed.

**Cancel:** Clicking this button cancels the selection of a new menu.

**Don't prompt for menu selection on startup:** Checking this box will hide the **Menu Selection** dialog when you start *Image-Pro Plus*. *Image-Pro* will start using the most recently selected menu.

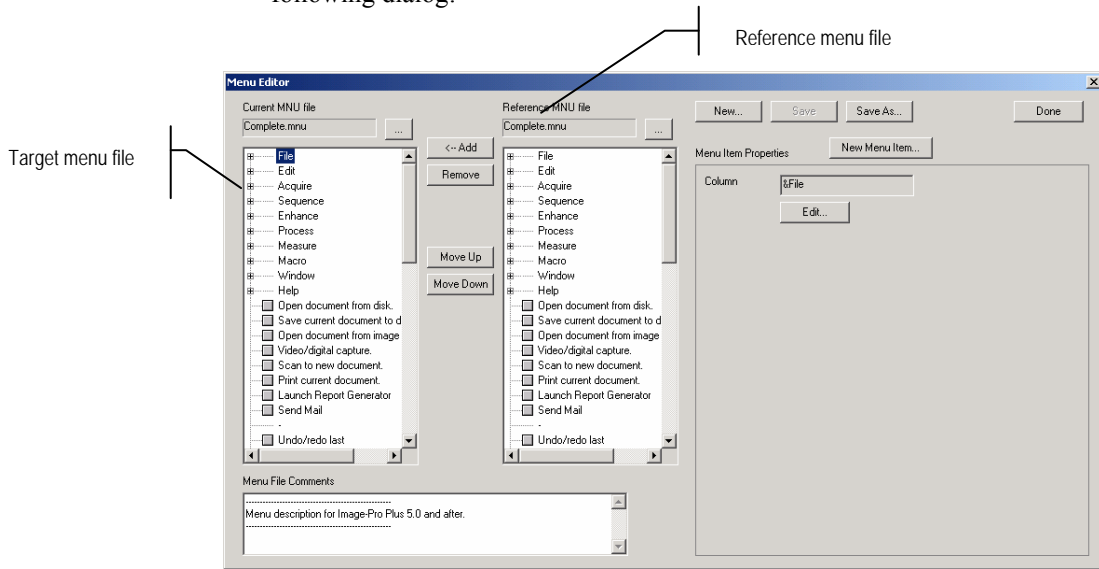
*Window:Select Menu*

Note that this option is also accessible from the *Edit:Preferences* dialog. The **Select Menu and Toolbar** dialog may be displayed at any time using the **Select Menu** item on the *Window* menu.

## Edit Menu

The new *Menu Editor* allows you to customize the *Image-Pro Plus* interface by adding or deleting items on the *Image-Pro Plus* menus.

When you select *Edit Menu* from the *Window* menu, will see the following dialog:



The two columns of tree controls on the left are the target and reference menu files ( \*.mnu ), respectively. The target (left) file is the one being edited, while the reference file is a source for additional menu items of all types.

## General Controls

**Add:** Adds a selected item from the reference file to the target file.

The editor will attempt to place it in a reasonable location in the target, near items it is close to in the reference. If a group or column is selected, all items in it will be added. Note that items not currently in the target are marked with green dots. If an item is already in the target, it will not be added again.

## Window:Edit Menu

**Remove:** Removes a selected item from the target. Groups and columns are removed in their entirety.

**Menu File Comments:** This displays any comments entered at the beginning of the MNU file. These are editable.

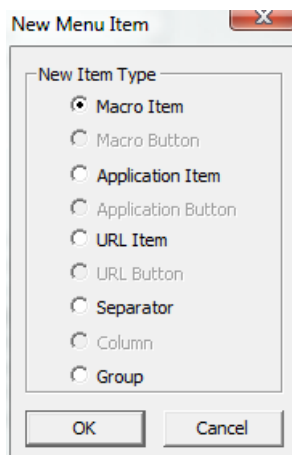
**New:** Creates a new MNU file. You will be prompted for a file name.

“...” – The browse buttons for the target and reference files allow you to select different target and references.

**Save:** Save the current target file.

**Save As:** Save the current target file under a new name.

**New Menu Item:** Create a new menu item in the target. This brings up the following dialog:



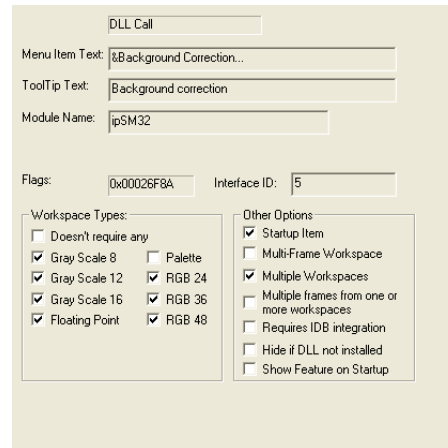
The permitted items are macro menu items, macro buttons, separators, and new columns or groups. In *Image-Pro Plus v. 7.0* you can also launch another application from the IPP menus using the Application Item selection, or launch the web browser to a specified web site using the URL Item choice.

You will have three choices for adding a new application item:

- Browse to the application executable
- Browse to the application shortcut in the Start menu
- Specify the application by the type of documents it handles

The **Menu Item Properties** section of the dialog displays appropriate controls for each type of menu item. For details on menu items and their types, see the SDK documentation.

As an example:



This is the **Sequence | Background Correction...** menu item. The various checkboxes control under what circumstances the menu item will be enabled. The **flags** code represents what the checkbox bitmap adds up to. The **module name** and the **interface ID** indicate which DLL will be called and with which code.

Program items (direct calls to IPP) do not have a module name. Help items (regular and HTML) have the help file names. Columns and Groups have just their names, while separators do not have any properties.

The following is an example of an added *Macro* menu item:

## Window:Edit Menu

Macro

Menu Item Text: Kymograph

ToolTip Text: Generate a kymograph

Module Name: L:\Scripts\kymograph.ipm ...

Method: Caliper\_kymograph

Flags: 0x00046B0A

Workspace Types:

- Doesn't require any
- Gray Scale 8  Palette
- Gray Scale 12  RGB 24
- Gray Scale 16  RGB 36
- Floating Point  RGB 48

Select All Types

Other Options:

- Startup Item
- Multi-Frame Workspace
- Multiple Workspaces
- Multiple frames from one or more workspaces
- Requires IDB integration
- Hide if DLL not installed
- Show Feature on Startup

This was generated by adding a new Macro Menu item, which is inserted after the active selection in the target menu. The browse (“...”) button in this window prompts the user to select a macro file and specific subroutine. After a macro is selected it can be named, given a tool tip, and workspace requirements can be set.

Note that the only editable items in the menu are macro items and macro buttons.

After a menu has been modified, it can be saved and the dialog editor exited. The menu can then be loaded using the **Windows | Select Menu...** item.

---

**Note:** If a *Help* menu is added using the **Menu Editor**, it must be the last menu.

---



## **Hide/Show Toolbar**

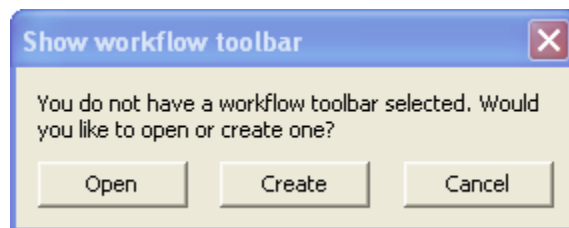
---

Use the *Hide/Show Toolbar* command (<F5>) to enlarge your imaging area by removing the toolbar from your application area, or to restore the toolbar to your application area. The command works as a toggle: if the toolbar is currently being shown, it hides it; if currently hidden, it restores it.

## Hide/Show Workflow Toolbar

---

Use the *Hide/Show Work Flow Toolbar* command to hide or display the work flow toolbar in your application area. The command works as a toggle: if the toolbar is currently being shown, it hides it; if currently hidden, it restores it. If you have not defined a workflow toolbar, you will see a message asking if you want to create one.



**Open:** Displays a workflow toolbar.

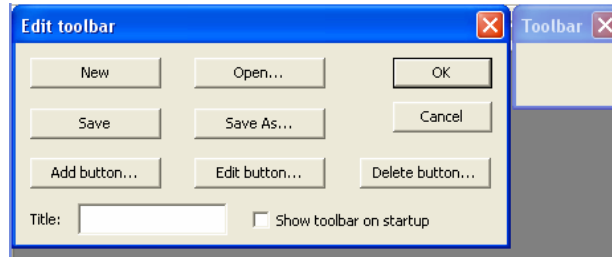
**Create:** Click **Create**, and you will see the **Edit Toolbar** dialog, described on the next page.

**Cancel:** Click **Cancel** to close this dialog.

## Edit Workflow Toolbar

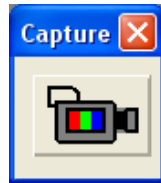
---

Use this feature to create or modify a workflow toolbar in the *Image-Pro* workspace. Selecting **Edit Workflow Toolbar** from the *Window* menu displays the **Edit toolbar** dialog:



This dialog is displayed alongside the toolbar that is being created or edited. The editor will always default to editing the current toolbar, or if one has not been selected, a new, empty toolbar. The right-hand illustration above shows a new toolbar with the default title next to the **Edit Toolbar** dialog.

The next illustration shows a toolbar with the Title changed to *Capture* and a single bitmap button added.



**New:** Click the **New** button to create a new, empty toolbar. You will be prompted whether to save or discard changes if the toolbar has been modified.

**Open:** Clicking the **Open...** button displays a standard common *File: Open* dialog that allows you to select and open a toolbar definition file (.IPT) from the toolbar folder.

**Save:** Clicking the **Save** button will save the edited toolbar back to its original file. If you have created a new toolbar, you will be prompted for a file name, as if you had used the **Save As...** button.

**Save As:** Clicking the **Save As...** button displays a standard **File: Save As** dialog that lets you save the toolbar to a new toolbar definition file.

## Window:Edit Workflow Toolbar

**Add:** Clicking the **Add** button... displays the **Add/Edit button** dialog, (described later in the Basic View and Advanced View sections).

**Edit Button:** The **Edit button**... button is only enabled when a button is selected on the current toolbar, and when clicked, will display the **Add/Edit button** dialog to edit the selected button.

**Delete Button:** The **Delete button**... button is also only enabled when a button is selected, and when clicked will delete the button.

**Title:** The **Title** box allows the user to provide a specific title for the workflow toolbar, such as the *Capture* title illustrated above.

**Show Toolbar on StartUp:** The **Show toolbar on startup** (which has a corresponding item in the **Application** page of the *Edit: Preferences* dialog) will set whether the currently selected toolbar is shown in the *Image-Pro* workspace when the application is launched.

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**Note:** When you are editing a toolbar, you can click on any button to select it. That button will have a blue frame to indicate that it has been selected. You can click and drag on the blue frame to move the button to a new location.

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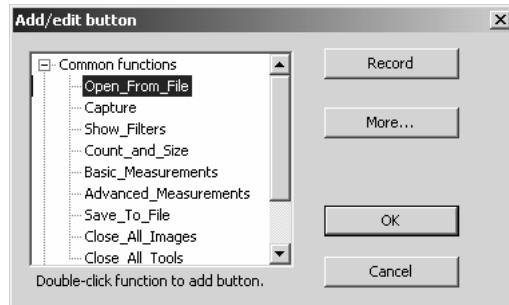
The **Add/Edit button** dialog has two views, a *Basic* view and an *Advanced* view, which are described below. The **Add/Edit button** dialog is always shown in its *Basic* view when used for the first time. In subsequent uses, the *Basic* or *Advanced* view will be displayed according to which was last used.

## Toolbars

The workflow toolbars themselves will have tool tips, which by default will display the function description provided by the toolbar script. The toolbars will remember the last position in which they were displayed.

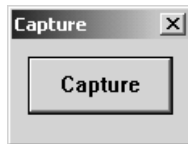
## Basic View

The *Basic View* of the **Add/edit button** dialog is shown here:



The *Basic* view allows you to create workflow toolbars very quickly. A list of functions from the current toolbar script is displayed in the list on the left-hand side of the dialog. The *Basic* view supports only text buttons, with no ability to edit or customize the button text or tool tip.

To add a button to the workflow toolbar, double-click on one of the functions in the list, or highlight a function and click **OK**. Your new button will appear in the workflow toolbar, as shown here:



**Record:** Clicking the **Record** button takes you to the macro recording dialog. You can record a set of *Image-Pro* operations here, exactly as if you were creating a new macro. However, your recorded actions will be saved in the toolbar instead of a macro file.

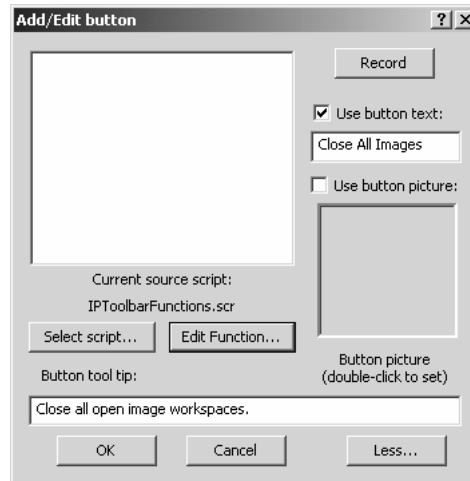
**More:** Clicking this button switches to the *Advanced* view, as shown below.

**OK:** Click this button to save your additions and changes to the workflow toolbar. This button is disabled until you have actually made a toolbar selection.

**Cancel:** Click **Cancel** to discard your changes.

## Advanced View

The *Advanced View* of the **Add/edit button** dialog is illustrated below:



The *Advanced* view of the dialog allows you to create more complex workflow toolbars. The *Advanced* view supports graphical buttons, and all of the aspects of the button may be edited.

The list of functions is similar to that of the *Basic* view, except that double-clicking a function is not supported. Clicking the function selects that function to be assigned to the button. This will also default the button tool tip.

**Record:** Clicking the **Record** button takes you to the macro recording dialog. You can record a set of *Image-Pro* operations here, exactly as if you were creating a new macro. However, your recorded actions will be saved in the toolbar instead of a macro file.

**Use button text:** Check this box to use the default text on the new button in your workflow toolbar.

**Use button picture:** Check this box to use the image bitmap on the new button in your workflow toolbar.

**Button picture:** Double-click here to display a standard *File:Open* dialog to select the desired BMP file. Graphical buttons can be created from any BMP file of a suitable size, e.g. not larger than 300 pixels wide by 100 pixels tall. When a picture is selected, the **Use button picture** option is selected automatically. Currently, the button can use text or picture, but not both simultaneously.

**Select script:** Click this button to change the script that is used to fill the list of functions. Note that the script can be changed as often as desired, with the resulting toolbar combining functions from many source scripts.

**Edit Function:** Clicking this button creates a temporary script containing the macro function that is currently assigned to the button. You can edit the script and save it to update the function assigned to the button, or just close the script if you are inspecting it. The button editor is disabled until you do one or the other. You can also use **Save As** in the Macro Editor to save a copy of the script; this could be used to document the function assigned to the toolbar button, or to make the function available as a script so that it can be assigned to other buttons in other toolbars. See “*Tips for Advanced Toolbar Functions*” below, for more information.

**Less:** Clicking the **Less...** button switches to the *Basic* view.

**OK:** Click this button to save your additions and changes to the workflow toolbar. The **OK** button will be disabled until the following prerequisites are met:

- A function is selected in the list AND
- The user has chosen to **Use button text** and there is button text provided, OR
- The user has chosen to **Use button picture** and a picture file has been selected.

**Cancel:** Click **Cancel** to discard your changes.

### **Tips for Advanced Toolbar Functions**

The workflow toolbar is generally oriented towards assigning one self-contained function to the button, in a macro script that is not directly editable in the **Macro Editor**. However, there are a number of techniques that can be used to assign more complex functionality to the toolbar buttons.

Common global variables and functions that you may want to use from your toolbar functions can be incorporated in a separate BAS file and saved in the BAS folder under the *Image-Pro Plus* application folder (typically C:\IPWIN70\BAS). Any global variables and functions can then be called from the self-contained function for the toolbar button.

### *Window:Edit Workflow Toolbar*

It is also possible to incorporate more complex coding into the toolbar's macro script. On the **Edit toolbar** dialog, click **Add** to add a new button. If you do not see the *Advanced View* described above, click **More....** Start by defining an empty function with the function name that you will want to use once the coding is complete. The easiest way to do this is to click **Record** and, in the **Record Macro** dialog, enter the function name. In the **Recording** dialog, click **Stop Recording**. Now click **Edit Function**.

The **Macro Editor** will open, editing a temporary script file that contains instructions for editing the toolbar function. If you already have the desired functionality prototyped in another macro script, you can load that script in the **Macro Editor** and copy and paste the variables and functions into the bottom of the temporary script. Otherwise you can add constants, variables, functions, subroutines and code within the toolbar subroutine manually within the editor.

The two keys to accomplishing this successfully are to make sure that you do not change the toolbar function name on the main button subroutine, and to make sure that all constants, variables and functions are inserted below the dividing line indicated in the temporary script. Note that any global constants and variables will not only be available to the functions and subroutines that you are adding for the current button, but will also be available to any toolbar buttons below the current button. So to define a variable global to all the workflow toolbar buttons, define it by editing the first button in the toolbar.



## **Sync Pan and Zoom**

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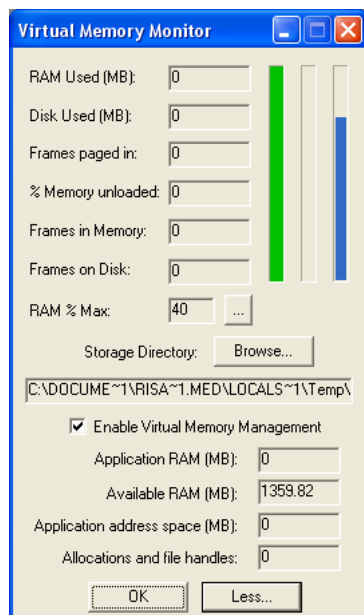
Use this command to synchronize the zoom, scroll, and pan movements in different open workspaces. When you select this feature, all the open workspaces will be set to the same size, zoom factor, and pan/scroll position as the active workspace. Any changes in the zoom, pan, or scroll of the active image will be mimicked in the other open workspaces.

Newly-created workspaces (regardless of their origin) will not automatically take on the characteristics (size, zoom, pan, or scroll) of the other, already synchronized workspaces; however, panning, zooming, scrolling or resizing any work space will affect all workspaces, including the newly-created one.

## Memory Monitor

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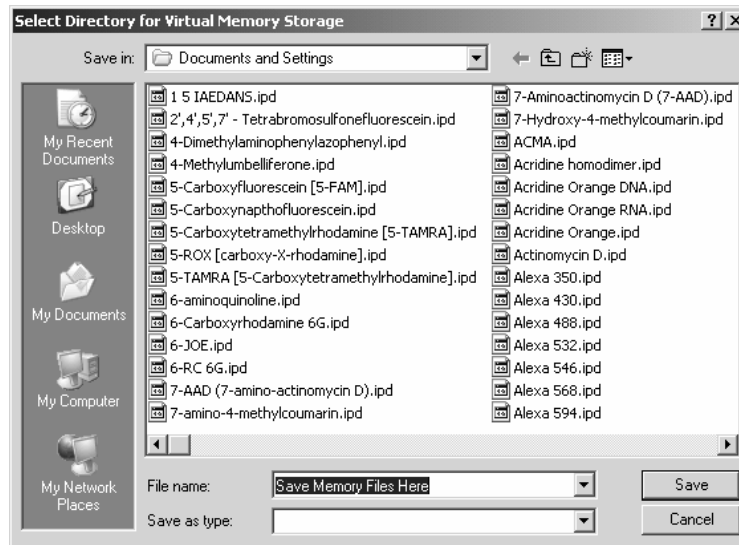
The *Memory Monitor* feature in *Image-Pro Plus 7.0* supports custom virtual memory management, allowing the application to exceed the 2GB process size. When you select **Memory Monitor** from the **Window** menu, you will see the following dialog:



The first bar (with the green background) indicates how much of the allocated RAM is being used. The second bar indicates the percentage of memory currently residing on disk. The third bar indicates how much of the disk used for virtual memory storage is currently free.

