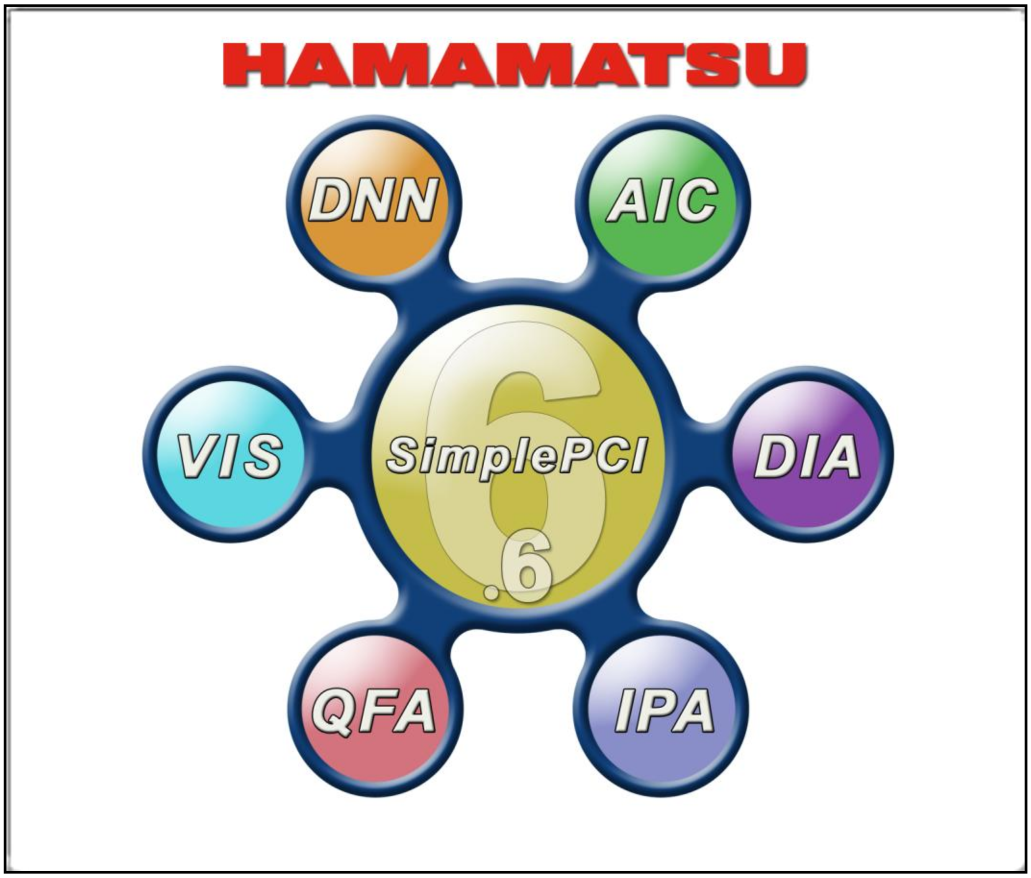
Welcome to *SimplePCI* © Help for Version 6.6!

The new SimplePCI 6.6 Splash Screen:



Version 6.6.0 - Help System Rev.0

Last updated: Tuesday, July 15, 2008

What's New?

Some exciting new modules are now available for SimplePCI...

SimplePCI
Help System

SimplePCI Core

At the core of SimplePCI are the Image Documents and Data Documents, allowing sophisticated image enhancements, presentation and analysis operations to be easily applied to single images or image sequences. Whether the application is in Life Science or Material Science, SimplePCI is the solution for your imaging needs.

Threshold objects or manually draw multiple regions for analysis, and then present the data as a list, statistical summary, or histogram. Field analysis of the data provides both summary and individual region information. Define ROI's by thresholding or drawn manually. ROI's can be moved manually during an experiment. Over 150 measurements are included, as is the ability to define custom measurements. Data can be viewed in 2D and 3D graphs, tables, or spreadsheets.

AVI movies can be created from Isometric plots, image sequences or 3D rendering. Image sequences can be processed as minimum/maximum projections or combined to create a montage.

Support for Image Enhancement filters and image registration included.

AIC: Automated Image Capture

Automated Image Capture module provides cost-effective image capture from a wide array of cameras, filter wheels, shutters and monochrometers.

- CD: Camera Devices

- Analog and Digital Cameras
- Time Sequences
- Image Processing

- SD: Standard Devices (includes -CD)

- Z-axis Motors
- Motorized XYZ Stages
- Filter Wheels
- Shutters
- RGB Tunable Liquid Crystal Filters
- Tunable Monochromators
- TTL Trigger Inputs and Outputs

- AD: Advanced Devices (Includes -CD and -SD)

- Microscope Inputs

- EP: Experiment Planner (Includes -CD, -SD and AD)

- Sophisticated Scanning

DIA: Dynamic Intensity Analysis

DIA Dynamic Intensity Analysis module provides full capability to measure the intensity of cells over time, either during an acquisition sequence (on-line) or within previously captured data (off-line.) DIA provides the means by which to work with large amounts of image data and keep the images strongly correlated with the relevant numerical values of measurements and the statistical analyses results derived from those measurements.

Intensity data collection monitor allows optimization of data throughput for given image parameters and system performance with defined Regions of Interest (ROI).

ROI's can be manually adjusted during an experiment by pausing the acquisition to conform to the changing ROI.

IPA: Image Processing and Analysis

IPA: Image Processing and Analysis

Image Processing and Analysis module provides features for making an incredibly flexibly and wide variety (over 150) of statistical measurements to quantify the count, size, shape, position, intensity, and color of automatically identified objects in images. Provisions are also made for allowing supervision and correction of the identification process as the identification algorithms as necessary when real-world applications cause changes in the conditions.

The IPA result-reporting features provide displays of statistical summaries, fully customizable 2D and 3D histogram graphs, and interactive object lists which can highlight identified objects to clearly convey which objects have been grouped within a particular histogram bin.

The measurement algorithm is set up by configuring an icon-driven workfile (macro), by adding steps in an interactive process and observing the effects on the identified image objects as the various steps are added and modified. The procedure is methodical, where the operator selects each option interactively. The steps used can be saved in a workfile (macro) for later reuse, review, or modification. Typical workfile steps include applying image enhancement algorithms (usually to better emphasize object boundaries), identify objects by interactively applying threshold criteria to various property values, improve the resulting binary mask defining identified objects by using various binary mask modification options, cull identified objects according to some qualification criteria, and finally, choose from the available built-in measurements or customize your own measurements from mathematical combinations of more than one hundred fifty image object property values.

- MTA: Motion Tracking and Analysis (Includes IPA)

Motion Tracking and Analysis module works in harmony with the IPA (Image Processing and Analysis) to record and display information on the motions of individually identified objects by tracking objects across multiple time-separated fields.

Fields are first collected and analyzed for size (Area), position (Center of Gravity), time (Field Time), and intensity. The **MTA** module then rapidly tracks objects and reports distance moved with straight and curve line velocity.

QFA: Quantitative Fluorescence Analysis

QFA

Quantitative Fluorescence Analysis module offers the ability to not only make measurements for performing quantitative fluorescence analysis, but for selected applications can also perform the calculations (in a customizable manner which allows for the convenient use of estimated quantities). When the calculations are for the purpose of compensating for some factor influencing image pixel intensity, ***SimplePCI*** can also provide immediate visual feedback on what the image would have looked like given those correction results.

- FRET: Fluorescence Resonance Energy Transfer

SimplePCI **FRET** correction helps to perform one or all of the following procedures:

- correct the image data to compensate for cross-talk situations, .i.e. when photons from the donor or acceptor fluorophores are visible while using the acceptor or donor filter set images, respectively
- compute the **FRET** efficiency
- measure the donor emission before and after acceptor photo-bleaching

FRET is the first sub-module to be made available for **Quantitative Fluorescence Analysis**; others may be added in a future release.

VIS: Multi-Dimensional Visualization

VIS Visualization Multi-Dimensional (VIS) module provides powerful visualization capabilities.

It is particularly well suited for displaying data documents which contain Z-scans, allowing the user to Rotate the data in three-dimensions by simply dragging the mouse. Data can be rendered using maximum, minimum, sum, and alpha blending projections. Data slicing capabilities include both orthogonal and oblique slice planes. Movies of the data rotating can be viewed and/or exported.

DNN: Deconvolution

DNN Deconvolution module improves the clarity of images by removing out-of focus blur and restoring vital image detail.

- D: Deconvolution No Neighbors/Nearest Neighbors

The No/Nearest Neighbor algorithms work by deblurring one 2D image slice at time. They use a subtractive approach based on the simplifying approximation that the out-of-focus contribution in the image slice is equal to a blurred version of the collected adjacent slices. These algorithms are fast, qualitative and work particularly well on images with strong signal to noise ratios.

- 2D: Deconvolution 2D Blind (Includes -D)

2D Blind Deconvolution is an adaptive method for 2D data that does not require microscope and image parameters. 2D Blind Deconvolution works by iteratively improving the data set. The algorithm works with time series image sets, individual color channels or intensity images. 2D Blind Deconvolution is capable of restoring features at a sub-pixel resolution level and can work with almost any 2D image.

Adding Hardware

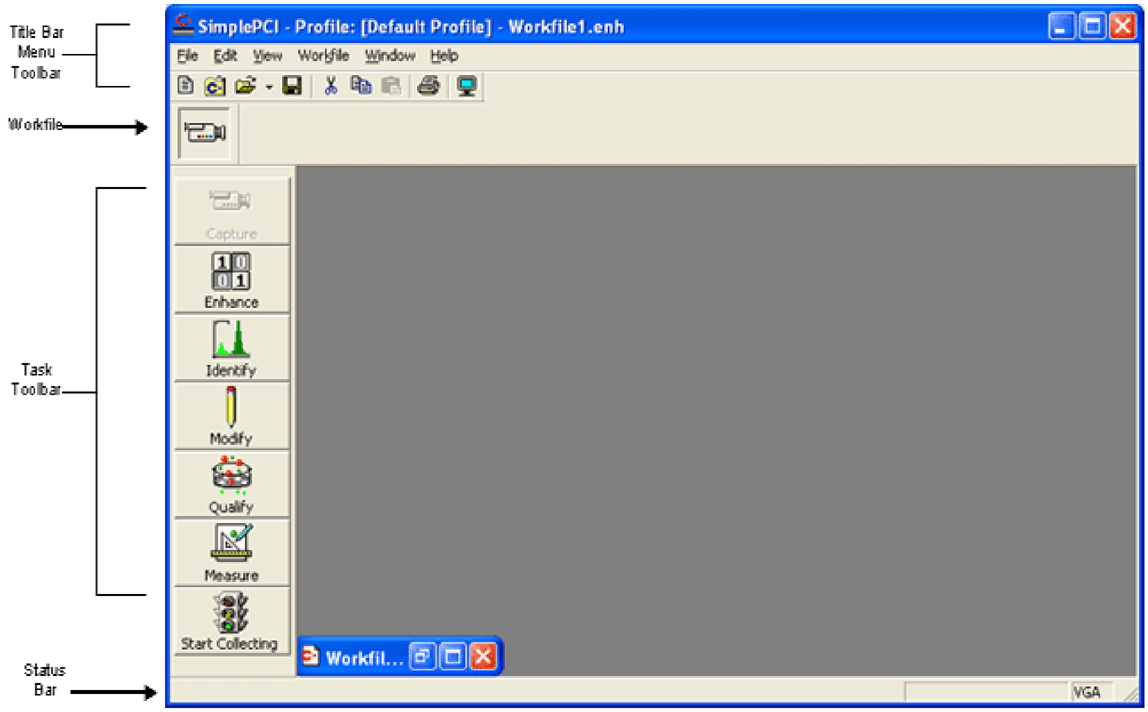
Hardware Devices can be added through the System Profile and saved in the Registry.

To add Hardware, click **Current Profile** in the **File** Menu. Select the **Device Control** Tab and begin adding your devices.

For best results, please ensure the device has been properly installed on your system before adding to the profile.

For more information, see System Profiles.

Understanding the Workspace



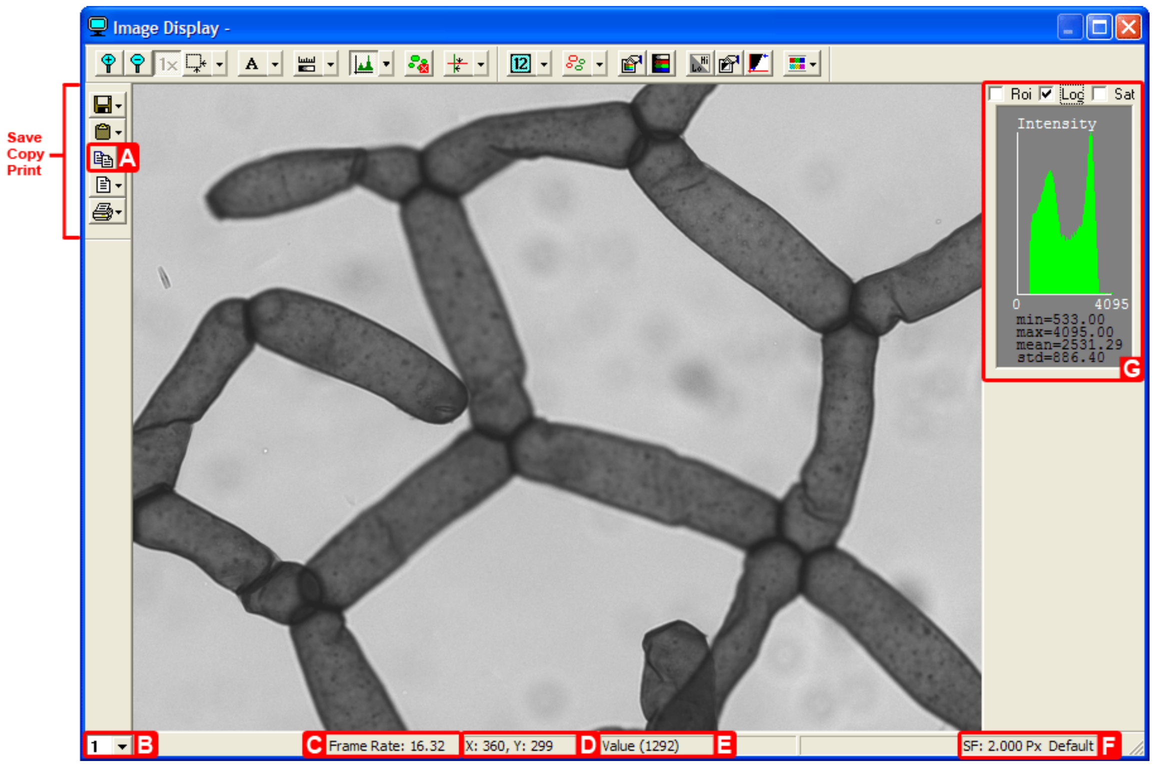
Title Bar	Displays current Profile and current Active Document.
Menu	Contains many software control commands. Menu may change depending on the Active Document.
Toolbar	For quick access to common **File** and **Edit** commands. Contains **Toggle Image Display** button**.**
Workfile	Used as an Icon-Based Macro in IPA. The capture icon can be used alone to initiate disk and camera capture in AIC.
Task Toolbar	For adding Tasks to the Workfile.
Status Bar	Provides Helpful Hints and Error Messages. Displays current Image Hardware.