**“... button”** : The **“... button”** next to the **RAM % Max** allows you to change the RAM percentage, up to 100%. Note that the actual RAM used is limited to 1GB, and that allocating all of the available RAM will cause thrashing between the IPP and Windows virtual memory mechanisms. The initial default is 40%.

**Browse:** The **Storage Directory** indicates where the virtual memory files are to be stored. Clicking the **Browse...** button allows you to change this. The default location is the *Documents and Settings* directory, as shown here:



In the above dialog, navigate to the desired folder of the desired drive, and select any file in that folder. Note that the file you select will not be opened or used by virtual memory management in any way - this is just a way of selecting a folder for the temporary files.

**Enable Virtual Memory Management :** Checking this box lets you disable the virtual memory module. Note that currently unloaded frames will remain unloaded until they are accessed.

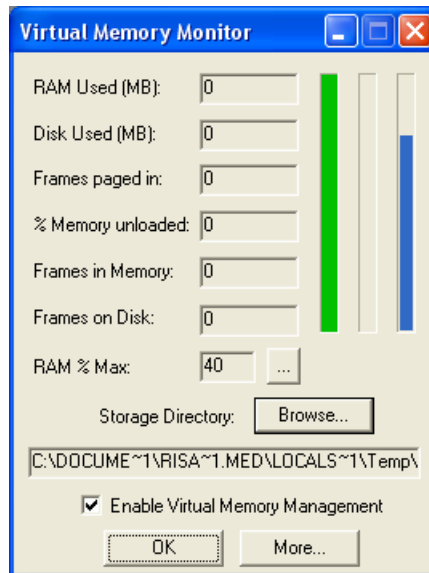
---

Note: Disabling virtual memory management can lead to memory errors managing large images, sequences or sets, and will greatly limit the ability of *Image-Pro* to display your data.

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*Window:Memory Monitor*

**Less:** Clicking this button displays a reduced form of the **Memory Monitor** dialog:



**OK:** Click **OK** to save your virtual memory settings.

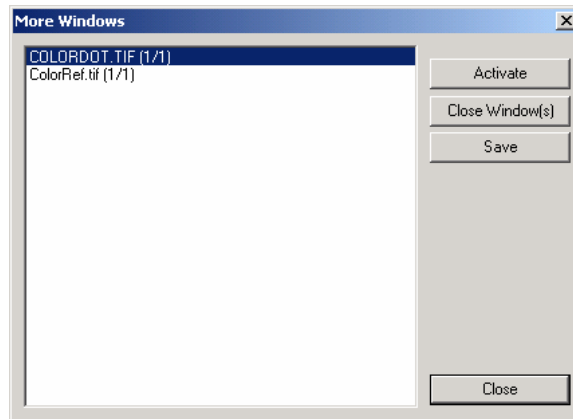
## Workspace List

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The workspace list appears at the bottom of the *Window* menu. It displays a list of the workspaces that you currently have open, with a checkmark next to the active one.

If there is at least one workspace open, a separator line is added to the *Window* menu, as well as a list up to 10 of the most-recently used workspaces, and finally the **Windows...** item.

**Windows:** The **Windows** item brings up a **More Windows** dialog (so named because it will list ALL open workspaces, regardless of how many there are), as shown here:



In this dialog, you can select one or more workspaces in the list and close or save them.

**Activate:** You can double-click a workspace, or select it (only one) and click the **Activate** button to activate a particular workspace (i.e. to see which one it is).

**Close Window(s):** Select one or more workspaces, and click this button to close them.

**Save:** Click this button to save the active workspace(s).

**Close:** Click this button to close the dialog and return to the main *Image-Pro* workspace.

*Window:Workspace List*

## The Help Menu

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The *Help* menu contains the commands for accessing the *Image-Pro Plus* and *Image-Pro* plug-in online help systems.

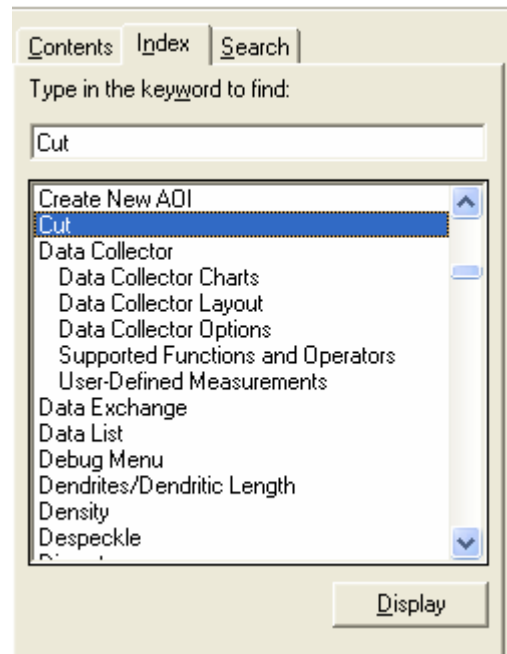


Selecting the name of a product or feature will bring you to the correct topic in the *Image-Pro Plus* online help.

## Index

---

Use the *Index* command to access the table of contents for *Image-Pro's* online help facility. Clicking on the **Index** tab displays the following dialog box:



To display help information on a specific topic:

1. Type in the first few letters of the word you're looking for.
2. Click on the index entry that most nearly corresponds to that topic.

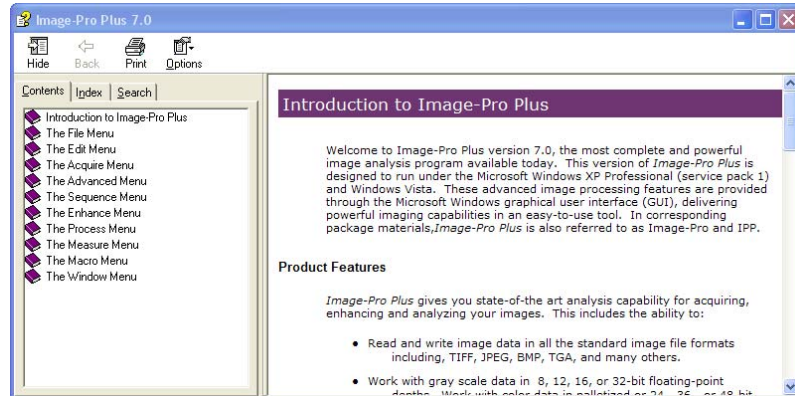
The **Help** window will pop up with the requested information.



## Image-Pro Plus

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Use the *Image-Pro Plus* command to access the table of contents for *Image-Pro's* online help facility. To access one of these topics, simply click on the topic of your choice.



Additional help for your other licensed features is available from the main help menu also.

## Macro Language

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Use the *Macro Language* command to access the table of contents for *Image-Pro's Auto-Pro* online help facility. The following topics are covered:

- ◆ **Functions:** this section describes each *Auto-Pro* function (e.g., IpLutShow, IpFltHiPass) and the options associated with each. *Auto-Pro Functions* contains all the information in *Section 2* of the *Auto-Pro Reference Manual*.
- ◆ **Data Types:** this section describes each *Auto-Pro* data type, as listed in *Appendix D* of the *Auto-Pro Reference Manual*.
- ◆ **Variable Names:** this section describes the rules for designating a variable name in *Auto-Pro*, as listed in the *Auto-Pro Reference Manual*.
- ◆ **Reserved Word List:** this sections lists the keywords reserved for *Auto-Pro* use, as listed in *Appendix B* of the *Auto-Pro Reference Manual*.
- ◆ **ANSI Character Table:** this sections lists the ANSI Character Table, as listed in *Appendix C* of the *Auto-Pro Reference Manual*.
- ◆ **Shortcut Key Assignments:** this sections shows the shortcut key combinations supported by *Auto-Pro*, as listed in *Appendix E* of the *Auto-Pro Reference Manual*.

To access one of these topics, simply click on the topic of your choice.

## Online Tutorials

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Clicking this menu item takes you directly to the *Image-Pro Plus* tutorials page of the *Media Cybernetics* web site. Here you can learn more about the features and functions of your *Image-Pro Plus* software.

## Technical Support

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The *Technical Support* menu contains links to more information about *Image-Pro Plus* and Media Cybernetics. From this menu, you can access:

### Technical Support On Line

Please refer to the Technical Support page, (accessible from the Media Cybernetics' home page on the World Wide Web at <http://support.mediacy.com> )where valuable technical information is available to you. This information includes:

- Answers to frequently asked questions
- A collection of technical notes, including 'How To...' tips, sample macros, and more.
- Technical information on built-in and third-party video acquisition hardware and software.
- A Technical Support E-mail form

You may obtain additional useful information via e-mail by joining the Image-Pro Users Group. This service facilitates information exchange among Image-Pro users. You will be able to learn how others use Image-Pro, and possibly find solutions to your needs. To join the Image-Pro Plus Users Group, visit our web page at <http://support.mediacy.com>.

## **Image-Pro on the Web**

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Media Cybernetics now has a presence on *YouTube*:

<http://www.youtube.com/user/MediaCybernetics>

We have a new Facebook Group for Image-Pro Plus Users. We plan to add Events, Recent News & Videos to this group. Join us! If you don't have a Facebook account, you may not be able to see this group.

Image-Pro Plus Users' Facebook Group:

<http://www.facebook.com/home.php?#/group.php?gid=44536513482>

## **Contacting Technical Support**

Before you contact Media Cybernetics' Technical Support for further assistance, please be prepared to provide the following information:

- Your registration information: the product serial number (\*), your name, phone number, organization name, mailing address, and e-mail address.
- The software version number (\*) and system information.
- A description of the problem, and any relevant information regarding prior technical support assistance.

(\*) These numbers are displayed on the 'About' screen (open from the Help menu)

You may contact Media Cybernetics' Technical Support in either one of the following ways:

- To speak with a technical support representative, please call 301-495-3305, and select the option for Technical Support. The Technical Support line operates on regular business days, between 9:00 a.m. and 5:00 p.m., EST.
- To use e-mail, you may fill out the e-mail form available from the Technical Support page on the World Wide Web (see above); or e-mail your message directly to: [techsupport@mediacy.com](mailto:techsupport@mediacy.com). Please remember to include your IPE serial number in your message.
- To send a fax, address your fax to Image-Pro Plus Technical Support, and send it to 301-495-5964. Please remember to include your IPE serial number in your message.

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**Note:** Technical support is available only to registered users, during the basic or extended warranty period. Please remember to return your registration card.

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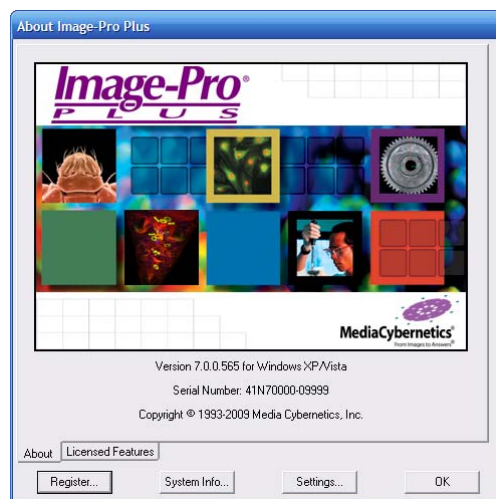
## About Image-Pro Plus

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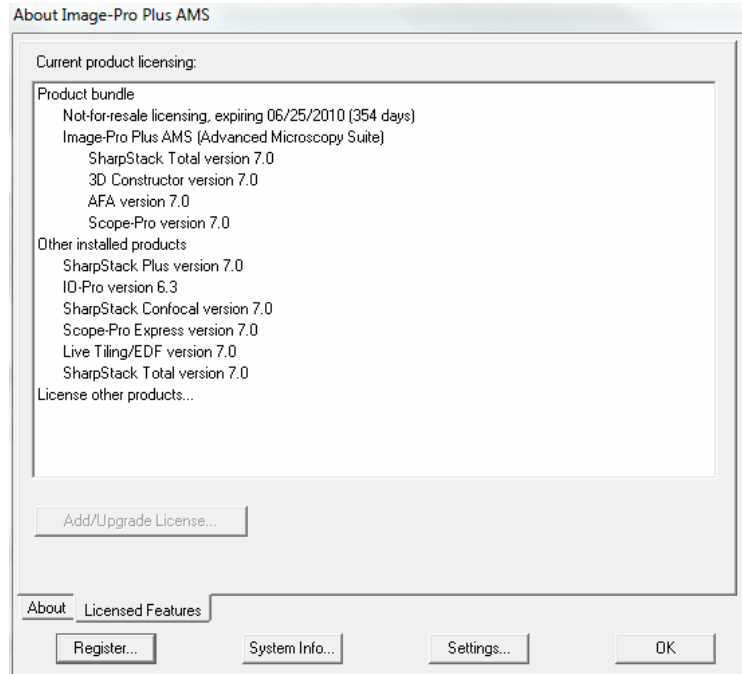
Use the *About Image-Pro Plus...* command to access *Image-Pro's* **About** information box.

**About:** The **About** tab page contains important version and release date information, as well as pertinent copyright information. You can register your product, get system information, and verify your settings from the buttons on the **About** dialog.

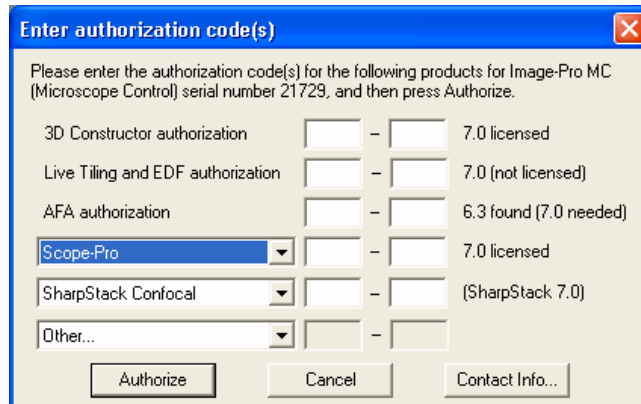
If you need to contact our *Technical Support Center*, you will need to provide the version information supplied by this box.



**Licensed Features:** This page displays a list of your current licensed *Media Cybernetics* products:



If you have upgrade codes to upgrade your *Image-Pro* to a new bundle, the possible bundles will be listed on this dialog. You can select the bundle and click **Add/Upgrade License**. If you have upgrade codes to license optional features, you can select **License other products...**, and click **Add/Upgrade License**. Either procedure will display the Enter authorization code(s) dialog illustrated below:



## Help>About Image-Pro Plus

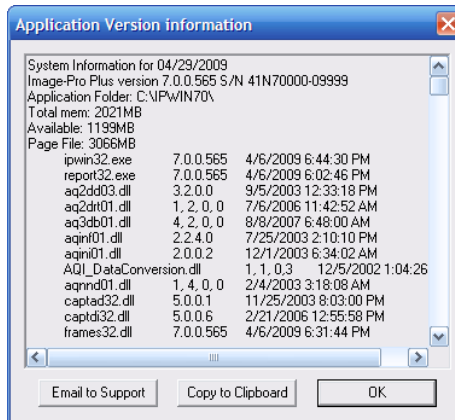
You will need to enter the codes supplied by *Media Cybernetics Technical Support* and then click the **Authorize** button to use the additional products.

**Register:** Click this button to register your copy of *Image-Pro Plus*. You will see the **Registration** dialog:



We strongly recommend that you register your copy of *Image-Pro Plus* so that you can receive all the benefits to which users of Media Cybernetics products are entitled. Please see the *Registration* section of your *Start-Up Guide* for more information.

**System Info:** Click this button to see the your system information. If you call for *Technical Support*, you will need this information. You will see the **Application Version** Information dialog:

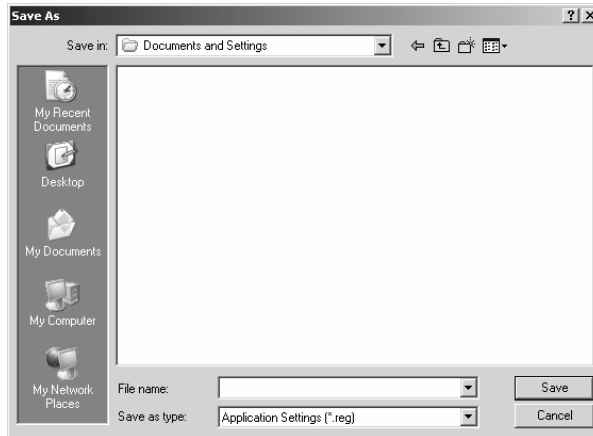


**Email to Support:** Click this button to bring up the **Send Mail** dialog. You can automatically email your system information to *Media Cybernetics Technical Support*.

**Copy to Clipboard:** Click this button to send a copy of your system information to the **Windows Clipboard**.



**Settings:** Click this button to save your current *Image-Pro* settings. You will see the **File: Save** dialog, enabling you to save your current *Image-Pro* settings to a file that can be sent to Technical Support.



*Help:About Image-Pro Plus*

## Appendix A - Glossary

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<b>Active Window</b>	The image window that is currently selected. If only one image window is open, it will always be the active window. If more than one image window is open, the active window is the frontmost window. Its title bar is highlighted.
<b>Angle dot screen</b>	A technique for representing gray tones through dots of varying sizes placed at regular intervals on a grid. The distance between the dots on the grid is the resolution in dots per inch (DPI). The positions of the dots on the grid fall on two sets of imaginary lines at 90 degrees to one another. The angle of these imaginary lines to the horizontal is 45 degrees, which is the conventional angle in printing processes using the screen technique.
<b>AOI</b>	“Area of Interest.” A contiguous subset of pixels defined within an image, which may be arranged in any polygonal shape. They are used to isolate the subset from the rest of the image.
<b>BCG</b>	Brightness, Contrast, Gamma
<b>Bilevel</b>	The simplest way to represent an image in digital terms, with each pixel represented by a single bit that is either on or off. Also referred to as “Black and White,” “Line art,” or “Halftone.”
<b>Bilinear Scaling</b>	A scaling technique that interpolates a pixel's rescaled value based upon its relationship to surrounding pixels. This technique creates smoother scaled output than traditional “factor” or “pixel replication” scaling methods.
<b>Bit</b>	The smallest unit of information recognized by a computer. A pixel is represented by one or more computer bits. The number of bits per pixel directly determines the number of colors or gray shades that can be represented. See <i>Bit Depth</i> .
<b>Bit Depth</b>	The number of pixels used to represent one pixel value. Also referred to as <i>Pixel Depth</i> and <i>bits-per-pixel (BPP)</i> .
<b>Bitmap</b>	A two-dimensional array used to represent an image. Each cell in the array contains a value that describes a sample of the image in terms of its color.  Contrast this with Vector graphics, where an image is created by describing its elements as geometric objects.