The Image Display

The **Image Display** is used to show the Image being acquired, processed and measured. The Image Display can be moved independently of the main application window. This allows a dual monitor display configuration that can maximize the Image Display area on a secondary monitor while the user interface is displayed on the primary monitor.

The Image Display can be shown and hidden using the Toggle Image Display button in the Main Toolbar.

The **Display Image** selection drop-down determines the image to be displayed during Capture. Up to five images can be defined in the Capture Dialog's Sensor Tab. See Define Capture Images. This selection can be changed during Capture for immediate visual feedback.



Understanding the Image Display

A. Copy Original Image: Copies the current Original Image to an Image Document.

> **Note:** This function is especially useful when focusing in Live and you want to capture an image.

B. Display Image: The drop-down menu determines the image to be displayed during Capture.

C. Frame Rate: The current frame rate that the camera is capturing images.

D. Location: Displays the X & Y coordinates of the cursor.

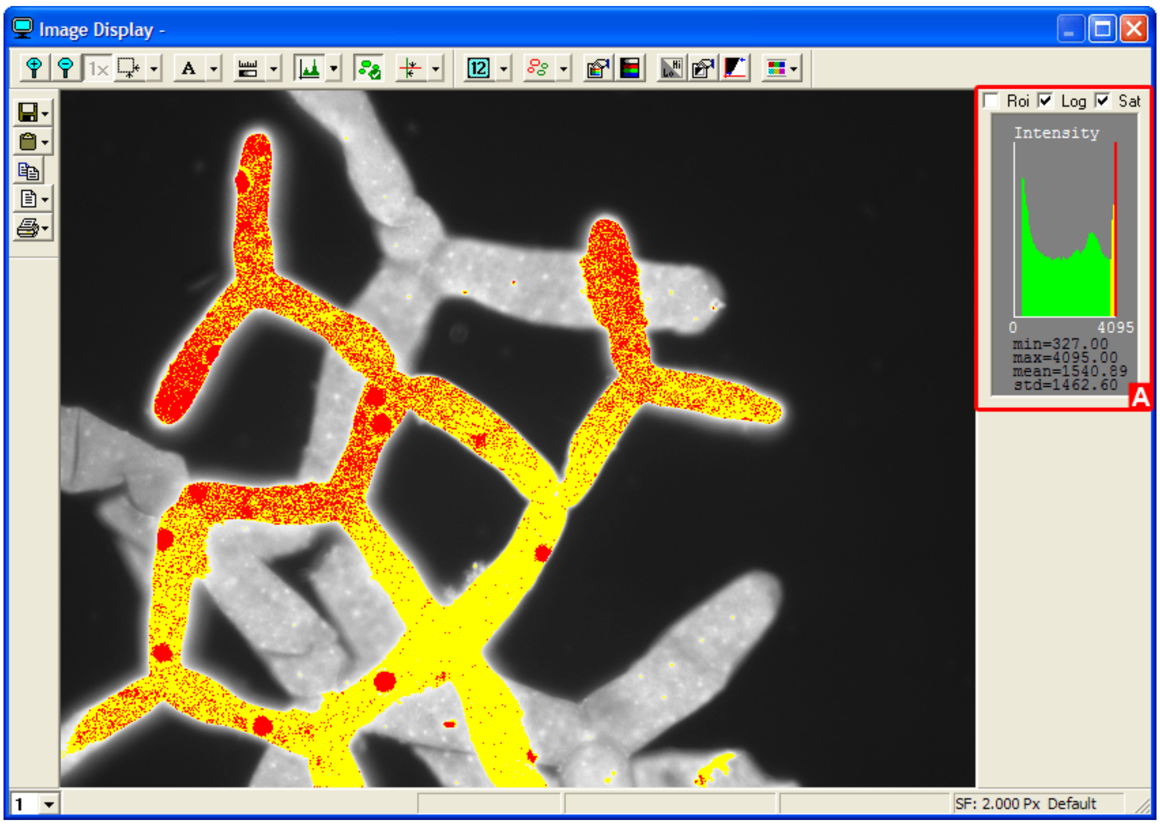
E. Intensity: Displays the intensity of the pixel at the cursor location.

F. Calibration: Displays the calibration factor for the current selected calibration.

G. **Intensity Histogram:** A live display of the image intensity in a histogram.

> **Note**: The Log function is checked and the Log of the Intensity Histogram is displayed. If the ROI box is checked, then the intensity of the ROI will be displayed in the Histogram.

The Saturation (**Sat**) function will display yellow representing a range of 4% from saturation. Red will be displayed when the image intensity reaches saturation. At the other end of the range when the image intensity is within 4% of zero (black) cyan is displayed. Blue will be displayed when zero is reached. The image below shows an image that is partially saturated. If you look at the Intensity Histogram (**A**) you can see that saturation is displayed in red.



Toolbar Management

Toolbar Menu

Right-click on an empty area of the toolbar (right under the title bar), or in the status bar (at the bottom of the window) to view the Toolbar Menu.

- Restore all the toolbars to their default settings, by choosing the **Reset All Toolbars** command.
- Add or remove a toolbar, by checking or un-checking the toolbar name in the list of available toolbars.