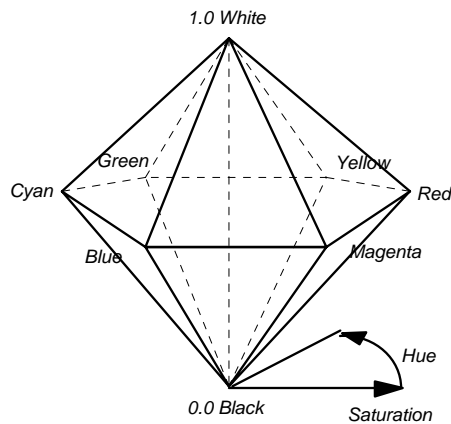
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<b>BMP</b>	Image bit-mapped format used by Windows and OS/2.
<b>BPP</b>	Bits Per Pixel. Describes the “depth” of an image. <i>Bilevel</i> images are only 1 BPP; <i>True Color</i> images are 24 BPP.
<b>Brightness</b>	The amount of white in an image. The brighter the image, the more white it contains, and, as brightness is increased, each color in the image is shifted more toward white.
<b>Calibration, Device</b>	<p>The process of setting Color Map attributes to compensate for the variations in quality of images due to differences in printers, scanners, and the materials associated with them.</p> <p>You can calibrate your printer(s) or scanner(s), save the calibration(s), then load them each time you use a different scanner or printer, or as your printer toner or ribbon age.</p>
<b>Calibration, Intensity</b>	The process of redefining the intensity scale associated with an image, to express intensity values in another unit of measure. For example, intensity values can be calibrated to reflect density, or temperature.
<b>Calibration, Spatial</b>	The process of redefining the spatial scale in an image to express distance between pixels in a unit of measure other than pixels. For example, you might calibrate the spatial scale to measure microns or kilometers.
<b>CCITT</b>	Consultative Committee for International Telegraph and Telephone (a committee of the International Standards Organization). The committee developed the CCITT compression methods for FAX data, which are used to compress <i>Bilevel</i> image files.
<b>Channel</b>	The luminance and color components associated with a color image. An RGB image contains 4 channels: Red, Green, Blue and Luminance. The Luminance channel being intensity irrespective of color.
<b>Chunk RGB Chunk</b>	The 3-byte (24-bit) group that represents the red, green and blue values for a pixel. A chunky image data line is made up of consecutive chunks, with no gaps or fillers between chunks.
<b>Class</b>	See <i>Image Class</i> .
<b>CMY</b>	Color model in which color is expressed in varying amounts of Cyan, Magenta and Yellow (CMY). CMY is the standard color model in printing.

<b>Color Correction</b>	Making changes to brightness, contrast, color, highlight, shadows in an image to compensate for perceived deficiencies.
<b>Color Model</b>	A mathematical model describing color. There are several <i>Color Models</i> are used today. <i>Image-Pro</i> supports the popular models. See the <i>Color Model</i> discussion in <i>Section 1</i> , and <i>CMY</i> , <i>HSI</i> , <i>HSV</i> , <i>RGB</i> , and <i>YIQ</i> entries in this Glossary.
<b>Color Plane</b>	See <i>Plane</i> .
<b>Color Reduction</b>	Optimization or reduction of the number of colors in a color image. Accomplished using the <i>Convert To</i> menu's <i>Palette</i> command. Often performed to reduce the amount of space require to store a color image.
<b>Compression</b>	Mathematical technique that allows an image to be stored with less memory. Redundancies in the internal representation of the image are identified and given a code; the data in the redundancies is then replaced by the code.
<b>Contrast</b>	The sharpness of an image. The higher the contrast in an image the larger the difference between white and black in the image, or (in color images) the more spread out the color range is. As contrast is increased, all the colors or gray shades in the image spread apart.
<b>DLL</b>	Windows Dynamic Link Library
<b>DPI</b>	Dots Per Inch. A unit of measure for resolution. DPI can refer to the resolution of the displayed image, or the output page. The output DPI does not necessarily map one-to-one to the image DPI. The number of dots per inch in a document determines its visual quality. A higher resolution document can appear smooth, realistic, and finely defined. A lower resolution document can appear blotchy, jagged, and ragged. (See also <i>Spatial Resolution</i> )
<b>Drag</b>	To press and hold down a button on your pointing device while you move the cursor on the screen. You “drag” something when you want to move it to a new location on the screen, or when you want to resize it. For example, you drag a document window to reposition or resize it.
<b>Error Diffusion</b>	A halftoning technique where the difference between the image pixel value, and the targeted halftoned pixel is added to the value of the next image pixel.

*Appendix A - Glossary*

- Factor Scaling** A scaling technique that uses a zoom factor. The zoom factor allows scaling to be accomplished quickly.
- File Format** The method with which an image is stored to disk, based on image class, compression type (if applicable), and halftone pattern (for bilevel images). TIFF, CUT, GIF, BMP and PCX are examples of file formats.
- Gamma** A nonlinear contrast correction factor which is used to adjust the contrast in dark or light areas of the image.
- Gray Level** In gray scale images, the brightness value assigned to a pixel. In an 8-bit image, this value ranges from 0 to 255 (from black, through shades of gray, to white).
- Halftoning** A method of simulating a gray scale image using patterns of very small black and white dots.
- Horizontal Differencing** Technique used for enhancing LZW compression. Horizontal differencing takes advantage of the fact that, in many images, the difference between adjacent pixels is frequently 0 or a small number. Instead of compressing the actual value, LZW then compresses the differencing values of the adjacent pixels.
- HSI** Color model in which color is expressed in varying amounts of Hue, Saturation and Intensity. The color space for the HSI model is normally represented by a double hexcone, with Intensity forming the primary axis as shown in the drawing below.



<b>HSV</b>	Color model in which color is expressed in varying amounts of Hue, Saturation and Value. HSV differs from HSI in the way Intensity is derived. See <i>HSI</i> above.
<b>Huffman</b>	See <i>Modified Huffman</i>
<b>Icon</b>	A symbol that represents a tool or item. For example, the symbols on the Ribbon are icons of the available tools.
<b>Image</b>	The “document” that <i>Image-Pro</i> works upon. Also may refer to the original artwork, graphics, or photo that you scan or import.
<b>Image Class</b>	Image category, determined by <b>bit depth</b> . Image classes supported by <i>Image-Pro</i> include <i>Bilevel</i> (1-bit), <i>Palette</i> (8-bit), <i>Gray Scale</i> (8-bit), <i>Gray Scale 12</i> (12-bit) and <i>True Color</i> (24-bit).
<b>Image Conversion</b>	The process of changing an image from one class and/or format and/or compression type to another.
<b>IMG</b>	Image file format developed by Digital Research, and used extensively by Ventura Publisher.
<b>Lossless</b>	Type of <b>compression</b> that allows full recovery of the original image and is fully reversible.
<b>Lossy</b>	Type of <b>compression</b> that degenerates an image during compression and is not fully reversible.
<b>Luminance</b>	Intensity irrespective of color. Usually derived from the mean of the RGB color values.
<b>LUT Lookup Table</b>	A table in which BCG adjustments are maintained, and through which the image bitmap is rendered. This allows you to experiment with the BCG qualities without affecting your actual image data. Also lets you improve the image for viewing purposes without affecting the integrity of your data for analytical purposes.
<b>LZW Lempel-Zif Encoding</b>	Lempel-Zif Encoding. A compression method which utilizes string encoding (assigning codes to groups of repeated data).

## Appendix A - Glossary

<b>MColor</b>	A method of converting a 24-bit color image to an 8-bit palette image. The MColor technique, developed by Media Cybernetics, uses a fixed 256 color palette, representing a range of values across the color spectrum. During MColoring, the 24 bit image is reduced to 8 bits using a dithering technique to convert the pixel values into MColor palette indices.
<b>Modified Huffman</b>	Compression type which is a variation on <b>RLE</b> . Modified Huffman uses a standard table of patterns that represent typical patterns in an image. These tables are usually tuned to the type of data they are used to compress.
<b>MSP</b>	Image file format developed by Microsoft Paint.
<b>Palette</b>	For a <i>Palette</i> class image, the 256 element array used to specify the RGB values associated with that image. Each pixel in the image contains an index to the palette, which contains the RGB value for that pixel.
<b>PCX</b>	Image file format developed by ZSoft Inc.
<b>Pixel</b>	Picture element. The smallest element in a digitized image.
<b>Pixel Depth</b>	The number of pixels used to represent one pixel value. Also referred to as <i>Bit Depth</i> and <i>bits-per-pixel (BPP)</i> .
<b>Plane Color Plane</b>	An arrangement of RGB image data such that all Red values are located in one array, all Green values in a second array, and all Blue values in a third. Contrast with <i>Chunk</i> above.
<b>Polyline</b>	A series of lines connecting pairs of points (vertices).
<b>Pseudo-color</b>	The process of assigning RGB color values to replace gray levels for display purposes. Usually used to highlight certain intensity ranges in a <i>Gray Scale</i> image, with color.
<b>Resolution</b>	See <i>Spatial Resolution</i>
<b>RGB</b>	Color model in which color is expressed in varying amounts of Red, Green and Blue (RGB). RGB is used by most digital imaging devices.
<b>RGB Chunk</b>	See <i>Chunk</i>
<b>Ribbon</b>	The graphic bar along the left side of some application windows that displays icon controls for some procedures..



<b>RLE</b>	Run Length Encoding. A compression technique that encodes data “runs” (lengths of identical information).
<b>Run Length Encoding</b>	
<b>Sharpen</b>	A filtering process that intensifies edges and details in an image by increasing the difference between gray values of neighboring pixels. Sharpen produces a crisper image.
<b>Smooth</b>	Bilinear scaling option that may be used during printing and resizing. This is a form of resizing that takes into account adjacent pixel values to generate in-between values for the resized image, so that the new image does not look as if pixels were arbitrarily removed or added. When this option is not selected, scaling is done using pixel replication (bigger) and decimation (smaller).
<b>Spatial Resolution</b>	Image attribute defined by a two-dimensional (width and height) grid of pixels.
<b>Threshold</b>	A value used to separate gray values into 2 values. Gray values less than the threshold will be set to 0. Gray values greater or equal to the threshold will be set to 255. Typically, the threshold is set to 128.
<b>TIFF</b>	Tagged Image File Format. A general-purpose file format recognized as a standard for image files.
<b>Tile</b>	One page panel in a multi-page poster-size print.
<b>TGA</b>	TARGA image file format developed by TrueVision Inc.
<b>True Color</b>	24-bit color. Image class in which each pixel contains 256 shades each of red, green, and blue, therefore the image can contain up to 16.7 million colors.
<b>YIQ</b>	A component analog color model wherein “Y” represents luminance value, and “I” and “Q” represent the color components. YIQ is the standard in broadcast television.
<b>VRI</b>	Virtual Raster Image. The memory-based copy of an image. It contains image data and the image attributes. (A raster image is formed by patterns of light and dark pixels in a rectangular array.)

*Appendix A - Glossary*

## **Appendix B - File Format Specifications**

---

### **Supported File Formats**

*Image-Pro Plus* supports the following file formats. A brief description of each format will list the name used by *Image-Pro* in the *File:Open* and *File:Save As* dialogs, the typical file extensions that identify the file format, and any limitations such as formats that are supported for *File:Open* only.

#### **AutoQuant ( \*.DEB, \*.AVZ, \*.HDR)**

---

*Image-Pro* supports many varieties of the *AutoQuant* file formats, which will have a \*.DEB, \*.HDR, or .AVZ file extension. *AutoQuant* files are supported in *File:Open* only.

#### **AVI (\*.AVI).**

---

*Image-Pro* supports many of the video varieties of the Microsoft™ *AVI* file format, which will usually have the \*.AVI file extension. The varieties that will be available in your copy of *Image-Pro* depend on the *AVI* file compression support that has been installed by the operating system and other multi-media components.

#### **BioRad (\*.PIC)**

---

*Image-Pro* supports many varieties of the *BioRad* confocal image file format, which will have a \*.PIC file extension. *BioRad* files are supported in *File:Open* only.

#### **BMP (\*.BMP, \*.DIB, \*.RLE)**

---

*Image-Pro* supports all known varieties of the Microsoft™ *BMP* file format, which will usually have the \*.BMP file extension but may also have a \*.DIB or \*.RLE extension.

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### **DeltaVision (\*.dv)**

---

*Image-Pro* now supports the *DeltaVision* file format, \*.dv, from Applied Precision, LLC. *DeltaVision* files are supported in *File:Open* and in the *Set Manager*.

### **Dr. HALO (\*.CUT)**

---

*Image-Pro* supports all varieties of the *Dr. HALO CUT* format that many previous *Media Cybernetics* applications supported. *CUT* files are supported in *File:Open* only.

### **EPS (\*.EPS)**

---

*Image-Pro Plus* will write (*File:Save*), but not read, Encapsulated Postscript Files with the extension \*.EPS.

### **EXIF**

---

*Image-Pro* now supports the EXIF format JPEG files, and extracting some of the useful information from the EXIF tags. Note that there is no way in IPP to generically support reading and reporting an indeterminate set of tags as IPP needs to know the meaning of the image and frame properties. At a minimum, tags that will be supported include date/time, description, copyright, exposure, make and model (reported as Camera properties). If a copyright tag is included in the file, *Image-Pro* will read it also.

### **Flat**

---

*Image-Pro* supports loading a wide variety of proprietary file formats using a configurable file description. This capability can also be used to define, load and save simple proprietary file formats of your own. For additional information on flat files, please see the section *Flat File Format* later in this appendix.

## **FITS (\*.FIT, \*.FTS, \*.FITS)**

---

*Image-Pro* supports reading files in the FITS (Flex. Image Transport System) format. A minimum amount of information from the FITS file format will be used directly by IPP (in image/frame properties), but all other FITS information found will be included in the file description so it will still be accessible.

## **Fuji (\*.INF)**

---

*Image-Pro Plus v. 7.0* supports Fuji image files with the extension \*.INF in *File:Open* only.

## **Gatan DigitalMicrograph (\*.DM3)**

---

*Image-Pro* supports many varieties of the *Gatan DigitalMicrograph* file format, which will have a \*.DM3 file extension. DM3 files are supported in *File: Open* only.

## **Graphics Interchange Format (\*.GIF)**

---

*Image-Pro Plus* allows you to read and write ( *File:Open* and *File:Save*) files with the extension \*.GIF.

## **Image Cytometry Std**

---

*Image-Pro Plus v. 7.0* supports Image Cytometry Standard image files with the extension \*.ICS in *File:Open* only.

## **Image-Pro Workspace (\*.IPW)**

---

*Image-Pro* supports a proprietary file format with the \*.IPW file extension.

## **In Vivo (\*.EXP, \*.QRS, \*.QCL)**

---

*Image-Pro* supports the \*.EXP, \*.QCL, and \*.QRS file types written by the *InVivo* products. These image sets can be opened directly by the *Set Manager*, which may give better

## *Appendix B – File Format Specifications*

results than using the *File:Open* command. Note that if all the tint in the QCL file are pure red, green, or blue tints, the image is loaded as a single-frame color image.

### **JPEG (\*.JPG)**

---

JPEG files will have a \*.JPG or JPEG file extension. *Image-Pro* supports all standard varieties of the JPEG file format, including JPEG 2000.

### **Kodak PhotoCD (\*.PCD)**

---

*Image-Pro* supports all known varieties of the *Kodak PhotoCD* file format, which will have a \*.PCD file extension. *PCD* files are supported in *File:Open* only.

### **Leica (\*.LEI, LIF)**

---

*Image-Pro Plus v. 7.0* will support Leica image files with the extension \*.LEI and \*LIF in *File:Open* only.

### **Molecular Dynamics (\*.GEL)**

---

*Image-Pro* supports many varieties of the *Molecular Dynamics* file format, which will have a \*.GEL file extension. *GEL* files are supported in *File:Open* only.

### **MetaMorph (\*.STK)**

---

*Image-Pro* supports many varieties of the *MetaMorph* file format, which will have a \*.STK file extension. *STK* files are supported in *File:Open* only.

### **Nikon**

---

*Image-Pro Plus v. 7.0* supports the *Nikon NIS-Elements ND2* file format in *File:Open* only.

## **NorPix (\*.seq)**

---

*Image-Pro Plus v. 7.0* supports the *Streampix* sequence file format in *File:Open* only.

## **Olympus FluoView 100 (\*.OIB, \*.OIF)**

---

*Image-Pro* supports the *Olympus FluoView* confocal image file format, which have a \*.OIB or \*.OIF file extension in *File:Open* only.

## **Park Scientific (\*.HDF)**

---

*Image-Pro* supports the *Park Scientific* image file format, which has an \*.HDF file extension in *File:Open* only.

## **PerkinElmer (\*.TIM)**

---

*Image-Pro* supports the *PerkinElmer* confocal image file format, which have a \*.TIMfile extension in *File:Open* only.

## **PICT (\*.PCT, \*.PICT)**

---

*Image-Pro* supports many varieties of versions 1 and 2 of the Macintosh *PICT* file format, which will usually have a \*.PCT or \*.PICT file extension. *PICT* files may contain vector graphs (e.g. lines and text) as well as raster image or bitmap data; only the bitmap data will be read.

## **PostScript (\*.EPS)**

---

*Image-Pro* supports *Encapsulated PostScript* files, which will have an \*.EPS file extension. *EPS* files are supported in *File:Save As* only.

## **RAW**

---

*Image-Pro Plus* will open and read Raw Binary Data files.

## **Sequence (\*.SEQ)**

---

*Image-Pro* supports multiple-image TIFF files with the .SEQ file extension. For additional information on TIFF files saved by *Image-Pro Plus*, please see the section, *Image-Pro Plus TIFF and SEQ Files* later in this appendix.

## **TIFF (\*.TIF)**

---

*Image-Pro* supports all known varieties of the TIFF (the *Tagged Image File Format*) file format, including most multiple-image varieties and *PowerPoint* TIFF files. For additional information on TIFF files saved by *Image-Pro Plus*, please see the section *Image-Pro Plus TIFF and SEQ Files* later in this appendix. If a copyright tag is included in the file, *Image-Pro* will read it also.

## **OME-TIFF (\*.TIF)**

---

OME-TIFF is a new standard file format for multi-dimensional file sets from the Open Microscopy Environment (OME) group. This file format uses the standard TIFF extensions, so the OME-TIFF format must be detected by the TIFF loader. OME-TIFF is supported for read-only. It supports the new delayed-loading feature of *Image-Pro Plus* v. 7.0 .

## **TrueVision (\*.TGA)**

---

*Image-Pro* supports all known varieties of the *Targa* and *TrueVision* file format with the \*.TGA file extension.

## **µManager (\*.TIF, \*.TXT)**

---

*Image-Pro Plus 7.0* supports \*.TIF and \*.TXT files from *µManager* in *File:Open* only.

## **Zsoft (\*.PCX)**

---

*Image-Pro* supports all known varieties of the *ZSoft PCX* file format, which will have a \*.PCX file extension. *PCX* files are supported in *File:Open* only.



## **Zeiss (\*.LIF, \*.LSM, \*.ZVI)**

---

*Image-Pro* supports many varieties of the *Zeiss* file format, which will have a .LSM, .LIF or .ZVI file extension. *Zeiss* files are supported in *File:Open* only.

## Calibration Files (\*.CAL files)

---

A calibration file is created when you store your *Calibration* command settings. A \*.CAL file is created using the *Save All* command from the *Calibration* command's pop-out menu.

A \*.CAL file is an ASCII file that contains the calibration values you specified in the *Intensity* and *Spatial Calibration* command windows. If values for only one of the two types of calibration have been specified, the file will contain values for only the one type.

---

An example of a \*.CAL file to which both intensity and spatial calibration values have been stored, is given below.

```
[SPATIAL]
CalibName=Spatial Cal 0
UnitName=Pixels
PixPerUnit=10,10
Origin=15,15
AngleOffset=20

[INTENSITY]
ImageClass=1
Name=Intensity Cal 0
UnitName=Gray Level
Type=0
InputRange=0,255
BlackIncident=0,255
NumPoints=3
Point0=0,0
Point1=10,50
Point2=255,255
NumSamples=256
```

## Measurement Files (\*.CNT files)

---

Measurement files are created when you store the measurement or statistical results of a *Count/Size* operation. They are created using the *Data to File* command from the *File* menu in the *Measurements* or *Count/Size* window.

A \*.CNT file is an ASCII file that contains the measurement data. Each line in the file is made up of the measurements for one object in the count. The measurement results for that object are separated by spaces within the line. The file contains header information describing the total number of records in the file, and the measurement types contained within it.

*Note - measurements for objects that were deactivated (hidden) when the Data to File command was performed, are not stored to the measurement file.*

---

An example of a \*.CNT file to which measurement results have been stored is given below.

```
#Total Count: 58
Obj#      Area   Centr-X  Centr-Y  Maj.Ax.  Min.Ax.  Perim.
  1        277   5.498195 19.76534 37.79405 11.87908   110
  2        375   180.20   15.25067 28.08347 27.37673   159
  3         10   193.70    .10     10.65680 1.148118    16
  4        152  250.1250 3.243421 25.75376 8.073408    56
  5        181  311.6077 6.624310 24.76410 11.92682    72
  6        349  35.99714 16.70201 25.30080 23.72677   126
```

---

An example of a \*.CNT file to which statistical results have been stored is given below.

```
Stats      Area   Centr-X  Centr-Y  Maj.Ax.  Min.Ax.  Perim.
Min         11   5.48276  .166667  3.81379  1.36119    8
(Obj.#)    11     37     4         11     4         11
Max        824  320.378  346.767  50.8240  31.7350   271
(Obj.#)    92     65    143     98     92     98
Range       813  314.895  346.60   47.0102  30.3738   263
Mean       288.194 161.479  142.390  23.8237  18.0395  102.274
Std.Dev    178.90  96.0388  100.414  9.85365  8.83737   56.0582
Sum        17868 10011.7  8828.15  1477.07  1118.45  6341
Samples     62     62     62     62     62     62
```

## Environment Files (\*.ENV files)

---

An environment file is created when you store your *Count/Save* command settings. A \*.ENV file is created using the *Save Settings* command from the *File* menu in the *Count/Size* window.

A \*.ENV file is an ASCII file that contains the values and parameters you specified in the *Count/Size* command window. Settings such as **Intensity Range**, **Select Measurements** are stored in this file. Loading an environment file lets you re-establish a *Count/Size* environment without having to reset all the parameters manually.

---

An example of a \*.ENV file to which *Count/Size* settings have been stored, is given below.

```
[BLOBLIB]
Start=189
End=255
Labels=1
DisplayMode=1
MinArea=0
FillHoles=0
CleanBorder=0
Smoothing=0
AutoRange=1
BrightOnDark=1
DispColor=0
LabelColor=1
Measure=1
Filter=1
FeatureSize=20
AddCount=1
NumClasses=10
Iterate=1

[BLOBENABLE]
Area=1
Aspect=1
Centr-X=1
Centr-Y=1
Max.Dia.=1
Min.Dia.=1
Perim.=1

[BLOBCLASS]
Area=1

[BLOBRANGE]
Area=10,1000000
Aspect=1,1000000
Centr-X=0,1000000
Centr-Y=0,1000000
Max.Dia.=0,1000000
```

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Min.Dia.=0,1000000  
Perim.=1,1000000

## Fast Fourier Transform Files (\*.FFT files)

*For versions prior to Image-Pro Plus 3.0:*

Fast Fourier Transform files are created when you save FFT data from the **FFT** window. The FFT file is a binary file containing the coefficients from the FFT array. If the **Full FFT** option in the **Forward FFT Options** dialog box has been selected, the entire set of FFT data will be stored. If the **Half FFT** option has been selected, only a little more than half of the array produced by the FFT (the left half) is stored in the file. However, the missing elements can be easily recreated because the data set is symmetrical.

All data stored within the FFT file are made up of 2-byte integers. The first 2-byte integer in the file defines the FFT array's width (referred to as "M") and the second 2-byte integer defines its height (referred to as "N"). M and N values will range from 16 to 512, and will be powers of 2.

The 4-byte header is followed by the Fourier coefficients. In an FFT file that contains only half the data, this 4-byte file header is followed by the Fourier coefficients which make up the *left half* of the FFT array (see below).

Each coefficient (array element) consists of a 2-byte, fixed-point REAL component, followed by a 2-byte, fixed-point IMAGINARY component. Within the file, each 4-byte coefficient is formatted as shown in the following example:

16-bit wide REAL component		16-bit wide IMAGINARY component	
0 1 1 0 0 0 0 1 0 (194)	0 1 0 0 1 1 1 (39)	0 0 0 1 1 1 1 0 0 (60)	1 1 0 1 0 1 0 (106)
9-bit mantissa	7-bit fraction	9-bit mantissa	7-bit fraction

Within each 16-bit component, the mantissa occupies the 9 most significant positions. Its value can range from -256 to +256. The fractional portion occupies the least significant 7 bits, and can have a value of 0 to 127/128.

*Note - FFT coefficients generated from a Floating Point image will be 8 bytes in length: a 4-byte, floating-point REAL component, followed by a 4-byte, floating-point IMAGINARY component.*

In a complete array, the FFT data are symmetrically organized around the central "zero frequency component". Except for two elements, every FFT element has a symmetric "opposite", which is made up of the same REAL value, but the opposite IMAGINARY value. The two exceptions are the first array element (at position 0,0) and the zero frequency component. These two array elements are unique.

## Appendix B – File Format Specifications

Also, the first row and column in the array are symmetrically arranged along their midpoints, rather than around the zero frequency component.

If only half the data have been stored, the file will contain  $(M/2 + 1) * N$  coefficients (where M and N are the FFT array's width and height, respectively). Remember, only the left side of the FFT array is actually recorded in the file, resulting in only  $(M/2 + 1)$  width elements.

The following figure illustrates the organization of a complete FFT array (for simplicity an 8 x 8 array is shown; however, the smallest array created by *Image-Pro* is 16 x 16). The figure also depicts the portion of the array that would be contained in the FFT file (the left-hand side, up to and including the column containing the "zero frequency component" (ZFC)) if only half of the data had been generated. The right-hand elements (shown in the de-emphasized grid) are the ones that must be recreated.

The labels within the array show how the elements are symmetrically related. Note that the first column and row are symmetrically "mirrored" along their middle elements, not around the ZFC like the remaining data.

		column (u)					M=8		
		0	1	2	3	4	5	6	7
0		1x	2x	3x	4x	5x	4x	3x	2x
1		2y A		B	C	D	X W		V
2		3y E		F	G	H	U T		S
row (v) 3		4y I		J	K	L	R Q		P
4		5y M		N	O	ZFC	O N M		
5		4y P		Q	R	L	K J		I
6		3y S		T	U	H	G F		E
N=8 7		2y	V W		X D		C B		A

The zero frequency component in an FFT array is located in the  $M/2$  column of the  $N/2$  row, where the first position in the array is 0,0.

Within the FFT file, this element will be found beginning at byte:

$$4 * (((M/2 + 1) * N/2) + M/2) + 4$$

where the file begins with byte 0.

$F(u,v)$  denotes the Fourier coefficient in column  $u$ , row  $v$ . It is a complex number that is represented by:

$$F(u,v) = \{R(u,v), I(u,v)\}$$

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where R is the real part, and I is the imaginary part.

Within the file,  $F(u,v)$ ,  $0 \leq u \leq M/2$ ,  $0 \leq v \leq N-1$ , would be located beginning at byte:

$$4*((v*(M/2 + 1)) + u) + 4$$

The first component of  $F(0,0)$  would be found at byte 4.

If you have stored only the half of the FFT array, use the following formulas to generate the Fourier coefficients for the right half.

**For the right side of row 0, use:**

$$F(u,0) = \{R(M-u,0), -I(M-u,0)\}$$

**For the right side of the remaining rows, use:**

$$F(u,v) = \{R(M-u, N-v), -I(M-u, N-v)\}$$

where, where  $M/2 < u < M$ .

**For Image-Pro Plus version 3.0 (and subsequent) :**

New features in the 3.0 version of the FFT module made it necessary to extend the FFT file format. The new format includes a version number which will enable us in the future to extend this format further while insuring backward compatibility. To differentiate the old files from the new ones, and since the old files did not have a version number, we set the first two short integers to 0. When we read a file with these two numbers set to a valid width and height, we know it is an old file. If the two numbers are (0, 0), we know it is a new file and that the format is as follow:

byte	type	description
0	short	0 (used to be the width of the FFT data)
2	short	0 (used to be the height of the FFT data)
4	short	version number
6	short	type: 5 = 32 bit floating point, 6 = 16 bit fixed point
8	double	start of spectrum display range (1)
16	double	end of spectrum display range (1)
24	double	start of phase display range (1)
32	double	end of phase display range (1)
40	short	0 = real/imaginary, 1 = spectrum/phase
42	short	0 = full data, not 0 = half data
44	short	width
46	short	height
48	short	class of the original image (2)



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<b>byte</b>	<b>type</b>	<b>description</b>
50	4*short	position of the unpadded original within the padded image (3)
58		Spectrum or real part
...		Phase or imaginary part.

short = short integer in C = integer in Basic = 2 bytes.

double = double precision floating point in C = double in Basic = 8 bytes.

(1): When displaying the 8-bit spectrum or the 8-bit phase, the real and imaginary parts are what gets saved. These are not displayed directly and do not require the floating point display ranges. When displaying the real spectrum and/or phase, the real and imaginary data are not maintained and the spectrum and phase are saved to file. These are displayed into floating point images which require a floating point display range. That range is visible in the Info dialog accessible from the Edit menu.

(2): 1 = 8 bits/pix, 4 = 12 bpp, 5 = 32 bpp, 6 = 16 bpp.

(3): When the sizes of the image are not powers of two, the image is padded into the next biggest power of two image sizes. The position of the original (smaller) image is kept in the fft data file in order to be able to inverse the fft data once it is read back. The position is stored as left, top, right, bottom.

## Flat File Format

---

*Image-Pro's* flat file format allows you to read and write image files in a format that you define. It provides a way for you to save *Gray Scale 12*, *Gray Scale 16*, and *Floating Point* images, since standard formats do not usually accommodate these classes.

When a flat file is read or written, *Image-Pro* looks for the format definition in a "description" file. The description file is a file that you create to define your image file format. It contains the following information about your file:

```
ClassName
{  bits:BitsPerSample
   width:ImageWidth
   height:ImageHeight
   start_offset:ImageOffset
   line_offset:LineOffset
   sample_offset:GreenOffset
   sample_offset1:BlueOffset
   next_sample:SameColorOffset
   byte:@Position=Value
   datatype:ByteOrder
   palette:PaletteOffset
}
```

where *ClassName* specifies the class of image contained in the file (e.g., *IFFCL\_BILEVEL*, *IFFCL\_GRAY*), and each of the definitions following class name provides a parameter (e.g., *ImageWidth*, *BitPerSample*) specifying information about the file (e.g., *width:512*).

Your parameter values in the description file may be entered as constant values, or can be specified as variables to be obtained from the image file itself. For example,

```
width:100
```

indicates that the width of the image is 100 bytes, while

```
width:@100=sshort
```

indicates that the width is specified by a standard (msb first), short value located 100 bytes into the image file.

If you specify a parameter as a constant, *Image-Pro* assumes it is a decimal value unless you use the *0xHEX\_NUMBER* notation. If you specify a parameter as a variable to be obtained from the image file, you must format the parameter as follows:

```
@Offset=Datatype
```

where: *Offset* is a decimal value specifying the position of the value in the file, expressed as an offset from the beginning of the file, in bytes, and

## Appendix B – File Format Specifications

*Datatype* specifies the format of the value located at that position as follows:

<b>A <i>Datatype</i> of...</b>	<b>Interprets the value in the file as...</b>
ishort	Intel short format. Two bytes, with the least significant byte first.
ilong	Intel long format. Four bytes, with the least significant bytes first.

<b>A <i>Datatype</i> of...</b>	<b>Interprets the value in the file as...</b>
sshort	Standard short. Two bytes with the most significant byte first.
slong	Standard long. Four bytes with the most significant bytes first.
byte Single	character

You may also include arithmetic operators and definition names in your parameter value, as long as the definition name you specify has been given a value.

For example, you might calculate line offset as `line_offset:width+10`. This will work as long as `width` has already been assigned a value (i.e., `width` must appear before `line_offset` in your definition).

The table on the following page explains the elements of a file format description. Note that not all elements are required.

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COMPONENT	DESCRIPTION														
<i>ClassName</i>	<p>Specifies the class of image contained in the file. Must be one of the following:</p> <p>IFFCL_GRAY IFFCL_PALETTE IFFCL_RGB</p> <p>This component is <u>required</u>.</p> <p><i>Note - if you are reading/writing an IMC_GRAY12, IMC_GRAY16, or IMC_FLOAT image, specify IFFCL_GRAY as your ClassName, then specify 12 (for IMC_GRAY12), 16 (for IMC_GRAY16), or 32 (for IMC_FLOAT) in the bits parameter.</i></p>														
<i>bits:</i>	<p>Bits per <i>sample</i> (not bits per pixel). The following <i>bits</i> values would be used for the standard <i>Image-Pro</i> image classes:</p> <table border="1" data-bbox="711 932 1094 1184"> <thead> <tr> <th>For image class...</th> <th>set bits to:</th> </tr> </thead> <tbody> <tr> <td><i>Gray Scale</i></td> <td>8</td> </tr> <tr> <td><i>Palette</i></td> <td>8</td> </tr> <tr> <td><i>True Color</i></td> <td>8</td> </tr> <tr> <td><i>Gray Scale 12</i></td> <td>12</td> </tr> <tr> <td><i>Gray Scale 16</i></td> <td>16</td> </tr> <tr> <td><i>Floating Point</i></td> <td>32</td> </tr> </tbody> </table> <p>This component is <u>required</u>.</p> <p><b>Important</b> – <i>The bits definition must be the first definition in your description file.</i></p>	For image class...	set bits to:	<i>Gray Scale</i>	8	<i>Palette</i>	8	<i>True Color</i>	8	<i>Gray Scale 12</i>	12	<i>Gray Scale 16</i>	16	<i>Floating Point</i>	32
For image class...	set bits to:														
<i>Gray Scale</i>	8														
<i>Palette</i>	8														
<i>True Color</i>	8														
<i>Gray Scale 12</i>	12														
<i>Gray Scale 16</i>	16														
<i>Floating Point</i>	32														
<i>width:</i>	<p>Width of the image, in pixels.</p> <p>This component is <u>required</u>.</p>														
<i>height:</i>	<p>Height of the image, in pixels.</p> <p>This component is <u>required</u>.</p>														
<i>start_offset:</i>	<p>Byte offset to first image line.</p> <p>This component is optional. Default is 0.</p>														

*continued on next page*

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COMPONENT	DESCRIPTION
sample_offset:	<p>Byte offset between the Red and Green channels. Valid only for IFFCL_RGB images. Used in color images when the Green sample does not immediately follow the Red sample. Also used to access the Green channel when reading planar images (an example of which can be found in the sample IFFFLAT.DES file that has been provided with <i>Image-Pro</i>).</p> <p>This component is optional. Default is 1.</p>
sample_offset1:	<p>Byte offset between Green and Blue channels. Valid only for IFFCL_RGB images. Used in color images when the Blue sample does not immediately follow the Green sample. Also used to access the Blue channel when reading planar images (an example of which can be found in the sample IFFFLAT.DES file that has been provided with <i>Image-Pro</i>).</p> <p>This component is optional. Default is sample_offset value.</p>
line_offset:	<p>Byte offset from one image line to the next. This parameter would be used if your application embedded additional information in an image line. If you specify a line_offset value, the value you specify must be greater than or equal to the width value. line_offset is combined with sample offset values to access data for the current channel on the next line.</p> <p>This component is optional. Default is:</p> $2 * \frac{width * bits + 15}{16}.$
next_sample:	<p>Offset to next sample of same color.</p> <p>Used when working with planar images, or to move from chunk-to-chunk in IMC_RGB images (useful in cases where there are 4 samples in a chunk -- see example in the sample IFFFLAT.DES file that has been provided with <i>Image-Pro</i>).</p> <p>This component is optional. Default is 1.</p>

continued on next page

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COMPONENT	DESCRIPTION
byte:	<p>Specifies a value and a position to which the value is to be written (on write), or from which the value is to be read (on read). Parameter is entered as follows:</p> <p><i>byte:@Position=Value</i></p> <p>where <i>Position</i> specifies an offset into the file, and <i>Value</i> specifies a 1-byte value to be written to (on write) or read from (on read) that position.</p> <p>This is usually used to read/write format-identifying, header information from/to an image file.</p> <p>While only a single byte can be defined in the <i>byte:</i> descriptor, you may include as many <i>byte:</i> descriptors as you need to read/write specific data to a file. For example, if you wanted to write "G12" in bytes 0-2 to mark your image file as a 12-bit gray scale class, you would use the following:</p> <p><i>byte:@0='G'</i>  <i>byte:@1='1'</i>  <i>byte:@2='2'</i></p> <p>Note that the <i>Value</i> can be expressed as a decimal value (default), a hex value (in 0xHEX_NUMBER form) or a character, as shown above.</p> <p>This component is optional.</p>
datatype:	<p>Specifies byte order in image data that are greater than 8 bits per sample (i.e., contains short or long integer values). Must be one of the following:</p> <p><i>msb_lsb</i>  <i>lsb_msb</i></p> <p>Where:</p> <p><i>lsb_msb</i> specifies that the least-significant byte(s) appear(s) first.</p> <p><i>msb_lsb</i> specifies that the most-significant byte(s) appear(s) first.</p>

*continued on next page*

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COMPONENT	DESCRIPTION
datatype: <i>continued</i>	<p>When writing a flat file, datatype specifies whether the most-significant byte of the short/long value will be written first or last. When reading, describes how the short/long values are presented in the existing file.</p> <p>This component is optional. If datatype is not provided, default is to write/read data as a binary stream.</p>
bit_offset	<p>Specifies how many low-order bits will be dropped when reading an N-bit-per-pixel image file into an M-bit-per-pixel workspace, where <math>M &lt; N</math>. If the bit offset is less than the difference between N and M, the remaining bits will be dropped from the high-order position.</p> <p>For example, if a 16-bit-per-pixel image file is to be read into a 12-bit-per-pixel workspace, then</p> <p style="text-align: center;">bit_offset: 3</p> <p>specifies that the 3 low-order bits of each 16-bit byte will be dropped. This also means that the high-order bit will also be dropped.</p> <p style="text-align: center;">16 = 1 + 12 + 3</p> <p style="text-align: center;">im age file msb workspace lsb</p>
palette:	<p>Offset to palette. Used with IFFCL_PALETTE class images, and optionally with IFFCL_GRAY class images. Palette will consist of <math>2^{**}bits</math> of RGB values.</p> <p>This component is optional.</p>

A description file can contain more than one description, *and* it can contain more than one description for a single class. For example, the following file contains two descriptions for IFFCL\_GRAY, one for 12-bit images, and one for floating-point image files.

```
IFFCL_GRAY
{
bits:12
width:@0=sshort
height:@2=sshort
byte:@3:12
}
```

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```
IFFCL_GRAY
{
bits:32
width:@0=sshort
height:@2=sshort
byte:@3=32
}
```

When a FLAT file is open for read and there is more than one format description in the description file, *Image-Pro* attempts to match each description to the image file (starting with the first description). It will use the first description it finds that agrees with the data in the file. This is why the `byte:` description, while not a technically a required component of the description file, is one that, for practical purposes, is required to ensure the correct format description is selected for a file. In the example above, the first IFFCL\_GRAY description will be used only if a value of 12 is found at byte 3 in the file. If this criterion was not included, every FLAT file would match this first description, and they all would be opened as 12-bit images.

When a FLAT file is open for write and there is more than one format description in the description file, *Image-Pro* will select the one with *ClassName* and `bits` values matching the Image Class and Bits elements in the image file's File Parameter Block.

For more examples of FLAT file descriptions, see the sample IFFFLAT.DES file that has been included with your *Image-Pro* files.



## Histogram Files (\*.HST files)

Histogram files are created when you store histogram or line profile data. They are created using the *Data to File* command from the *File* menu in the *Histogram* or *Line Profile* window. A \*.HST file is an ASCII file that contains the values that have been charted along the X and Y axes.

---

An example of a \*.HST file created by the *Histogram* command is given below. A \*.HST file that contains histogram data will contain a record for each intensity level (X-axis) value. The record will contain the calibrated intensity value in the first field, followed by the calibrated spatial measurement value. If the data are from a color image, the record will contain 3 measurement values; one for each of the RGB channels, as shown below. Data fields are separated by spaces.

The file contains several lines of header information that provides information about the contents of the file. It also lists the histogram statistics *before* the individual measurement records. If the file contains histogram data for a color file, statistics will be listed for each of the color channels in Red, Green and Blue order.