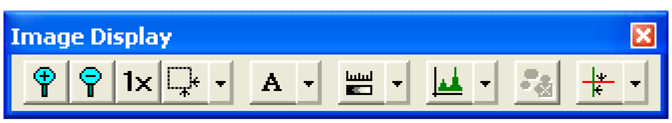
Whether a given toolbar appears in the list of available toolbars will vary depending on the type of document and its current view mode.

Toolbar Positioning

To change the position of a toolbar, left-click on an empty area in the toolbar, and drag it to the new desired position

Note: You can prevent the toolbar from docking by either holding down the CTRL key or dragging the toolbar completely outside the document window before releasing the mouse button. Putting the toolbar in its undocked state is the easiest way to figure out which toolbars correspond with each toolbar name in the right-click rest/visibility control menu.

Toolbar Customization



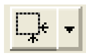
Some Toolbars include buttons which combine the functionality of a Toolbar with a drop-down Menu. The Toolbar/Menu combination can be customized to provide one-click access to commonly used functions, while allowing other items to be hidden when they are not immediately useful.

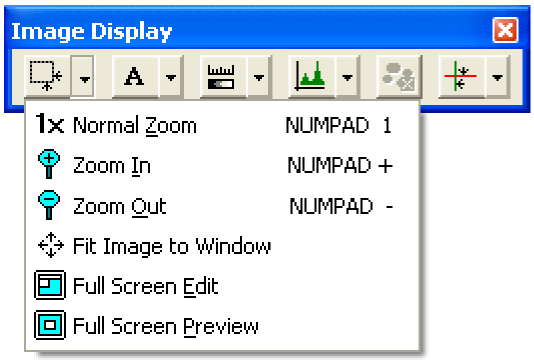
This is an example of the Image Display Toolbar.

 ***Drop-down Menu Anchor-icons***

Certain icons have an attached down-arrow button on their right side. These icons are anchor points where a group of related function icons can be demoted into a single drop-down menu to better preserve the valuable real estate on the computer display. This is a smart way to reduce the size of toolbars by piling up the icon functions, especially those which are seldom used.

Example: Zoom-functions anchor icon:





On clicking the down arrow in the button group a drop-down menu appears showing any items not already visible in the toolbar. Items can be activated from the toolbar or from the drop-down menu as normal.

To Promote a Button

To promote a button from the drop-down menu to the toolbar, click the button while pressing the CTRL key.

To Demote a Button

To demote a button from the toolbar to the drop-down menu, click the button while pressing the CTRL key – the same method is used in both cases.

To Reset the Default State

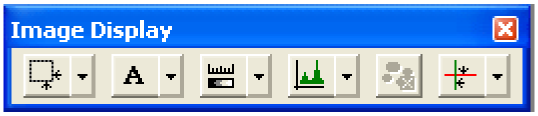
The toolbar has a default state, which can be reset by clicking on the background of the toolbar and selecting **Reset Toolbar** option.

Note: The button with the adjacent arrow button cannot be promoted or demoted. This button may display the current selection for cases where the drop-down menu is a list of choices for a particular setting, e.g. color mask.

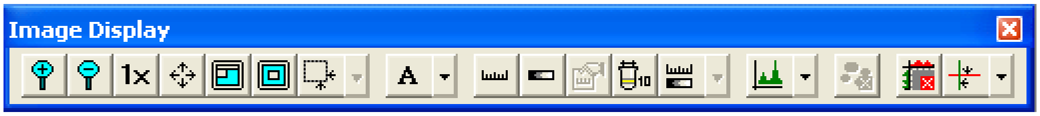
Some menu choices do not have buttons and cannot be promoted, for example checkable items which display only a check mark.

The settings for each customizable toolbar are saved on a per user basis in the Current Profile, and are saved at the end of the session and re-loaded with the profile.

Customizable Toolbar Examples:



This example shows the Image Document Display Toolbar in its fully-demoted state.



This example shows the same toolbar with all display items promoted to the toolbar.

This example shows the same toolbar **Reset** to the default state.

SimplePCI Documents

To understand the workings of the application it is important to be familiar with ***SimplePCI*** proprietary document types:

Workfile Documents are specific to IPA and AIC. **Workfiles** contain the steps for capture, processing and measurement of images for particular applications. The instructions can be saved and reloaded for routine operation.

Image Documents, **Data Documents**, and **Summary Documents** are at the core of *SimplePCI*.

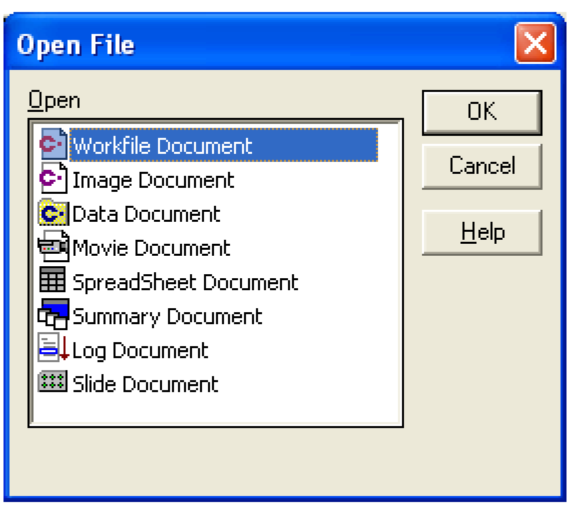
- **Image Documents** contain Image Data. This Image Data may have been acquired from a Capture Device or loaded from an image file on disk.
- **Data Documents** can also contain **Image Data**. Thus, data documents provide a way to manage and organize multiple images. Image Data within a Data Document can be manipulated in the same ways as Image Data in an Image Document.
- **Summary Documents** are basically containers for **Data Documents**. They provide a way to manage and organize multiple Data Documents.

Log Documents and **Slide Documents** are specific to the **Experiment Planner**. Please see the Experiment Planner documentation for more information.

File Open

In the **File** menu, select **Open**...

A variety of document types are supported by ***SimplePCI***.



Along with proprietary documents, many commonly used documents can also be manipulated in ***SimplePCI***.

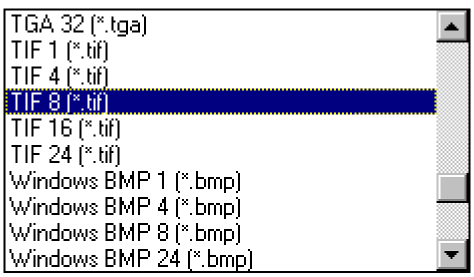
Most Image Formats can be opened in ***SimplePCI*** as **Image Documents** or imported into **Data Documents**. See Supported Image Formats.

Standard Movie (**.avi**) files can be loaded as **Movie Documents**. Movies are used to store, display and manipulate image sequences. Standard AVI files can be created from **Data Documents** and image sequences. Movie files can be loaded and played using convenient playback controls including forward and backward play, stepping and speed control.

Spreadsheets can be opened and manipulated using an embedded spreadsheet application containing many useful spreadsheet functions for flexible data customization. Spreadsheet Documents are used to store, display, print and manipulate numerical data and allow export to compatible file formats. Spreadsheet files are Microsoft Excel™ compatible.

Convert Image Format

Images can be converted from an Image Document to a variety of image file formats. This can include the conversion of the image color depth, for instance from a 24-bit color image to an 8-bit or 1-bit image file format, and also data compression.



In the **File** Menu, select **Convert Image File**. Choose new format from a list of supported image file formats.

Image Save, Copy, Print

Image data in an Image Document can be output to a variety of places, for interacting with other applications and devices. For each destination the source data can be specified to include the various image components, additional display layers and contrast enhancements.

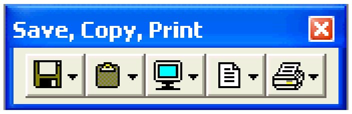


Image Source Data may include:

For Monochrome Images:

- **Original Image -** a copy of the original image data (with Image Region applied)

For Color Images, access individual components:

- **All components** - a copy of the original image data (with Image Region applied)
- **Red Component** - only the Red component of the **Image Data** (for color images)
- **Green Component** - only the Green component of the **Image Data** (for color images)
- **Blue Component** - only the Blue component of the **Image Data** (for color images)

For all Images:

- **Processed Image -** a copy of the original data after processing (Enhance, Registration, ...)
- **Display Image** - the **Image Data** as displayed. Contrast, Merge and Tinting will be applied. Remember, a Display Image is only 8-bit so for complete access to 16-bit data use Original or Processed source data.
- **Display Image + Layers** - the **Image Data** as displayed including additional Display Layers. Display Layers include Scalebars, Annotations, Shapes, Intensity Profile, ...

Importing Files

Multiple Images may be imported into a Data Document. Images may be imported from multiple image files or from a single multi-image file. Supported multi-image file formats include multipage TIFF, MetaMorph® Stack (.stk) and Hamamatsu Image Sequence (.his). It is also possible to import/export from one Data Document to another.

1. To Import Images to a Data Document (.cxd) file, do one of the following:

    - Use **File Open** to open a supported multi-image file. You will be prompted to open each image individually or to import those images.

    - Use **File Open** to select multiple image Files. You will be prompted to open those files individually or to import those images.

    - In the **File** Menu, select **Import Images**.

2. Then, choose your import preferences in the **Import Images to a cxd file** dialog.

    - Click **Source** and choose your source file or files: the files you are importing.

    - Click **Dest** and choose your destination: the file receiving the new images.

    - Under **Items to Import**, you may choose to import **All** images or a select image **Range.**

    - If you are importing from a data document with multiple images per field, you may choose the image number you wish to import..
        1. Select the right arrow button  next to image number.
        2. Un-check **All Images** from the drop-down menu.
        3. Choose the image number you wish to import.

Controlling Import Order

1. With Multipage TIFs, images will be imported in sequential page order.

2. During Multiple selection, images will be imported in the order in which their titles appear in the **File Name** Edit Box in the **File Open** Dialog. As each image is clicked, its title is added to the beginning of the title list. (**TIP**: When using CTRL-CLICK, select the images in reverse order. When using SHIFT-CLICK add an extra dummy image to the directory of interest. Select the first image. Hold down the shift key. Select the extra dummy image. All items, except for the dummy image, will be added in the order in which they appear in the directory. The dummy image title will appear first in the **File Name** Edit Box. Remove the dummy image title from the Edit Box.)

3. When using the Import Images Menu item to import as sequence of individual images, the images will be imported by numerical order. (Image1.TIF, Image2.TIF, Image3.TIF....) To import properly, the individual images must share a name prefix and they must be numbered sequentially. (FITC1.TIF, FITC2.TIF, FITC3.TIF.....)

Exporting Files

SimplePCI manages the exporting of Images and the exporting of Data.

Images can be exported to Data Files (.cxd), Movie Files (.avi), individual Image Files, OME TIFFs and multipage TIFFs.

Data can be exported to spreadsheet files.

- How to Export a Collection

    1. Open a Summary Document
    2. In the **File** Menu, select **Export Collection**

- How to Export Images

    1. Open a Data Document
    2. Select an **Image** in the Data Tree
    3. In the **File** Menu, select **Export Image Sequence**

- How to Export Graphs

    1. Open a Data Document
    2. Select an **Image** in the Data Tree
    3. Change to Graph View (2D, 3D, etc.)
    4. In the **File** Menu, select **Export Image Sequence > Display Image**

How to Export a Group Montage

1. Create Group Montage.
2. Right-click on Montage and select **Export Group Montage Sequence.**
3. Select the type of file (datat doc, image document, avi, etc.) to export the montage to.
4. Choose a location to save new file(s) and click OK.

Type your drop-down text here.

- How to Export Object Measurements

    1. Open a Data Document
    2. Select **Object Data** in the Data Tree
    3. In the **File** Menu, select **Export**

- How to Export Tracking Information

    1. Open a Data Document
    2. Select **Track Info** in the Data Tree
    3. In the **File** Menu, select **Export**

Export preferences are set in the **Export Dialog** and may include:

- File Type, Path, Name

    - Select File **Type** from the Drop-Down Box.
    - Click **Browse** to choose File path and name.

- Field Range

  - **Select All to Export All Fields.**
  - Select **Current** to Export only the current Field.
  - Select **Define** and click the right arrow  to Export a defined range.
  - Select **Fields** and enter specific field numbers and/or ranges

- Convert 1 plane to 2 color planes

  For Monochrome Image Export to Data Files, you may choose to **Convert 1 plane to 2 color planes** by splitting the image in half and merging each half into one color image.

- Video Compression

  For Image Export to Movie Files, you may choose to **Use Video Compression** in the exported movie. Movie speed (**Frames per second**) may also be entered.

- Export Item Numbering

  For Image Export to individual Image Files, you may choose to keep the original image field numbers or to renumber beginning with an entered value.

  The difference between these two methods becomes apparent when exporting selected fields from a data document.

- More Options

  For Object Data Export, choose to export current class or all classes.

- Config OME
  For OME TIFF Exporting, define the domain name of your company or institution.

Supported Image Formats

The following image formats and capabilities are supported for image saving and loading.