```
# Image-Pro Plus Histogram Data
# Time: Mon Aug 23 09:40:45 1996
# Image: color.tif
# Spatial Unit: Pixels
# Intensity Unit: Pixels
# Number of Data Sets: 3
# Set Size: 256
# Channels: Red Green Blue

#      Mean      Min      Max      Std.Dev
      118.2758      33      255      71.77151
      82.77801      31      226      32.74552
      80.31753      25      212      31.88052

# Gray Level      Pixels
      0              0              0              0
      1              0              0              0
      2              0              0              0
      3              0              0              0
      4              0              0              0
      .
      .
      253           993              0              0
      254           175              0              0
      255            18              0              0
```

---

An example of a \*.HST file created by a *Line Profile* command is given below. A \*.HST file that contains line profile data will contain a record for each pixel position (X-axis) along the line. The record will contain the calibrated spatial measurement value in the first field, followed by the calibrated intensity value. If the data are from a color image, the

## Appendix B – File Format Specifications

record will contain 3 intensity values; one for each of the RGB channels, as shown below. Data fields are separated by spaces.

The file contains several lines of header information that provide information about the contents of the file.

```
# Image-Pro Plus Line Profile Data
# Time: Mon Aug 23 09:59:58 1993
# Image: colordot.tif
# Spatial Unit: Pixels
# Intensity Unit: Pixels
# Number of Data Sets: 3
# Set Size: 22
# Channels: Red Green Blue

#      Mean      Min      Max      Std.Dev
248.4545      244      252      1.751033
84.27273       79       95      3.967846
148.1364      136      167      7.706523
1      249       95      167
2      249       91      160
3      250       88      154
4      247       88      156
5      250       86      152
6      250       85      149
7      246       86      151
8      249       84      149
9      249       86      152
10     249       86      152
11     249       86      151
12     245       86      155
13     252       82      143
14     248       83      146
15     249       79      138
16     244       81      143
17     249       81      143
18     250       81      143
19     248       80      144
20     249       80      138
21     248       80      136
22     247       80      137
```

## **Lookup Table Files (\*.LUT files)**

---

A lookup table file is created when you store your the current LUT values using the *Contrast Enhancement* command's *Save* option.

A \*.LUT file contains the intensity table through which your image bitmap values are interpreted. This file is not in ASCII format.

## Outline Files (\*.SCL files)

Outline files are created when you save object outlines using *File* menu's *Save Outlines* command from the *Count/Size* or *Measurement* commands. An Outline file is a binary file containing a list of polygonal shapes. All elements of this file are made up of 2-byte integers.

The first 2-byte integer defines the total number of polygons contained in the file. This is followed by the polygon definitions themselves. A polygon is represented as a 2-byte integer defining the number of points in the polygon, followed by the XY coordinate-pairs for each of these points. Each coordinate pair is made up of 2, 2-byte integers; the first 2 bytes define the X position, and the next 2 bytes, the Y position.

The following table illustrates the structure and contents of a file containing 2 polygons, one with 3 points, and one with 4 points:

16-BIT INT #	BINARY/DECIMAL CONTENTS OF THE 2-BYTE INTEGERS		DESCRIPTION
#0 - #13	String		"IpWin_Blob_Outline" carriage return,line feed in next 2 bytes "v30" carriage return,line feed in next 2 bytes
#14	00000000 00000010 (2)		Indicates that 2 polygons are contained in this file
#15	00000000 00000011 (3)		Indicates that Poly1 has 3 points
#16 & 17	00000000 00001010 (10)	00000000 00010100 (20)	Defines XY location of Poly1's 1st point (10,20)
#18 & 19	00000000 00001010 (10)	00000000 00101000 (40)	Defines XY location of Poly1's 2nd point (10,40)
#20 & 21	00000000 01000110 (70)	00000000 00101000 (40)	Defines XY location of Poly1's 3rd point (70,40)
#22	00000000 00000100 (4)		Indicates that Poly2 has 4 points.
#23 & 24	00000000 01100100 (100)	00000000 01000000 (64)	Defines XY location of Poly2's 1st point (100,64)
#25 & 26	00000000 01100100 (100)	00000000 10010110 (150)	Defines XY location of Poly2's 2nd point (100,150)
#27 & 28	00000000 01111000 (120)	00000000 10010110 (150)	Defines XY location of Poly2's 3rd point (120,150)
#29 & 30	00000000 01111000 (120)	00000000 01000000 (64)	Defines XY location of Poly2's 4th point (120,64)

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The following example shows how an Outline file would be accessed and used within a C program:

```
typedef struct {short x;short y;} COORD;
#define MAXPOINTS 4000

int
LoadOutlines(char *outfile)
{
    int i;
    short count;
    short num_obj = 0;
    COORD *Points;
    FILE *fp;

    /* open outline file */
    if((fp = fopen(outfile, "rb"))==NULL)
        return -1;

    /* allocate space for polygon */
    if((Points=malloc((long)MAXPOINTS*sizeof(COORD)))==NULL)
        return -1;

    /* reads in number of polygons */
    fread((char *) &num_obj, sizeof(short), 1, fp);
    if(num_obj <= 0)
        return -1;

    /* for each polygon...*/
    for(i = 0; i< num_obj; i++){

        /* reads in number of points in polygon */
        fread ((char *)&count, sizeof(short), 1, fp);
        if(count > MAXPOINTS || count < 0)
            break;

        /* read points*/
        if(fread((char *)Points, sizeof(COORD), count, fp)==count{
            :
            :
            do something with the polygon
            :
            :
        }
        else
            break;
    }
    free(Points);
    fclose(fp);
    return 0;
}
```

## **Pseudo-Color Files (\*.PSC files)**

---

A pseudo-color file is created when you store your *Pseudo-Color* palette. A \*.PSC file is created using the *Save Pseudo-Colors* command from the *File* menu in the *Pseudo-Color* dialog box.

A \*.PSC file is an *Image-Pro* file that contains the color assignments that are to be applied to the intensity range. This file is not in ASCII format.

## Range Files (\*.RGE files)

---

A range file is created when you save the color selections you have made using the *Save Ranges* command on the *Color Segmentation Input/Output* tab. The range file contains all of the colors that are to be extracted from an image. If the file has been saved in RGB format, the values it contains will be RGB values. If the file has been saved in HSI format, it will contain HSI values.

---

The example file below contains the RGB values extracted from an image using the *Color Segmentation* command.

```
# IPWindows Color Segmentation ranges
# V.1
# Divisions: 6
# Color Model: RGB
200 55 100
200 55 111
200 55 122
200 67 100
200 67 111
200 67 122
200 78 100
200 78 111
200 78 122
211 55 89
211 55 100
211 55 111
211 55 122
211 67 89
211 67 100
211 67 111
211 67 122
211 67 133
211 78 89
211 78 100
211 78 111
211 78 122
211 78 133
211 78 144
211 78 155
211 78 166
```

## **Manual Tag Files (\*.TAG files)**

---

A Manual Tag file is created when you store your class and/or tag marker information in the *Manual Tag* selection from the *Measure* menu. A \*.TAG file is created using the *Save Points* command on the *File* menu in the *Manual Point Count* dialog box.

A \*.TAG file is an *Image-Pro* file that contains the class and marker information that is to be applied to the objects in an image. This file is not in ASCII format. Users should not attempt to modify a \*.TAG file



## Kernel Files (\*.?x? files)

---

A kernel file contains the coefficients used by the convolution filtering process. A number of pre-defined kernel files are provided with *Image-Pro*. You can also develop your own filters by creating a kernel file like the ones shown below. A kernel file can be created using any ASCII file editor. It is made up of 5 header lines, followed by the kernel itself.

The file must contain the following information:

```
line1: # Image Pro Plus Kernel
line2: Name Your Kernel Name
line3: Size XX
line4: Offset XX
line5: Data
line6: kernel coefficients, 1st row
line7: kernel coefficients, 2nd row
:
```

where:

Size *XX* defines the size of your kernel array  
 Offset *XX* defines the position of the pixel to be replaced

---

Two kernel file examples are shown below:

```
# Image Pro Plus Kernel
Name HiPass3x3
Size 3 3
Offset 1 1
Data
-1 -1 -1
-1 9 -1
-1 -1 -1

# Image Pro Plus Kernel
Name HorzEdge7x7
Size 7 7
Offset 3 3
Data
-1 -1 -1 -1 -1 -1 -1
-1 -1 -1 -1 -1 -1 -1
-1 -1 -1 -1 -1 -1 -1
0 0 0 0 0 0 0
1 1 1 1 1 1 1
1 1 1 1 1 1 1
1 1 1 1 1 1 1
```

## Image-Pro Plus TIFF and SEQ Files

---

*Image-Pro Plus* will open all known varieties of the *TIFF* file format. The *TIFF* file format supports multiple images and thumbnails. Images and thumbnails can be in different image formats (e.g. pixel type and bit depth) and/or sizes. Multiple-image files where all the images are the same image format and size will be opened as *Image-Pro* multi-frame sequences, where you will be prompted to decide how many frames to open. Multiple-image files where the images are the same image format but different sizes will be loaded as multiple-resolution files, where you will be prompted to decide which image resolution to open. Multiple-image files where the images are saved in different image formats will be treated as a single-image file and the first image in the file directory will be opened.

When *Image-Pro* saves an image document (or workspace) to a *TIFF* or *SEQ* file, it will always write the *TIFF* file in a standard manner that follows the *TIFF 6.0 Specification*. This discussion assumes the reader is familiar with the *TIFF* standard and the organization of *TIFF* files. For detailed information on the *TIFF* standard, please consult that document, which is available on-line from Adobe at:  
<http://partners.adobe.com/asn/developer/pdfs/tn/TIFF6.pdf> .

How the *TIFF* or *SEQ* image is saved depends on whether the image is a multi-frame sequence workspace or single-frame, and on the other information that is known about the image. Single-frame images will be saved as a single image directory. Multiple-frame images will be saved as a multiple-image file, with one image directory for each frame of the active portion of the sequence.

Additional information known about the image is saved in custom *TIFF* tags. *Image-Pro* and other Media Cybernetics' products may write one or more proprietary *TIFF* tags along with the image(s). Frame-dependent information will be written in each image directory, while general information about the image as a whole will be written only to the first image's directory. Media Cybernetics' proprietary *TIFF* tags generally use tag numbers in the range between 40100 and 40110.

## Appendix B – File Format Specifications

*TIFF* tags from 50288 through 50297, inclusive, are reserved by Media Cybernetics from Adobe. Unless otherwise indicated, the following tags will be present on each image of multiple-image files. The following tags are currently in use.

Tag number: **50288**

Purpose: Media Cybernetics ID

Type: ASCII

Length: Varies according to ID string.

Value: Currently, all Media products will write the following ID string: MC *TIFF* 4.0. Future products may write a new version number. Presence of this tag containing an ID string containing MC *TIFF* indicates that the file was written by a Media product and the rest of the tags should be valid, if present.

*Note:* It is still recommended that a *TIFF* reader check the type and length of each tag prior to assuming that it is valid.

Tag number: **50289**

Purpose: X/Y position

Type: DOUBLE

Length: 2

Value: X/Y position of the image, where the first value is the position along the X axis, and the second value along Y.

*Note:* These positions are in the current calibration units (see tag **50291**).

Tag number: **50290**

Purpose: Z position

Type: DOUBLE

Length: 1

Value: Z position of the image, which is always expressed in microns.

Tag number: **50291**

Purpose: X/Y calibration

Type: DOUBLE

Length: 3

Value: This tag contains values that can be used to spatially calibrate the image. The first value is a conversion factor from the native calibration units to millimeters (e.g. for microns this value will be 1000). The second value is the size of a single pixel along the X axis in the native calibration units, and the third value the size along the Y axis.

*Note:* This tag is written only to the first image of multiple-image files as it is constant for all images in the file.

## *Appendix B – File Format Specifications*

Tag number: **50292**

Purpose: Lens characteristics

Type: DOUBLE

Length: 3

Value: The following lens characteristics, in order

- Emissions wavelength in nanometers
- Numeric aperture
- Refractive index

Tag number: **50293**

Purpose: Channel name

Type: ASCII

Length: Varies with length of channel name, will not exceed 260 characters.

Value: The name of the channel, often the name of the dye used to stain and filter the image.

Tag number: **50294**

Purpose: Excitation wavelength

Type: DOUBLE

Length: 1.

Value: The excitation wavelength in nanometers

Tag number: **50295**

Purpose: Time stamp

Type: DOUBLE

Length: 1.

Value: The time point that the frame belongs to in V/OLE/DATE format.

Appendix B – File Format Specifications

Tag number: **50296**

Purpose: Frame properties

Type: Byte

Length: 2054

Value: A proprietary structure containing information about other frame properties.

(defined in C/C++ defines and structures):

```
#define FRAME_PROPS_VERSION 1
#pragma pack(1)
typedef struct
{
    DW        ORD        dwVersion;
    B         OOL        bHasMagnification;
    doubl     e          dfMagnification;
    B         OOL        bHasBinning;
    W         ORD        wBinning[2];
    BOOL      bHasSiteName;
    char      szSiteName[_MAX_PATH];
    B         OOL        bHasExposure;
    doubl     e          dfExposure;
    BOOL      bHasGain;
    doubl     e          dfGain;
    BOOL      bHasOffset;
    doubl     e          dfOffset;
    BOOL      bHasGamma;
    doubl     e          dfGamma;
    BOOL      bHasWhiteBalance;
    doubl     e          dfWhiteBalance[3];
    BOOL      bHasCaptFrame;
    RECT      rCaptFrame;
    B         OOL        bHasChipCoord;
    R         ECT        rChipCoord;
    BOOL      bHasDriverName;
    char      szDriverName[256];
    BOOL      bHasDriverVersion;
    char      szDriverVer[32];
    B         OOL        bHasCameraModel;
    char      szCameraModel[128];
    BOOL      bHasCameraID;
    char      szCameraID[128];
    BOOL      bHasCaptureFeatures;
    char      szCaptureFeatures[512];
    BYTE      bReserved[1024]; // not used at this time
} // end of version 1 info
```

## Appendix B – File Format Specifications

```
} FRAME_PROPS;  
#pragma pack()
```

Media Cybernetics' software will also write the following proprietary/private *TIFF* tags:

Tag number: **40000**

Purpose: Flag on palette TIFF images to indicate that the palette is actually a Pseudo-coloring lookup-table

Type: Short

Length: 1

Value: The value is actually not used. The presence of this tag with the right type and size is used as an indication to Image-Pro that while the TIFF file contains a "palette" image, it should be opened as a grayscale image, and the "palette" used as a pseudo-coloring lookup table.

Tag number: **40001**

Purpose: X/Y axis spatial calibration

Type: Byte

Length: 307

Value: A proprietary structure containing the X/Y spatial calibration settings:

```
#pragma pack(1) // very important!!
```

```
#define MAXCALNAME 128
```

```
typedef struct
```

```
{  
    char    Sig[10]; // s/b the nul-terminated string IPWINSICAL  
    BYTE    Ver;    /      / s/b 1  
    char    Name[MAXCALNAME]; //      calibration name  
    char    CUName[MAXCALNAME]; // calibration units name  
    doubl   e    XPixPerCu;  
    doubl   e    YPixPerCu;  
    doubl   e    XCuOrigin;  
    doubl   e    YCuOrigin;  
    doubl   e    AngleOff;
```

```
} SCALTAGDATA;
```

```
    #pragma pack()
```

Tag number: **40002**

Purpose: Display settings (black and white levels)

Type: Unsigned short

Length: 6

Value: A proprietary structure containing the display settings:

```
#pragma pack(2)      /* I want to save this to a file... */
```

```
typedef struct
```

```
{
```

*Appendix B – File Format Specifications*

```
W      ORD start;          // black level
      WORD end;           // white level
      WORD inverse;       // typically not used
W      ORD d isable;      // typically zero
      WORD start8;       // can be set to zero
      WORD end8;        // can be set to 255
} DISPRANGE;
```

Tag number: **40003**

Purpose: Floating-point dynamic range

Type: Double

Length: 2

Value: The first value is the minimum value for the dynamic range of a floating point image, and the second value is the maximum value. This interacts with the display settings above, as the black and white levels are specified using a 0-65535 range that is normalized to the floating point dynamic range.

Tag number: **40010**

Purpose: Pseudo-color lookup table (LUT)

Type: Byte

Length: 768

Value: An array of 256 red values, followed by 256 green values, followed by 256 blue values. The pseudo-color LUT is applied to the entire image, so this tag is usually found only on the first image of multi-image TIFF or SEQ files.

Tag number: **40011**

Purpose: Frame tint lookup table (LUT)

Type: Byte

Length: 768

Value: An array of 256 red values, followed by 256 green values, followed by 256 blue values. A frame tint can be applied to each frame of the image, so this tag is usually found only on all of the images of multi-image TIFF or SEQ files.

*Appendix B – File Format Specifications*



## Appendix C - Source Code

---

### Working with Fast Fourier Transform Data Files

---

If you plan to develop programs that read *Image-Pro* FFT files, the following source code shows you how to work with integer-based, FFT data files, to which only half of the FFT data have been stored.

In this example, the program reads the *Image-Pro* FFT data file and stores it in a  $[width] \times [height]$  buffer, which is pointed to by [FFT]. Then, the program recreates the missing half of the data.

Floating-point FFT data are treated in a similar manner, but use 4-byte, floating-point data instead of data that are in the 2-byte, fixed-point format.