File Format Description	Read Bits per pixel	Write Bits per pixel
JFIF This is the JPEG File Interchange Format. Supports YUV 4:4:4, 4:2:2, and 4:1:1 color spacing, and YUV 4:0:0 for gray-scale.	8 for gray-scale 24 for color	8 for gray-scale 24 for color
Progressive JPEG This is a JFIF format that is useful for transmitting images, because the first part of the file contains the full dimensions of the image. Therefore, in a paint-while-load routine, you can display the whole image, then progressively clarify it as the rest of the file loads. Supports YUV 4:4:4, 4:2:2, and 4:1:1 color spacing, and YUV 4:0:0 for gray-scale.	8 for gray-scale	Read only
JTIF This is the JPEG Tagged Interchange Format. Supports YUV 4:4:4, 4:2:2, and 4:1:1 color spacing, and YUV 4:0:0 for gray-scale.	8 for gray-scale 24 for color	8 for gray-scale 24 for color
TIFF This is a tag-based file format designed to promote universal interchanges of digital image data. Because TIFF files can include images defined according to multiple image-encoding schemes including CMYK, LZW and RLE, there are many versions of TIFF. Supports the most common TIFF formats.	1, 2, 3, 4, 5, 6, 7,8,16,24,32,48	8,16,24,48
MPT This is a multi-page TIFF format that enables a file to contain more than one image. It is handled the same as a regular TIFF file, except for the multi-page LZW and RLE features.	1, 2, 3, 4, 5, 6, 7,8,16,24,32	8,16,24
TIFF LZW These files use the Tagged Image File Format with LZW compression See Legal notice below	1, 2, 3, 4, 5, 6, 7,8,16,24,32	1, 2, 3, 4, 5, 6, 7,8,16,24
TIFF CCITT These are compressed TIFF files that are commonly used for FAX transmission and document imaging.	1	1
TIFF CCITT Group 3 These are TIFF CCITT files in a format that is more advanced and more compressed than TIFF CCITT. Supports both one-dimensional and two-dimensional	1	1

variations of this format.		
TIFF CCITT Group 4 These are TIFF CCITT files in a format that is more advanced and more compressed than TIFF CCITT Group 3.	1	1
IOCA (ICA) This is the Image Object Content Architecture developed by IBM. Supports these files in an MO:DCA wrapper with embedded single-bit CCITT Group 3 or Group 4 images. Also supports IOCA files without an MO:DCA wrapper.	1	1
WinFax Group 3 and 4 This is a FAX format created by Delrina for Group 3 or 4 support.	1	1
FAX Group 3 This is a raw FAX format (without a header) for Group 3 support. Supports both one-dimensional and two-dimensional variations of this format.	1	1
Fax Group 3 This is a raw FAX format (without a header)	1	1
Truevision TGA (TARGA) This is a file format created by Truevision Inc. Supports all uncompressed and RLE compressed TGA file formats.	8, 16, 24, 32	8, 16, 24, 32
GIF This is the Graphics Interchange Format created by CompuServe for storing and exchanging color raster images. This format compresses its image data with the LZW compression technique.* See Legal notice below	1, 2, 3, 4, 5, 6,7,8	1, 2, 3, 4, 5, 6,7,8
Animated GIF This is a multi-page GIF format that can be played as an animation by displaying the images sequentially. LEADTOOLS lets you create and play the animations. This format compresses its image data with the LZW compression technique.* See Legal notice below	1, 2, 3, 4, 5, 6,7,8	1, 2, 3, 4, 5, 6,7,8
PNG (Portable Network Graphics) This is a replacement for the GIF format. It is a full-featured (non-LZW) compressed format intended for widespread use without legal restraints.	1, 4, 8, 16, 24, 32	1, 4, 8, 24
Photoshop 3.0 (PSD) This is the format produced by the Adobe Photoshop graphics editor.	1, 8, 24	1, 8, 24
Windows Bitmap (BMP)	1, 4, 8, 16, 24, 32	1, 4, 8, 16, 24, 32

This is a file format created by Microsoft. Some BMP images are compressed with RLE- type compression.	1, 4, 8 RLE	
Windows Metafile (WMF) These files are not bitmap based images. A Windows meta-file consists of a collection of device independent functions that represents an image. When a program loads a meta-file, these functions are executed to obtain the image.	8, 24	8,24
PCX This is a file format created by ZSoft. This format compresses its image data with the RLE type compression.	1, 4, 8, 24	1, 4, 8, 24
DCX This is a multi-page PCX format that enables a file to contain more than one image. It is handled the same as a regular PCX file, except for the multi-page feature.	1, 4, 8, 24	1, 4, 8, 24
PostScript Raster (EPS) (Encapsulated PostScript) These files are used primarily on PostScript printers. These printers usually offer more variety of fonts and higher resolution than standard laser printers. EPS files will work on any PostScript compatible printer and any end user application that supports placement of EPS files in its work space.	1, 8 (raster only) 1, 4, 8, 16, 24, 32 (embedded TIFF)	8 gray-scale (raster)
OS/2 Bitmap (OS/2 BMP) These are files created on an OS/2 operating system. LEADTOOLS supports both 1.x and 2.x formats.	1, 4, 8, 24 1, 4, 8 RLE	1, 4, 8, 24
CALS Raster These are single-bit CCITT Group 4 CALS raster files. CALS is a United States government standard.	1	1
MacPaint (MAC) These Macintosh Paint files are commonly used for monochrome clip art.	1	1
GEM Image (IMG) These files are native to the Graphical Environment Manager developed by Digital Research.	1	1
Microsoft Paint (MSP) These files from early versions of Windows are used for black-and-white drawings and clip art.	1	1
WordPerfect (WPG) These are WordPerfect raster files	1, 4, 8 (raster)	1, 4, 8 (raster)
SUN Raster (RAS) These files are native to Sun UNIX platforms.	1, 4, 8, 24, 32	1, 4, 8, 24, 32

Macintosh Pict (PCT) These files, produced using Macintosh QuickDraw, are used in desktop publishing and imaging applications.	1, 4, 8, 24	1, 4, 8, 24
LEAD This is the LEAD CMP compressed format for gray-scale and color images. This format results in smaller files and better image quality than industry-standard formats.	8 for gray-scale 24 for color	8 for gray-scale 24 for color
LEAD 1-BIT These are single-bit LEAD compressed files.	1	1
PCD These are Kodak PhotoCD files	All	Read Only
FPX This Kodak format is newer than PhotoCD. This format can also contain more than one physical resolution, but without fixed dimensions. You can choose which resolution to load, and thus control the size of the resulting bitmap. FPX can be uncompressed or JPEG compressed.	8,24	8,24
RBF This Hamamatsu image file format is used in Wasabi.	8, 16, 24, 48	Other file formats

Profile Overview

System Profiles allow rapid configuration of the system for multi-user or multi-project installations.

A System Profile includes configuration information detailing

- Default File Paths File Names and File Types to be loaded on application start-up.
- Image Hardware selection.
- Device Control of attached devices including cameras, stages, filters, shutters, I/O and microscope devices.