```
#include "fft.h"

/*
Usage:
    complex *fft;
    char *filespec;
    int width;
    int height;
    .
    .
    fft = C_ReadFFTData(filespec, &width, &height);
    fft: pointer to a struct complex. Memory is allocated by
        routine.
    filespec: name of the file containing the FFT data.
    width: width of the fft array (power of 2). Initialized by
        routine.
    height: height of the fft array (power of 2). Initialized by
        routine.
*/

complex *
C_ReadFFTData(filespec,width,height)
char *filespec;
int *width, *height;
{
    complex *fft;
    FILE * DataFP;
    int row,col;
    complex *RowData = NULL;
    FFTHEADER fffthead;
    int temp = 0;
    int retcod;
    DataFP = fopen(filespec, "rb");
    if (fread((char *)&fffthead, sizeof(FFTHEADER), 1, DataFP)< 1)
    {
```

## Appendix C – Source Code

```
        printf("File I/O Error\n");
        return ((complex *)NULL);
    }
    *width = ffthead.Size;
    *height = ffthead.Height;
    RowData = (complex*)malloc((*width/2 + 1)*sizeof(complex));
    fft = (complex*)malloc((*width)*(*height)*sizeof(complex));
    for(row=0;row<*height;row++)
    {
        if(fread((char*)RowData, sizeof(complex),
                *width/2 + 1, DataFP) == *width/2 + 1)
        {
            C_PutRowData(RowData,fft,row,*width,*height);
        }
        else
        {
            printf("File I/O Error\n");
            break;
        }
    }
    free((unsigned char *)RowData);
    fclose(DataFP);
    return(fft);
}
C_PutRowData(dataline,fft,row,width,height)
complex dataline[];
complex *fft;
int row, width, height;
{
    int col;
    unsigned int offset1, offset2;
    offset1 =row * width;
    if (row!=0)
        offset2 = (height - row) * width;
    else
        offset2 = 0;
    fft[offset1].x = dataline [0].x;
    fft[offset1].y = dataline [0].y;
    for (col = 1; col<width/2; col++)
    {
        fft[offset1 + col].x = dataline [col].x;
        fft[offset1 + col].y = dataline [col].y;
        fft[offset2 + width - col].x = dataline[col].x;
        fft[offset2 + width - col].y = dataline[col].y;
    }
    fft[offset1 + width/2].x = dataline[width/2].x;
    fft[offset1 + width/2].y = dataline[width/2].y;
    return(0);
}
```

The structure, "complex," is made of two signed integers (2 bytes each), representing, respectively, the real and imaginary parts. Each integer is, in fact, a fixed-point number in which the decimal part is contained in the 7 lowest bits. The following example transforms a fixed-point "complex" into a floating-point "complex."

## Appendix C – Source Code

```
typedef struct COMPLEX {int x; int y;} complex;
typedef struct FCOMPLEX {float x; float y;} fcomplex;

fcomplex
FixedToFloatingPoint(number)
complex number;
{
    fcomplex fnumber;

    fnumber.x = (float)number.x / 128.0;
    fnumber.y = (float)number.y / 128.0;
    return(fnumber);
}
```

The contents of FFT.H follow:

```
#define N 512L
#define DIRECT 1
#define INVERSE 2
#define FFT_MAX_SIZE N
#define MAXINDEX 255
#define SCALE 30
#define FIXEDPOINT
#ifdef FIXEDPOINT
#define FFTCLASS int
#define FFTLCLASS long
#else
#define FFTCLASS float
#define FFTLCLASS float
#endif
typedef struct COMPLEX { FFTCLASS x; FFTCLASS y;} complex;
typedef struct LCOMPLEX { FFTLCLASS x; FFTLCLASS y;} lcomplex;
typedef struct fftheader {
    unsigned int Size;          /* in pixels per line */
    unsigned int Height;      /* number of lines */
} FFTHEADER;
struct FFT {
    FFTCLASS huge *Rp;
    FFTCLASS huge *Ip;
    unsigned int Length;
    unsigned int Height;
    unsigned int *Bit;
    complex *W;
    int LogN;
    RECT sourcearea;
    RECT spectrumarea;
    RECT inversearea;
    int NoDisplay;
};
#define SPECTRUM 1
#define PHASE 2
#define REAL 3
#define IMAGINARY 4
#define AOI_FFT (AOI_USER<<4)
```

## Appendix C – Source Code

```
int C_StoreDataInFile(MENU menu, char *filespec, struct FFT *fft);
int C_GetDataFromFile(MENU menu, char *filespec, struct FFT *fft);
int C_FreeData(struct FFT *fft);
int C_AllocateData(struct FFT *fft);
int C_TwoDimFFTd(struct FFT *fft);
int C_XorScanLine(int row, RECT *bounds, int horiz, int paneid);
int C_DisplayFFTline(lcomplex *Cout, RECT *lineaoi, int height, int flag);
int C_TwoDimFFTi(struct FFT *fft);
int C_DisplaySpectrum(struct FFT *fft);
int C_SetCoefTable(struct FFT *fft, int length);
int C_SetBitOrderTable(struct FFT *fft);
int C_SetDataEnviron(struct FFT *ptr1, int *ptr2, int *ptr3, char *ptr4);
int C_FreeData(struct FFT *fft);
int Menu_Pop();
int C_SetFFTEnviron(struct FFT *data);
#define SCAN_HORIZ 1
#define SCAN_VERT 2
#define MAX_SPECTRUM_SIZE 256
#define LOGTABLELEN 512
```

## Appendix D - References

---

If you would like more information about digital image processing, consult the following sources:

- Baxes, Gregory A., *Digital Image Processing: Principles and Applications*, John Wiley & Sons, Inc., New York, NY, 1994
- Castleman, Keneth R., *Digital Image Processing*, Prentice-Hall, Inc., Englewood Cliffs, NJ, 1979.
- Conrac Corporation. *Raster Graphics Handbook*, 2nd Edition, Van Nostrand Reinhold Company, New York, 1985.
- Foley, J. D., et al. *Fundamentals of Interactive Computer Graphics*, 2nd Edition, Addison-Wesley Publishing Company, Reading, MA, 1990.
- Gonzalez, Rafael C. and Woods, Richard E., *Digital Image Processing*, Addison Wesley Publishing Company, Reading, MA, 1992.
- Newmann, W. M. & Sproul, R. F. *Principles of Interactive Computer Graphics*, McGraw-Hill, New York, 1973.
- Pratt, William K., *Digital Image Processing*, John Wesley and Sons, New York, NY, 1978.
- Rosenfield, A. and Kak, A. *A Digital Picture Processing, Volumes I and II*. Academic Press, San Diego, 1982.
- Russ, John C., *The Image Processing Handbook*, 4rd Edition, CRC Press, Inc., Boca Raton, FL, 2002.
- Russ, John C., *Computer-Assisted Microscopy, The Measurement and Analysis of Images*, Plenum Press, New York, NY, 1990.
- Russ, John C., *Practical Stereology*, Plenum Press, New York, NY, 1986.

*Appendix D - References*

## Appendix E – Command Line Options

---

*Image-Pro Plus* can accept arguments on the command line to open images, run macros or modify the normal configuration of the program. You might use these command line options in writing a batch file (or script) to execute *Image-Pro Plus* with your chosen configuration, or to customize a desktop shortcut. You can use command line options in the **Target :** field of desktop shortcut or **Start** menu properties, from a DOS command prompt window, from the **Run** window, from batch files or scripts, or from any other application or programming environment that supports invoking an application with command line options.

The basic syntax of the command line is as follows:

```
<PathToImagePro>\<ExecutableName> [options] [images]
```

For a typical *Image-Pro 6.2* installation, <PathToImagePro> will be C:\IPWIN62, and <ExecutableName> will be ipwin32.exe.

Each option has the following syntax:

```
[ / ] [ - ] <C> <value>
```

This means that the option has to start with a '-' or '/' and be followed by a single character indicating one of the options documented below, followed by one or more space, followed by an appropriate value for the option.

As an example, make a copy of your *Image-Pro* desktop icon, then right-click on the copy and select properties. The command line options (which will be explained below) can be added after the executable name in the **Target :** field, so you could add " /n basic.mnu" to always invoke *Image-Pro* with the *Basic* menu:  
C:\IPWIN62\ipwin32.exe /n basic.mnu

The command line can also include the names of one or more images to open. These image file names should be fully specified (with a complete path to the image to open) and should be specified after all other options. Note that it is less useful to invoke *Image-Pro* with startup images from a desktop shortcut, but this can be very powerful when invoking *Image-Pro* from a dynamically written script or from another application.

## Supported Options

**Note:** Option letters are not case sensitive, and the '/' can be replaced with '!'. Unrecognized options will be ignored.

`/S <scriptfile>`

The `/S` option specifies a script file to be loaded on startup. The `<scriptfile>` value can be a fully qualified pathname to the script file (e.g. "C:\IPWIN62\Scripts\Demo.ipm"), or pathname that is relative to the *Image-Pro* folder (e.g. ".\Scripts\Demo.ipm").

`/M <macroname>`

The `/M` option specifies a macro to be run on startup. The `<macroname>` value can be any macro subroutine from the current script file, which is usually specified with the `/S` option.

`/N <menufile>`

The `/N` option overrides the currently selected menu file for the *Image-Pro* session if the specified menu file can be found in the *Image-Pro Resources* folder. If this option is used and the specified menu found, then the **Select menu** dialog will not be displayed. The selection of this menu for this session does not persist, so the currently selected menu file is not changed, nor the state of the **Don't prompt for menu selection** option (also called **Prompt for menu selection on startup** in the **Preferences** dialog).

`/T <toolbarfile>`

The `/T` option specifies a toolbar to be loaded and displayed on startup. The `<toolbarfile>` value can be a fully qualified pathname to the script file (e.g. "C:\IPWIN62\Scripts\PC\_LUT.ipt"), or pathname that is relative to the *Image-Pro* folder (e.g. ".\Scripts\PC\_LUT.ipt").



## **Specifying Images**

One or more images can be specified in the command line, after all other options. The images can be specified by a fully qualified pathname to the image file (e.g. "C:\IPWIN62\Images\SPOTS.tif"), or pathname that is relative to the *Image-Pro* folder (e.g. ".\Images\SPOTS.tif"). If the image name or path name contains spaces, the full path should be surrounded by quotes, and multiple image file names should be separated by spaces.

*Appendix E – Command Line Options*

## Appendix F – Scope-Pro/Stage-Pro Hints and Tips

The following are hints and tips that may assist you with your results when working with *Scope-Pro* and *Stage-Pro*.

### A. Capturing multiple tiled images from a microscope slide and working with *Scope-Pro*.

- 1) After you have defined the Scan Area, move the stage to the position where you want to start the image acquisition. Go to the *Stage* tab page and click **Current Position** (located in the upper right **Set XY Origin** group box).
- 2) To capture the images, click the **Acquire** button on the *Acquire* tab page. If you do not select the **Current Position**, *Scope-Pro* will begin the image acquisition at the currently set origin.
- 3) If you wish to capture another tiled image from the same slide, move the stage to where you wish to start the image acquisition first. Go to the *Stage* tab page and click **Current Position** to create the new origin position. Press **Acquire** to start the image acquisition.

### B. If you are planning to use a multiwell plate or a microscope slide holder containing multiple slides:

You will need to create:

- a Scan Area (capturing images from an individual well or microscope slide) and
- a Sample Pattern (i.e., for use with a 96-well plate or a 4-position microscope slide holder).

It is preferable to create the Scan Area *first*, and then the Sample pattern. Although it can be set in either order, setting the Area Scan and then the Sample pattern follows a more natural workflow.

### **C. Defining and refining a Sample Pattern for multiwell plates on multiple slide holders.**

You may want to develop a protocol that involves the use of a Sample Pattern with the use of “grouping” wells or slides within the pattern. Before you define your groups, you may want to verify that the pattern aligns well with the actual well plate or slides. The easiest way to do verify pattern alignment is to:

- 1) Define a group containing only the top left well or slide (A1) and another group containing only the bottom right well or slide.
- 2) Then select the first group in the **Groups Within Sample Pattern** list, go to the **Acquire** tab page, click **Tile Images**, and then click **Acquire** (now).
- 3) Go back to the **Sample Pattern** tab page and select the second group (of the bottom right well or slide) and once again acquire a tiled image of that well or slide.
- 4) Compare the two tiled images and verify that the well or slide is well centered in both images (remembering that you may need extra area on the bottom and/or right depending on the magnification and the coverage of a single capture frame).

If the both images cut off on the top and/or left, you will:

- 1) First correct the origin of the sample pattern to make sure that the pattern as a whole is aligned correctly.
- 2) Once the pattern is aligned, you will verify the well or slide spacing (set by the Delta X and Delta Y controls on the **Outer Sample Pattern** dialog box) by checking how the second image (of the bottom right well or slide) is aligned.
- 3) If the second image is cut off at the left, then the Delta X is a little too large. If it is cut off at the top, then the Delta Y is a little too large. If there is too much area of the previous well or slide shown on the left side of the second image, then the Delta X is a little too small, and if there is too much shown on the top, then the Delta Y is a little to small.

Remember that the Delta X and Delta Y are accumulated over the number of columns and rows (respectively) in the pattern, so with large patterns (e.g., a 384-well plate) very small changes can multiply quickly.

*Appendix F – Scope-Pro/Stage-Pro  
Hints and Tips*

If your protocol also includes grouping of wells or slides (for example, setting a group as “control” or “standards”, etc.), then you will want to define your groups next.

- 1) On the **Sample Pattern** tab page, click **Add Group** and name your first group.
- 2) Then click and drag on the sample pattern representation on the bottom of the page to define the dimensions of this first group.
- 3) You can continue to add new groups, name them, and define them by clicking and dragging the rectangular area to be grouped.

**D. Archiving Sample Patterns for repetitive use in the lab.**

If you are developing a protocol that involves the use of a Sample Pattern (with or without the use of “grouping” wells or slides within the pattern), you will want to use the **Save As Template** control on the **Outer Sample Pattern** dialog.

- 1) When you first define the Sample Pattern (whether you use the Pattern Configuration Wizard or not), you will want this control to be unchecked.
- 2) Set up the dimensions of the pattern and display options, and then click **Save**.
- 3) Next, verify the pattern alignment and define any groups that you want to use (as described in **Option 1** above).
- 4) Now that the pattern is complete and correct for your protocol, you can use the **Save As Template** control. Use **Edit Current Pattern** to revisit the **Outer Sample Pattern** dialog, click **Save As Template**, and then click **Save**. The pattern will be marked as read-only so that it cannot be accidentally modified.

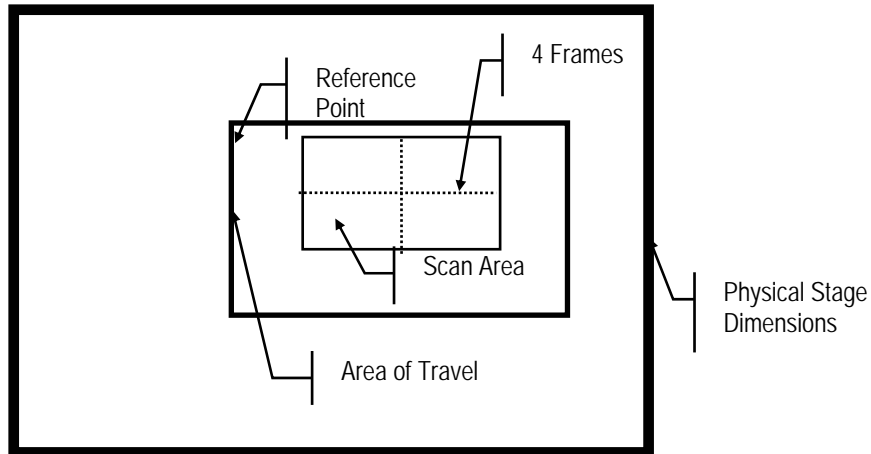
---

**Note:** *You will still be able to modify any groups defined on the pattern, so that you can do any experimentation or verification that you need to at any time. However, any such changes will be **temporary** during that Image-Pro session and will **not** be saved to the pattern template.*

---

## E. Notes on Area of Travel

This is the only dialog that affects the travel area of the stage (i.e., the physical limits of stage movement). The following diagram illustrates the “area of travel.”



Either **Set Area of Travel by corners**, **Use physical limits of stage**, and **Use Current Position** define the allowable limits of motion for *Stage-Pro*. Since stages have powerful motors, they can damage your microscope, especially the optics, if they travel too far. Hence, setting limits of travel can help you avoid costly damage to your microscope.

If you have adjustable limit switches on your stage, set them to the limits where your samples will lie. For slides, this might be the corners of the slide; for well plates, the corners just beyond the corner wells. If you can vary the microswitches, then you can choose **Use physical limits of the stage** to set the area of travel.

If your limit switches are non-adjustable, set the area of travel with the **Set Area of Travel by corners** selection.

If you choose **Use Current Position**, then the **mm in X** and **mm in Y** edit boxes will be enabled. If the edit boxes contain numbers, the **OK** button will be enabled. When you choose this option, the current stage position will be used as the origin of the scan area and the center of the travel area. The extents of the travel area will be “**mm in X**” and “**mm in Y**” on both sides of the current position. **No checking will be performed.** We will assume that the user gave us good information and that there is room to move in that area.

The seven choices shown in the **Select Area of Travel** dialog box are divided into three groups. The top three options (**Set Area of Travel by corners**, **Use physical limits of stage**, and **Use Current Position**) in **Determine New Area of Travel** will define the area position

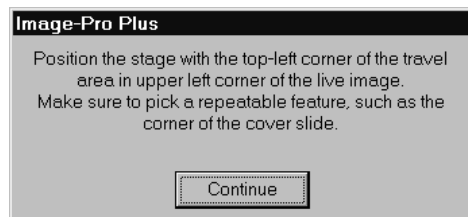
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and extent. The next two, in **Previously Defined Area of Travel**, will only determine the origin for the previously defined extent. You must previously have set one of the top three options in order to enable one of the bottom two options.

Only one choice can be selected among the following describes the options available for determining the active area of travel:

- a. **Use Joystick to Set Area of Travel by corners:** User-determined origin. This option will be the default selection if the active area has not previously been determined.

You will be prompted to move the stage to an easily reproducible top-left corner position (i.e., a position that displays a recognizable feature in the top-left corner of the capture area; for instance, the corner of the cover slip). This top-left corner position will then become the reference point for all subsequent scan areas and patterns.



You will then be prompted to move the stage to the bottom-right corner of the area desired. The extents determined by this operation will be retained for future *Stage-Pro* sessions.

- b. **Use physical limits of stage:** Automatically determines the maximum limits of the stage by driving the stage up and to the left until it hits the limit switches; then it moves the stage down and to the right until hits the lower right limit switches. The origin is set to a point half-way between the limit switches on each axis, and the stage is left in this position. Be *very* careful that there is nothing in the way of the stage when you are using this option. The extents determined by this operation will be retained for future *Stage-Pro* sessions.

---

*Note: If your stage is not equipped with limit switches, the **Use physical limits** option will not be available.*

---

- c. **Use Current Position as the center of the Area of Travel:** The current stage position will be used as the origin of the scan area and the center of the Area of Travel. The extents of the travel area will be “**mm in X**” and “**mm in Y**” on both sides of the current position. **No checking will be performed**, but

## Stage-Pro/Stage-Pro

physical limit switches, if present, will be respected. We will assume that the user gave us good information and that there is room to move in that area.

- d. **Use Joystick to Move to origin of existing Area of Travel:** This option prompts you to move the stage such that the original of a previously-defined Area of Travel is in the top left corner of the Live Preview image. Once you have located the Origin of the Area of Travel, the stage will move to the origin (top left corner) of the last scan area. If you wish to use this option, be sure to use an easily-reproducible landmark, such as the corner of your slide, as the origin (top left corner) of the Area of Travel when you first define it.
- e. **Use current position (parked) as origin of the existing Area of Travel:** This options uses the current position of the stage as the origin (top left corner) of the stage, and then moves the stage to the origin of the last scan area.

---

*Note:* For this option to work properly, you must do three things:

1. Use either option **a** or **b**, above, to set the **Area of Travel** the first time that you use *Stage-Pro*.
  2. Enable the **Park at Shutdown** option on the *Stage* tab to allow *Stage-Pro* to park the stage in a known location
  3. DO NOT move the stage unless *Stage-Pro* is active. If you do move the stage, you will have to reset the **Area of Travel** again before this option will function correctly.
-



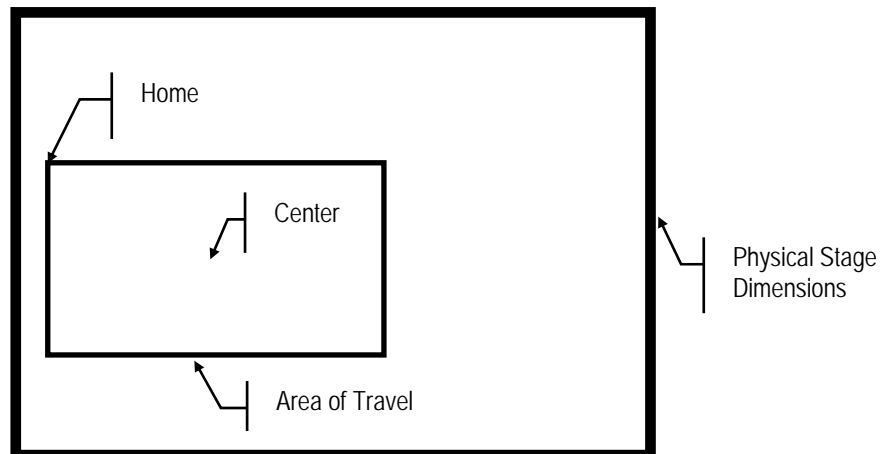
Appendix F – Scope-Pro/Stage-Pro  
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**Important:** The *top-left* corner of the area of travel is the reference point for all subsequent scan areas and patterns. If you define your area of travel using **Set Area of Travel by corners**, you will need to use the *same* reference point consistently, or your scan areas will not be properly located.

To insure that you use the correct reference point, you should “park” the stage before exiting *Stage-Pro* (use the **Park at shutdown** option on the **Stage** tab page; refer to page 7-**Error! Bookmark not defined.**).

You can also select the **Set origin of Area Travel** and use a slide bearing a registration mark as a reference point. The easiest and most consistent reference point is to use the extreme top-left corner of the stage travel area, but lenses and other equipment under the stage can make it difficult to use the maximum limits of stage travel safely.

The “*origin*” of the Area of Travel will be the “Home” position as illustrated here in the below diagram, and the origin of the scan area will be the “Center” position.

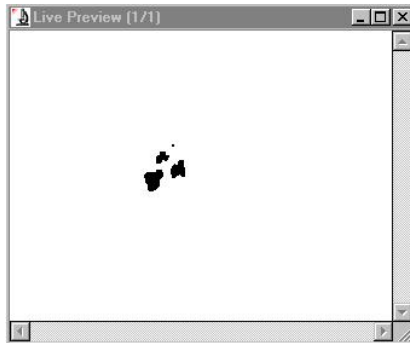


## F. Notes on Stage Squaring

The **Stage Square** function is used to square the stage movement with the video camera. To get accurate results from *Stage-Pro* you must have the stage and the camera aligned. This means that when the stage moves in only one axis, the video image moves only in *that* specific axis. If you move the stage in the X-axis, you should see no movement or drift in the Y-axis of the video image.

Because the mechanical axis of rotation of the video camera is usually not the same as the pixel center of the video image, the first steps involve finding the camera's axis of rotation.

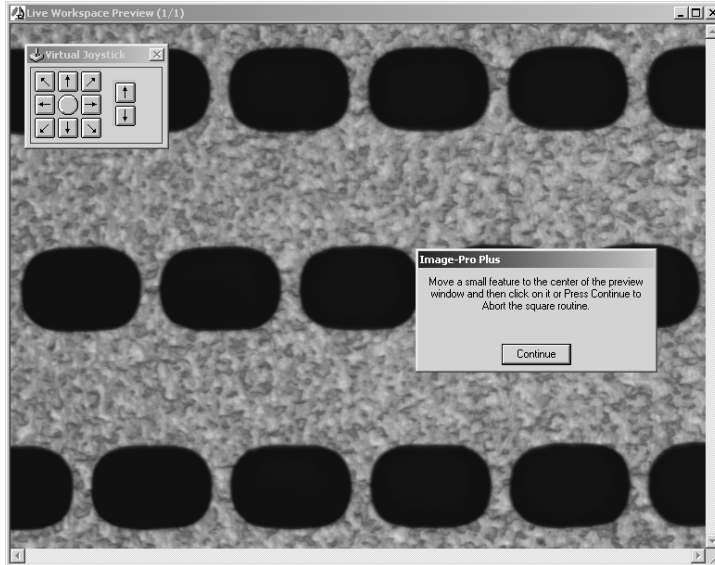
Below is a step-by-step example of how to square the stage movement using the following image:



### Procedure For Squaring the stage movement

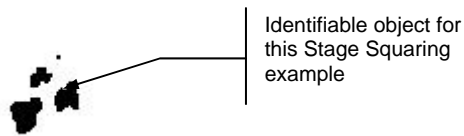
#### 1. Position the stage using the joystick.

When you first press the **Square** button *Stage-Pro* will bring up the following dialog and live workspace preview.



#### 2. Use the joystick to move the stage so that some small identifiable object is near the center of the stage.

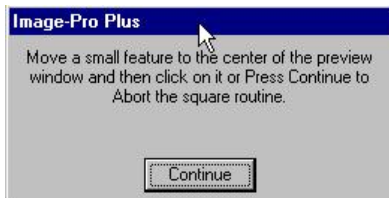
In this example, the object we will use is the smallest of the four objects shown in the live preview screen.



Remember that this is a *workspace* preview, which is not as smooth as a normal preview. You will have to move fairly slowly when you do this.

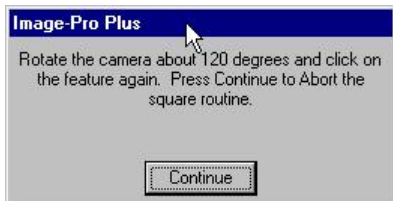
## Stage-Pro/Stage-Pro

If you wish to exit out of the Stage Squaring routine, press the **Continue** button on the dialog box shown below.



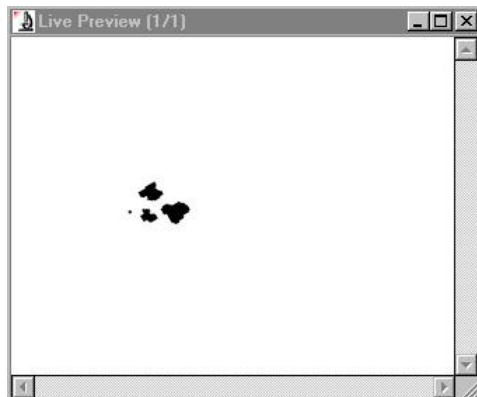
### 3. Click on your identifiable object in the workspace preview.

After you center and click on the small object, the following dialog box appears.



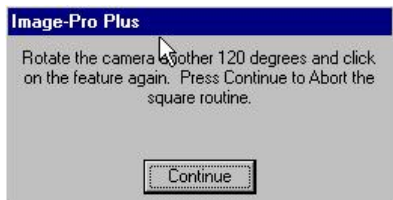
### 4. Follow the instructions and rotate the camera 120° (about a third of the way around).

Note that the your identifiable object has moved from the top to the left, and that an object can move a considerable distance in the workspace preview after rotation. Do **not** move or reposition the feature by moving the joystick!

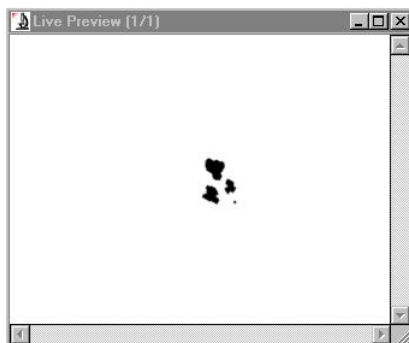


**5. Click on your small identifiable object again.**

The following dialog box appears with another set of instructions to rotate the camera.



**6. Rotate the camera another third of the way around (120°).**



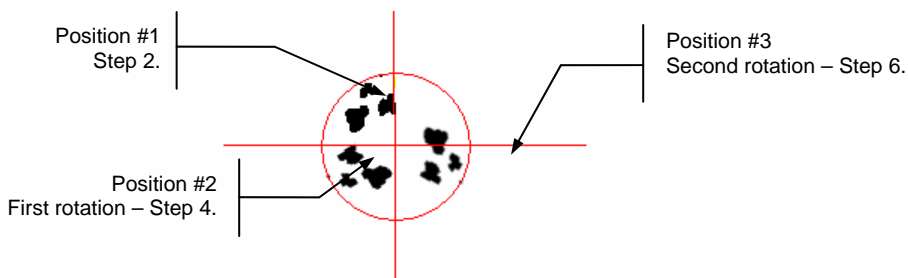
The above figure shows the camera rotated for a third time, and your small identifiable object is now on the lower right.

## Stage-Pro/Stage-Pro

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**Note:** The image shown in the diagram below demonstrates how **Stage-Pro** finds the mechanical center of the video image, based on the camera rotations you have just done. You will **NOT** see this diagram; it is for demonstrative purposes only.

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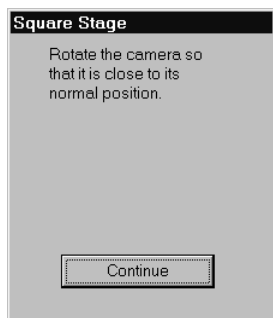


The above shows the images at the three camera positions superimposed on each other. **Stage-Pro** finds the circle that contains the three points that you clicked. The center of this circle is the camera's mechanical axis of rotation, and this point will be used to square the stage.

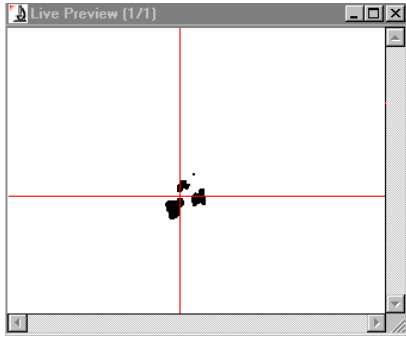
---

### 7. Once you have rotated the camera, click again on the previously selected object.

The **Stage Square** dialog box appears.



8. Follow the prompt and rotate the camera back near its normal position.

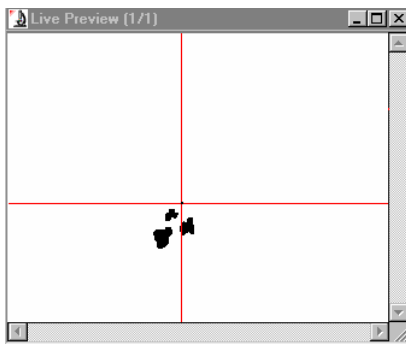


9. Click on the **Continue** button in the **Stage Square** dialog box.

The following dialog box appears with the next set of instructions.



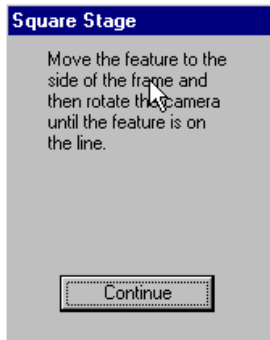
10. Move your small identifiable object under the crosshair.



11. Click on **Continue**.

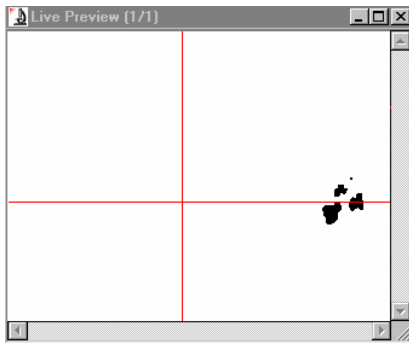
The last **Stage Square** dialog box appears.

## Stage-Pro/Stage-Pro



### 12. First, move the object to one side.

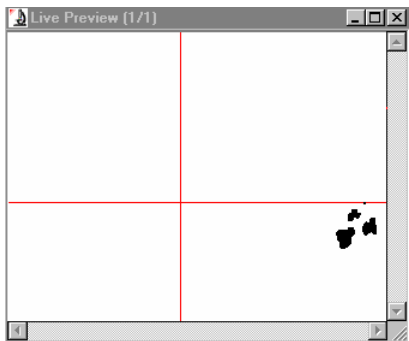
Make sure you only move the stage in the *X*-axis, as shown below.



Note that because the stage and camera are not square, the object appears to have moved up.

### 13. Finally, rotate the camera.

Rotate the camera until the object moves down to the *X*-axis.



In the figure above, the camera has been rotated so that the small object is back on the line. Tighten the camera being careful not to disturb the alignment.



*Appendix F – Scope-Pro/Stage-Pro  
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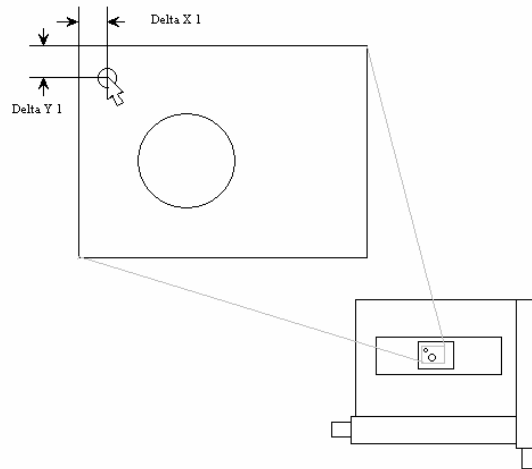
At this point the camera and the stage should be square. You can test this by moving the stage back and forth in the X-axis. The object should remain on the line as you move it across the workspace preview. When done, press **Continue** to dismiss the **Stage Square** dialog.

## **G. Notes on XY Calibration**

**Calibration** is the process of determining the size of a pixel in some absolute measurement (*Stage-Pro* supports millimeters (mm), microns, and mils). Once a pixel's dimensions are calibrated, that spatial calibration can be used to calculate the size of an image feature or the distances between those features.

In *Stage-Pro*, a calibration is maintained for each objective, but you should also be aware that changing other optics between the sample and the camera may affect the calibration.

Below is an example of a lens calibration in *Stage-Pro* using the **Calibrate Lens (XY)** button. Here, you have been prompted to move the stage so that a recognizable object (the small circle in the diagram) is in the upper left corner of the live preview image.



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**Note:** *Most capture devices, when set up correctly, should display an aspect ratio very near 1.0. The inability to calibrate your lenses to a 1.0 aspect ratio may indicate that the camera needs to be aligned with the axis of the stage movement. See the **Stage Square** button on Error! Reference source not found...*

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## Stage-Pro/Stage-Pro

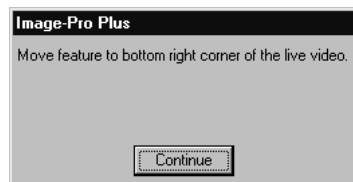
Clicking the **Continue** button on the message box below sets the current stage position.



*Stage-Pro* captures an image and prompts you to click on the same object previously selected in the acquired image. Pick a sharp corner or recognizable feature of the object and click on the object in the captured image. The object will appear larger in this image and may appear less sharp and not as well-defined as in the live preview. (If the object was not close to the top-left corner, you may need to use the scroll bars to bring the object into view.)

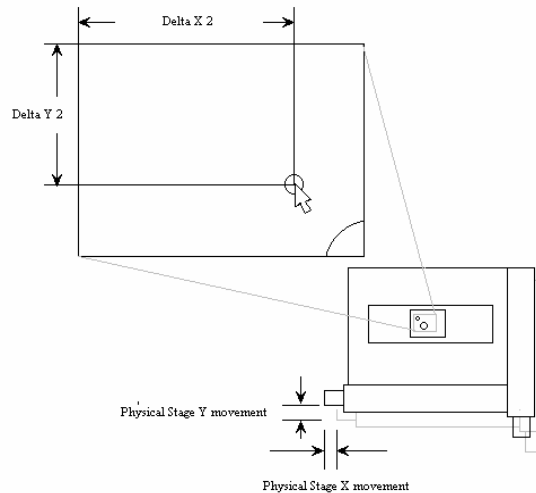
When you click on the object, *Stage-Pro* calculates the X/Y pixel offset of that position (labeled Delta X1 and Delta Y1).

Next you are prompted to move the stage to some position such that the recognizable object appears in the lower right corner of the live preview window.



## Appendix F – Scope-Pro/Stage-Pro Hints and Tips

After you move the stage and click **Continue**, *Stage-Pro* captures a new image into the workspace, as shown below.



When you click on the object's new position in the captured image, *Stage-Pro* calculates the XY pixel offset of the new position (labeled Delta X2, Delta Y2).

*Stage-Pro* now asks the stage where it is and gets a physical distance moved (labeled Physical Stage X and Y movement in the diagram). The **Accept Calibration** dialog box appears, and *Stage-Pro* can now calculate all of the numbers shown on the **Lens** tab. For example, pixel per millimeter in X would be calculated as: the absolute value of (Delta X2 minus Delta X1) times Physical Stage X movement. Note that in this example, the stage was in fact moved in the negative X and Y direction.

As you can see from the above example, the size of the stage movement is dependent on the currently selected lens or objective. That is, with a higher power objective, you will need a smaller movement to move the object from corner to corner of a fixed size video frame. This is the reason that a separate calibration is needed for each objective you plan to use.

These numbers are used to build an *Image-Pro* spatial calibration, which is passed from *Stage-Pro* back to *Image-Pro* for use in *Image-Pro's* measurement functions. The current *Stage-Pro* calibration will be loaded as *Image-Pro's* current system calibration for any image captured through *Stage-Pro*.

There are a number of factors that determine the accuracy of your calibration. Two of the most common errors come from stage squaring and object selection. To get an accurate calibration, the stage and the video camera must be square. This means that when the stage is moved in one axis the image from the video source moves only in that axis. If the stage is moved only in the X-axis you should not see any drift of the video image in the Y-axis. Please read the previous section on the stage squaring routine.

## Stage-Pro/Stage-Pro

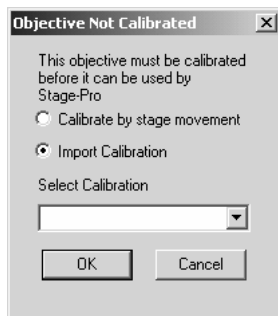
The second common error is object selection. You need to pick something as small and as distinct (“recognizable”) as possible. Because of lighting and other factors, the object will never look quite the same captured on one side of the video frame as it does captured on the other. If you pick an irregular-shaped object, it will be difficult to find the same place to click on. Try to pick something with a sharp corner or the intersection of two lines. The closer you click to the same place on your object, the better your calibration. With a little care aspect ratios should be within a few thousandths of 1.0.

## H. Importing an *Image-Pro* Calibration for Stage-Pro

This feature allows you to select an existing *Image-Pro* spatial calibration instead of calibrating an objective by stage movements.

When ever you select a lens that is not calibrated, such as the first time that you are running *Stage-Pro*, you will get a message box telling you that the lens is not calibrated and asking if you want to calibrate it now. At least one lens must be calibrated.

The dialog pictured below will come up when ever you select an un-calibrated lens or press the **Calibrate Lens XY** button. It will default to the first choice, *Import Image-Pro calibration*. If you choose the default, calibration will proceed as normal.



**Selecting Calibrate by stage movement** uses the current method described under the **Lens/Magnification** tab page..

**Selecting Import Calibration** is the default selection. Clicking this button displays a drop-down list box. You can select a current *Image-Pro* reference calibration setting to load as the current system calibration, and you will be asked to fill the number of pixels per unit. You should be sure that you have already created reference calibrations in *Image-Pro*, using the *Calibration Wizard* or the **Calibration** dialog.

To use, **Import calibration**, you must have a good *Image-Pro* calibration from an image captured through the objective that you want to create a *Stage-Pro* calibration for, and from the camera that you plan to use with *Stage-Pro*. There are a number of ways to get that *Image-Pro* calibration, but the obvious one follows:

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1. Move the objective you want to use into position.
2. Set the camera to the way you intend to use it. If you are using a digital camera make sure that the binning and frame size are set to what you intend use when capturing the real images.
3. Capture a frame of something of known dimensions, i.e. a stage micrometer.
4. From the *Image-Pro* **Spatial Calibration** dialog create a new calibration with the **Image** function of the **Pixel/Unit** group box.
5. From the *Stage-Pro* **Lens** tab select the objective you are creating the calibration for and choose **Import Calibration**.
6. Select the calibration you just created (in *Image-Pro*) from the drop down list box and press **OK**.
7. *Stage-Pro* will respond with the normal dialog showing calibration info and giving you the option to accept or cancel. If you accept, a *Stage-Pro* calibration will be created in the normal *Stage-Pro* manner, i.e. 2X. *Stage-Pro* will use this name and not the one created with *Image-Pro*.

Stage-Pro/Stage-Pro

## I. Notes on Scan Area

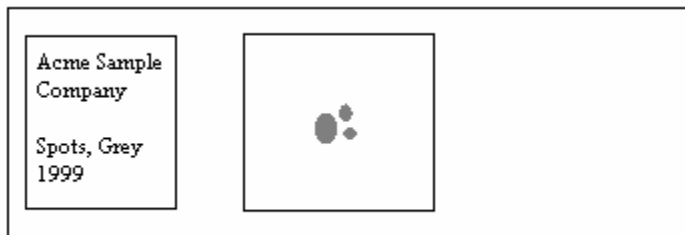
The basic purpose of using *Stage-Pro* with a motorized stage is to move a sample under an objective in an orderly, accurate, and repeatable manner, such that you can cover all of the area that you are interested in. First some basics.

*Stage-Pro* defines the “**Area of Travel**” as the limits of stage movement in X and Y. There are physical limits on the range of movement of the stage. The length of the drive mechanism and the placement of the end switches determine these limits.

Stages come with either adjustable or fixed end switches. *Stage-Pro* refers to the area between these switches as the “**Physical Area of Travel**”. Because there can be obstructions such as objectives or other hardware, sometimes it is not possible for the stage to move to its physical limits. In this case, the user defines a subset of the physical Area of Travel and *Stage-Pro* restricts its movements to that subset. *Stage-Pro* calls this a “**User-defined Area of Travel**”.

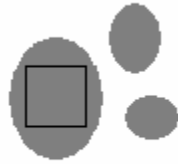
Within this Area of Travel is usually a smaller area that the user is actually interested in; *Stage-Pro* refers to this as the “**Scan Area**”. (Note: In previous versions of *Stage-Pro*, the Scan Area was referred to as the “area of interest” or “AOI”.)

For this example, we will use the slide in the diagram below. We will assume that the Area of Travel is large enough that any part of the slide (including the label) can be moved under and viewed through the objective. The user is interested in the gray objects underneath the cover slip.



*Appendix F – Scope-Pro/Stage-Pro  
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What the camera sees in one video frame will depend on the currently selected objective. Shown below, a 4X objective (on the left) video frame will “see” an area smaller than the large gray object. By switching to a 2X objective (below right), however, the camera can “see” the whole object.



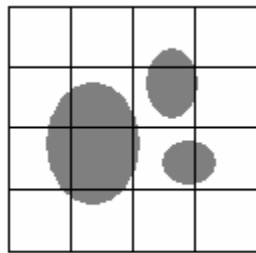
Sample seen at 4X



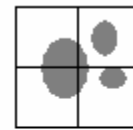
Sample seen at 2X

The above example demonstrates that to represent the whole sample, you will need more video frames with a 4X objective than you will with a 2X objective.

*Stage-Pro* defines this number of frames as the “**Scan Area**”. The diagram below illustrates Scan Areas for the sample with 4X and 2X objectives.



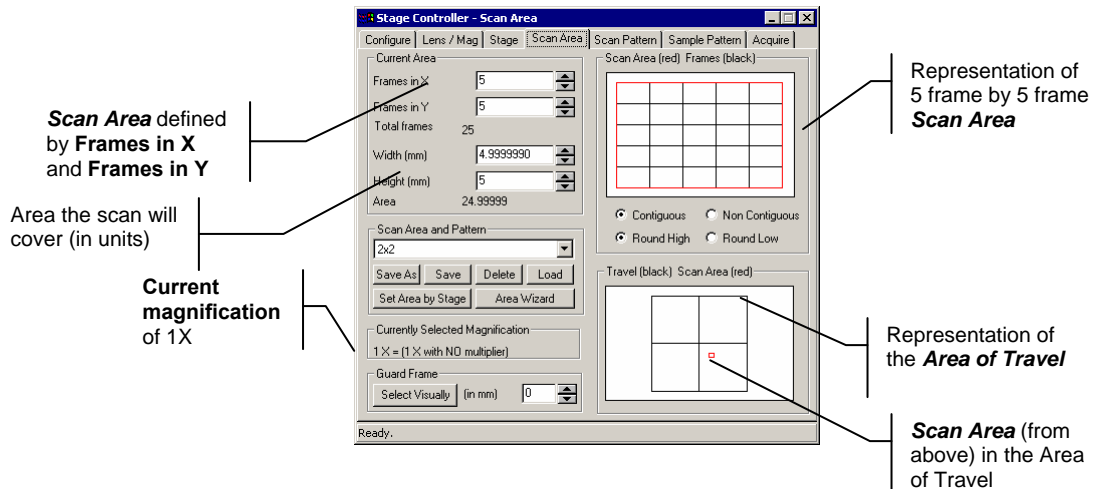
Sample seen at 4X



Sample seen at 2X

## Stage-Pro/Stage-Pro

Now, we'll take look at the *Scan Area* tab page to see how *Stage-Pro* shows this:



In the *Scan Area* tab page above, you can see that the currently selected magnification is **1X**. The **Frames in X** and **Frames in Y** are both set to 5, and the area the scan will cover is about 4.999 x 5 mm. The (red) box on the upper right side of the tab shows a representation of the 5 frame by 5 frame *Scan Area*.

In this example, the *Scan Area* was defined by frames in X and Y; therefore the area is an even multiple of the frame size. If the *Scan Area* had been defined by millimeters in X and Y, there would probably be some overlap. For more details on this, see the section on **Round High** and **Round Low** in **Relative Position of Frames**.

The box in the lower right side of the tab shows a representation of the *Area of Travel*. In the center of the *Area of Travel* box is a smaller (red) box, which corresponds to the *Scan Area* from above.

Note that the *Area of Travel* is much larger than the *Scan Area*. The smaller *Scan Area* (the area you are interested in) can be anywhere within the larger *Area of Travel*. The (red) *Scan Area* box is near the center in this example but will probably have to be moved, depending on where the slide is physically located.



*Appendix F – Scope-Pro/Stage-Pro  
Hints and Tips*

The upper left corner of the Scan Area is called the **XY origin** or the **Scan Area origin** on the **Stage** tab page will let you manually set the origin. Set this origin to make the upper left corner of our 4 x 4 pattern correspond with the physical location of the sample.

In the next example below, the magnification has been changed to **2X**. With a 2X objective the camera will “see” more of the sample in one video frame than with the 4X shown previously.

**Frames in X and Y** are now set to 2

Area the scan will cover is still approximately the same size as before

**Current magnification** of 2X

Representation of the **Area of Travel**

Representation of the **Scan Area**

**Scan Area** in the Area of Travel has moved

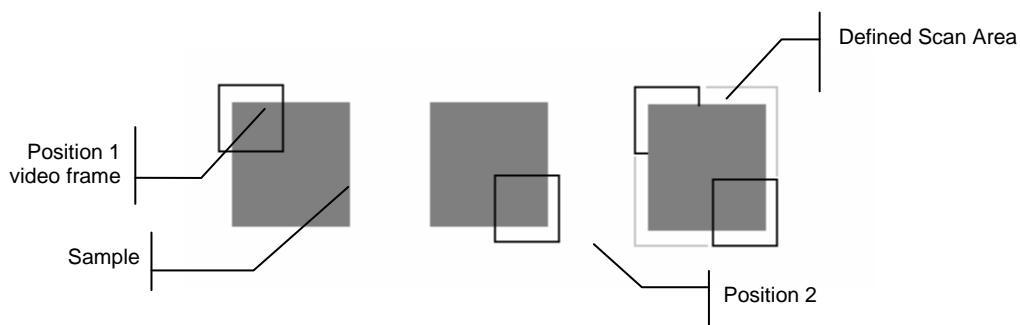
The **Frame X** and **Frame Y** values are now set to 2 frames each. The width and height are still about 1.6 x 1.2 mm. The point here is that we will still scan the same physical area on the slide, we will now just do it in fewer frames. Note also that the Origin has been moved. The Scan Area is now above and right of its previous position in the Area of Travel box.

## Stage-Pro/Stage-Pro

There are a number of ways to set the *Scan Area*. In the example above, we manually set the area to some number of video frames in X and Y and then moved the stage to where we want to start and manually set the origin.

In most cases, it more convenient to set the area by moving the stage and viewing the area with the camera. When you use the stage to set the Scan Area, you can define the extents by defining two opposite corners or by defining the four sides. The current magnification and the shape of the sample will determine the method you choose.

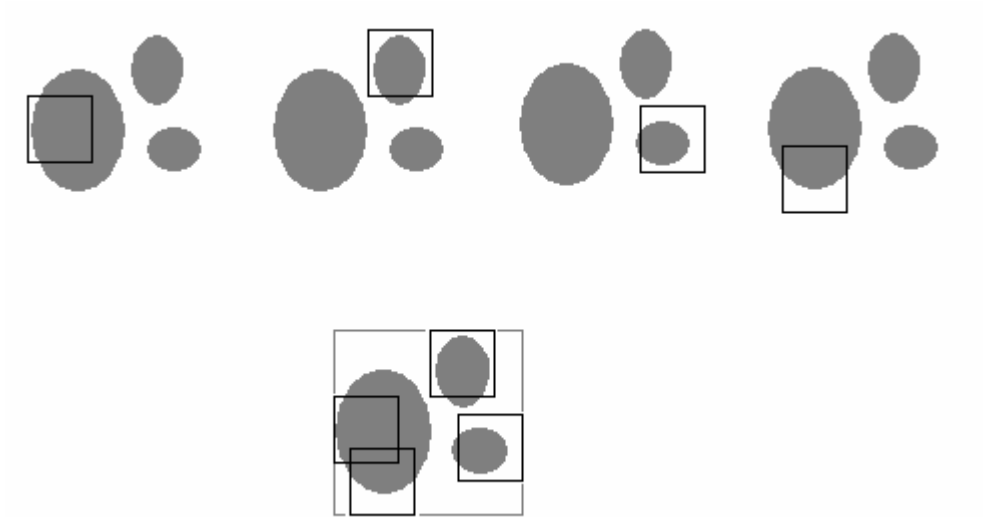
The simplest example is defining a Scan Area for a rectangular sample by using the *corners*.



Because you can only see the area inside of the video frame, it is difficult to define a Scan Area by corners for a round or irregular sample. In this case, it makes more sense to define the Scan Area extents by four sides.

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Hints and Tips*

The figures below show the stage movement for defining the Scan Area extents by the four *sides* of the sample



The stage is moved first to the left, then the top, then the right, and finally to the bottom. The last figure shows the extents defined by the four positions.

Remember that when defining a Scan Area with the stage, you are inherently defining it by physical measurements and *not* by video frames. Since we only capture in multiples of video frames, the end result will be determined by the **Round Low/Round High**.

Stage-Pro/Stage-Pro

## **J. Notes on Sample Pattern**

*Stage-Pro's Sample Pattern* functions let you use a given **Scan Area** multiple times, over different positions on the stage. In other words, instead of capturing a single frame at each position in the sample pattern, the sample pattern can be used to define a set of Scan Area origins. At each point of the Sample Pattern (at the top-left corner of the well or slide), the origin is set and the Scan Area traversed.

As an example, assume that the stage will hold four slides.

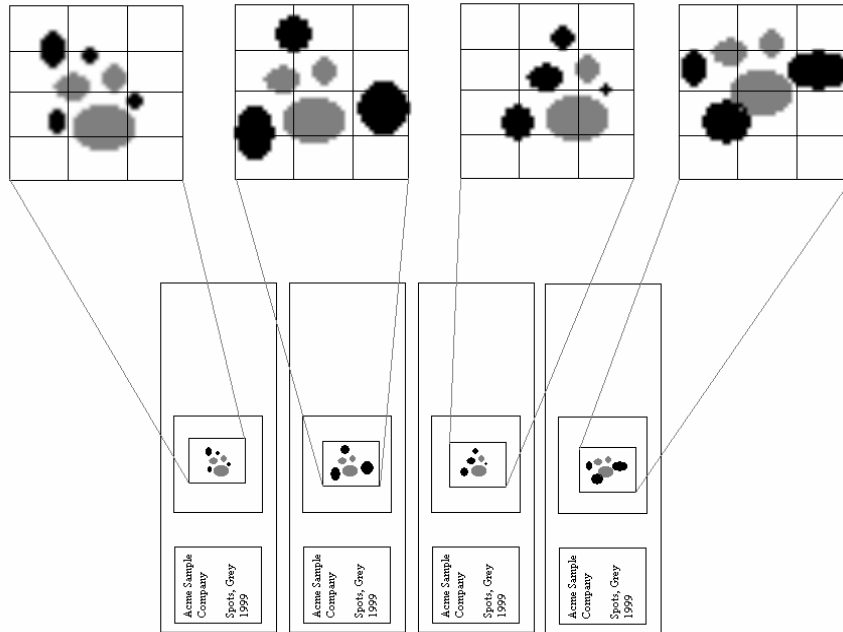
### **1. Define an appropriate Scan Area for one slide.**

First you would define an appropriate **Scan Area** for one slide. In this example below, a 3 x 4 Scan Area will cover the entire sample on an individual slide. After *Stage-Pro* has moved through the Scan Area on the first slide, the origin will be moved to the right and reset. The process repeats where the same Scan Area pattern is repeated at a new position on the next slide, and continues until the last slide has been covered.

### **2. Set the Sample Pattern origin.**

Move the stage to the position of the Scan Area origin for Well A-1 and click the **Set Sample Pattern Origin** button.

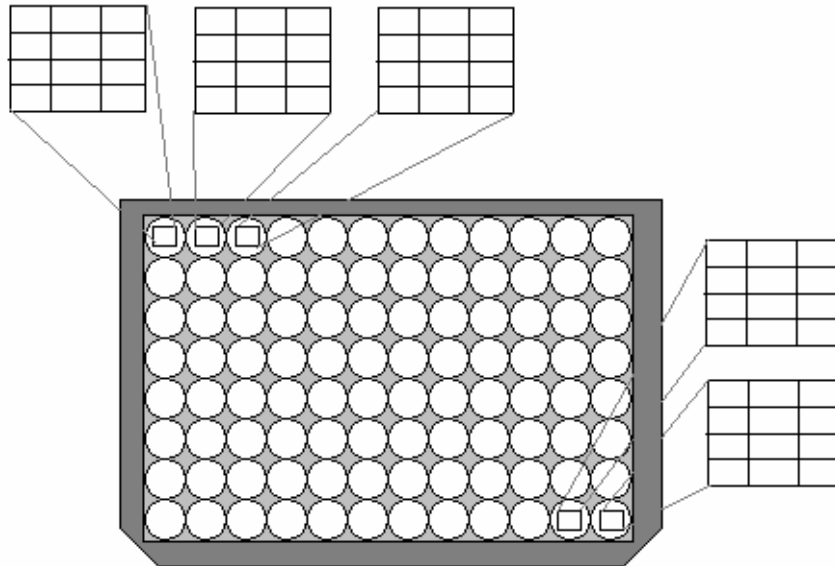
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In the above example, the outer **Sample Pattern** was traversed in just the X direction (a “row” of Scan Areas), but *Stage-Pro* can handle both rows and columns of Scan Areas.

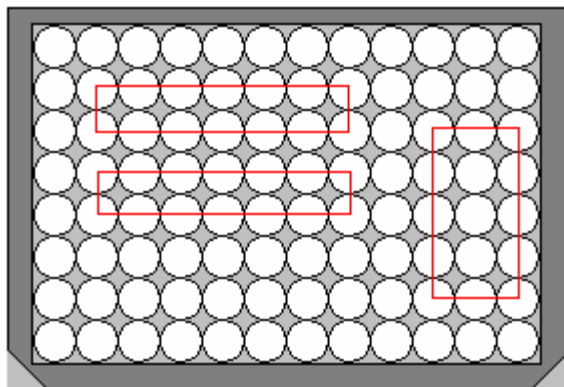
In the next example below, we will assume that the stage is set up to hold a multiwell plate.

## Stage-Pro/Stage-Pro



This example shows a 96-well plate. The example shows a 3 x 4 Scan Area that will be repeated 96 times over the surface of the whole plate. The above diagram leaves out the intermediate 91 positions but *Stage-Pro* will move through them all. This brings up the next feature in the Sample Pattern functions; the idea of a **Group**.

In some instances you may not want to scan through all the positions on something like a large well plate. *Stage-Pro* will let you define rectangular sub sets of the plate and access them as a group. For example, the first row could be defined as Group 1 and the second row as Group 2. Groups are not restricted to single rows. A **group** could be defined as any smaller rectangular area or sub area.



Appendix F – Scope-Pro/Stage-Pro  
Hints and Tips

The figure above shows some possible groups. To define a group, click and drag the cursor over any rectangular sub area.

New Outer Sample Pattern

Pattern Configuration Wizard

Name: Costar96WellPlate

Number of columns in X: 12

Number of rows in Y: 8

Delta X: 90

Delta Y: 90

Gap  Round  Slide

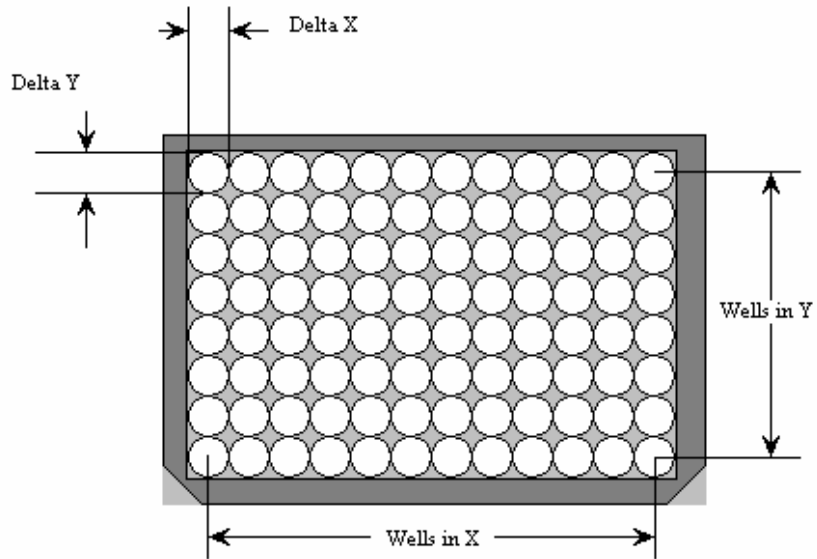
You have opened this dialog box to edit an existing outer sample pattern.

Save as Template (read-only)

Save Cancel

To define an *outer* Sample Pattern, you will use the dialog box above. The edit boxes for rows and columns determine how many times the *inner* Scan Area will be repeated. The **Delta X** and **Delta Y** values are the distances (in current units, set in *Error! Reference source not found.*) that the origin of the Scan Area will be moved with each iteration of the outer Sample Pattern loop. *Stage-Pro* will reference the wells with a letter for the row and a number for the column. In this example, the wells range from A1 to H12 (rows A through H, and columns 1 through 12).

Stage-Pro/Stage-Pro



The last concept in the layout is the Sample Pattern origin. Just as the user needs to tell *Stage-Pro* where to start for a Scan Area, the user must tell *Stage-Pro* where the Sample Pattern will start. The Sample Pattern origin will be the same as the Scan Area origin for well **A1**.



## Default Settings

In addition to setting the correct **COMM** port, the communications settings on the **Configure** tab must agree with your controller's settings. The following are the default settings:

Microscope/Stage Type	Baud Rate	Stop Bits	Parity
<i>Cairn Optoscan</i> <sup>1,4</sup>			
<i>CRI Micro*Color filter</i> <sup>2</sup>			
<i>Leica 2 Series</i>	19200 1		None
<i>Leica DMRXA</i>	9600 1		None
<i>Ludl BioPoint</i>	9600 2		None
<i>Ludl Mac 2000/2002/5000</i> <sup>3</sup>	9600 2		None
<i>Märzhäuser LStep</i>	9600 2		None
<i>Märzhäuser/ITK</i>	9600 1		None
<i>Media Cybernetics Stage Controller</i>	9600 1		None
<i>Nikon E1000</i>	9600 1		None
<i>Nikon Focus</i>	9600 1		None
<i>Nikon TE2000</i>	9600 1		None
<i>Olympus BX2</i> <sup>4</sup>	19200 1		Even
<i>Olympus IX</i> <sup>4</sup>	19200 1		Even
<i>Olympus Provis AX-70/80</i>	9600 1		None
<i>Prior H128</i>	9600 1		None
<i>Prior OptiScan</i>	9600 1		None
<i>Sutter Lambda 10-2</i>	9600 1		None
<i>Sutter Lambda DG – 4/5</i>	9600 1		None
<i>Vincent Associates D3</i>	9600 1		None
<i>Vincent Associates T1 or D1</i>	9600 1		None
<i>Zeiss AxioPlan 2</i>	9600 1		None
<i>Zeiss AxioVert</i> <sup>5</sup>	9600 1		None
<i>Zeiss MCU-27</i>	9600 2		None
<i>Zeiss MCX2 Stage</i> <sup>5</sup>	9600 1		None

<sup>1</sup> The Cairn Optoscan uses a Analog to Digital converter board. See the device specific documentation for configuration details.

<sup>2</sup> The CRI Micro\*Color filter's communications parameters are hard coded at 9600, 1, None.

<sup>3</sup> The Ludl Mac 5000 can use the USB port instead of a serial port, if necessary.

<sup>4</sup> This driver requires operating system drivers that are installed by Scope-Pro but may be updated by the hardware manufacturer at any time.

<sup>5</sup> This driver also requires the Zeiss Micro Toolbox, available from our web site, or from Zeiss.

Stage-Pro/Stage-Pro

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