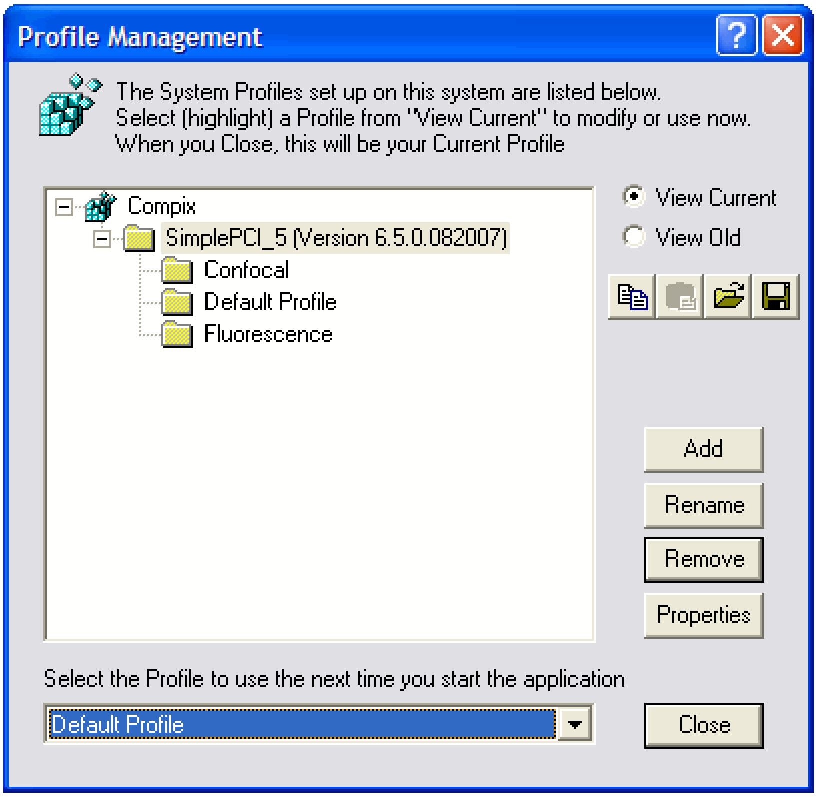
Multiple Profiles can be configured and saved. One profile is used as the default and loads automatically when the application is started.

Select **Manage Profiles** in the **File** menu to configure, save and load System Profiles.

Select Current Profile in the **File** menu to modify the current settings in effect. You may also select **Manage Profiles** in the **File** menu and click **Properties** to access these settings.

Manage Profiles (File menu)

The **Profile Management** Menu enables new profiles to be added, removed, copied and modified. It also allows you to perform profile file I/O and to carry forward your profile information from previous versions of software installation to the current version.



Click on a profile in the **Profile Selection** box to select it. The selected profile is highlighted in the list, and can be modified by clicking on the Properties button. Upon exiting this dialog, the selected profile (in this example "Fluorescence") will become the Current Profile. The Current Profile's name is displayed in the main window's titlebar.

Click Add to create a new profile. Each profile must have a unique name. You can make the names descriptive and include spaces. When the profile is created, the profile properties are set to default values.

Click Remove to permanently delete a Profile.

Rename allows you to name each profile to suit your needs.

Click Properties to review or modify the current selected profile.

The **View Old/View Current** radio buttons give you control whether to view profiles corresponding to older or current versions of the device profiles registry section. This can allow you to copy your old profiles forward to the current version of the software.

The Copy button allows you to copy the profile to the clipboard. Click on Paste and you will be prompted to rename the profile and then it is saved in the into the current version's folder, you will answer a rename dialog and have **Paste** Upon applying the button allows the selected profile to be copied to the clipboard.a copy of your old profile (but with the new name and/or upgraded version). This is useful if several devices and other properties have been set which are common to different projects or users.

The **Load** and **Save** buttons can be used for storage and retrieval of profile information to file storage. The profiles are normally stored as part of your computer's registry; these commands simplify making a registry backup file of the registry subset that is the selected profile information. This file is useful for disaster recovery, troubleshooting, and to transfer profile information to another system.

Note: When loading profiles across users, please be sure to reboot between logins. Merely switching users (logging off and then logging back in) creates extra registry protections in Windows and your profiles will not load properly.

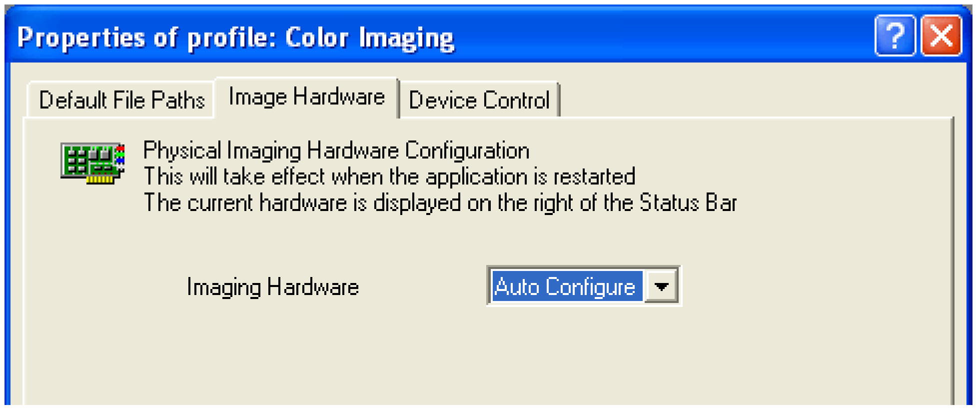
The drop down list at the bottom of the Menu is used to select a Start-up Profile for automatic loading when the application is run. This is useful to automatically configure the system for routine work, and includes loading a Workfile, setting up the Capture Device and initializing the imaging hardware.

The Close button will exit the Menu applying the currently selected profile from the list.

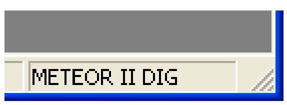
Note: This may be different to the profile selected for Start-up.

Image Hardware

Select Imaging Hardware.



Auto Configure automatically detects and initializes installed hardware. Detected components are shown in the bottom right hand corner of the application window.



A variety of Image Hardware types are supported:

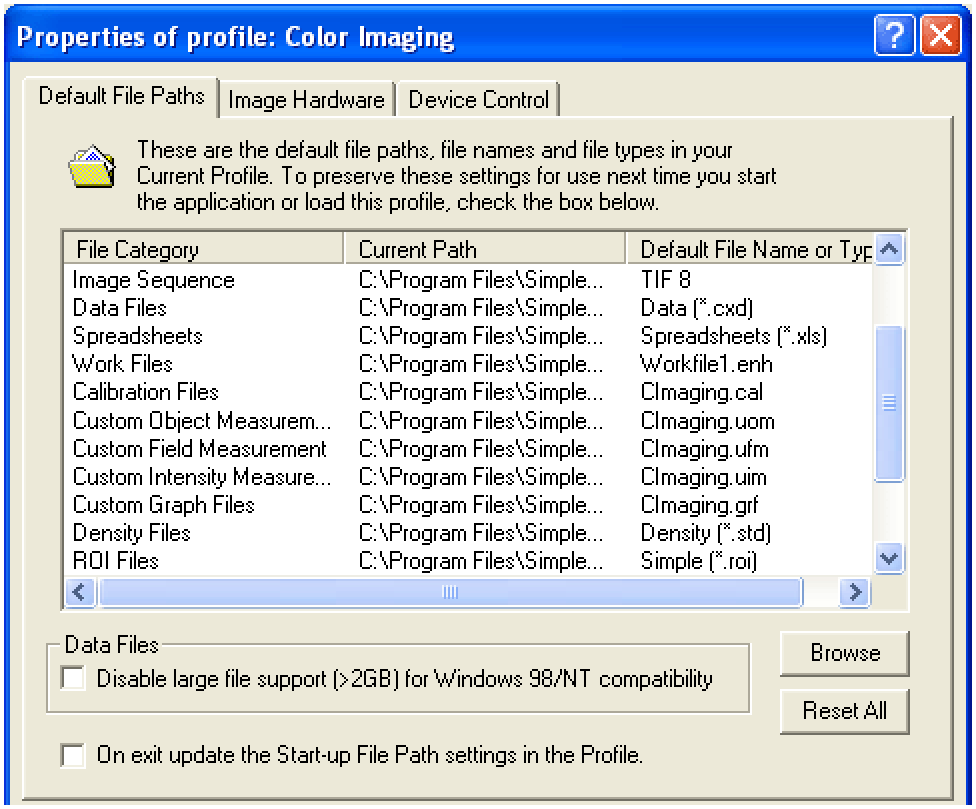
- The **Meteor2/Multi-Channel** Hardware supports high resolution Capture of monochrome and 24-bit color images. This device uses the Host Computer VGA card for image display. This device can capture both single channel and up to 3-channel video data (e.g. RS170 and RGB video formats).
- The **Meteor2/Standard** Hardware supports high resolution Capture of monochrome and 24-bit color images. This device uses the Host Computer VGA card for image display. This device can capture up to 2-channel video data (e.g. RS170 and SVHS video formats) using the on-board decoder.
- The **Meteor2/Digital** Hardware supports high resolution Capture of monochrome digital images using RS-422 communication. This device uses the Host Computer VGA card for image display. This device can capture from specific digital devices (e.g. the Hamamatsu 4742-95 cooled CCD Camera).
- When no Matrox frame grabber is installed, the software defaults to **VGA.** A variety of non-Matrox capture devices are available.

Switching between Image Hardware devices requires a restart of the application.

For optimized image transfer with the Meteor2 series, use Matrox MGA graphics products.

File Paths

Set default path, name and type for various file categories.



Files in each **Category** use the **Current Path** and **Default File Name or Type** when saving or loading. These settings are loaded with the profile the start of the application and when switching profiles.

The **Current Path** and **Default File Name or Type** will be updated as files are loaded and saved.

> **TIP**: For best results when saving Mono and Color Images, it is recommended that you choose **TIF (Auto-Depth)**.

On exit update the Start-up File path settings in the profile: All updates made to the profile will be saved permanently and reloaded with this profile.

> To start in a fixed state: Enter Profile. Modify settings. Check **On exit update...** Click **OK.** Reenter Profile. Uncheck **On exit update...**

Modify Settings:

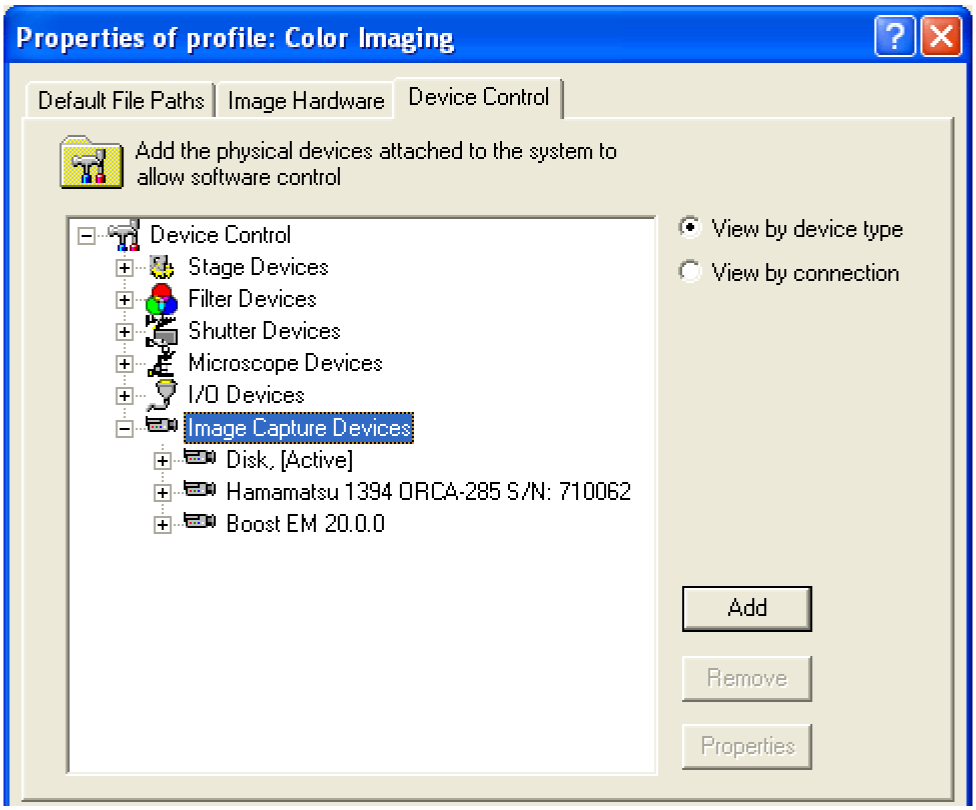
- Select an item in the **File Category**; click on the **Browse** button;
- OR Select an item in the **Current Path** list;
- OR Select an item in the **Default File Name or Type** list;
- THEN Navigate to the required location; Select the proper name or type.

Press **Reset All** to specify and apply a path to all file categories.

Disable large file support if you expect to create data intended for users of operating systems which are not capable of handling files larger than 2GB (such as Windows 98/ or Windows NT).

Device Setup

Add devices to the System Profile for ***SimplePCI*** control.



Device Types:

- **Stage Devices** - for X,Y and Z controlled movement of the sample
- **Filter Devices** - for wavelength control of the sample illumination
- **Shutter Devices** - for exposure control of the sample illumination
- **Microscope Devices** - for control of computer-interface-equipped microscopes
- **I/O Devices** - for custom pin-by-pin I/O configuration of serial ports, parallel ports, or general I/O devices
- **Camera Devices** - to allow multiple devices to be installed and configured for easy selection in the Capture Menu.

Device Setup:

- Click on the **Device Type**, then...
  - Click **Add** to add a new Device to the Profile.
- Click on the Device Name, then...
  - Click **Remove** to delete the selected device from the Profile. This will free any associated COM port if it is the last device using the port.
  - Click **Properties** to activate the configuration menu for the selected device.
  - Click **Apply** button to activate the operational menu for the selected device.

- Expand the device tree to show detailed information.

View by Connection to show the Installed Devices from a port perspective.

> **Note:** The number of devices using Serial RS232 control may be limited by the available COM ports on the host computer. Some controllers are multi-function, and allow sharing of the port by using the same controller.

Device Port Types

Each device connects to the system through a port. Except in very unusual cases, all interaction between the system and each device occurs through a single port.

Some devices can be connected to the system through more than one type of port, but this does not necessarily imply that more than one port should be connected to that device, as this will almost always cause severe problems.

SimplePCI can interact with devices through three types of ports:

- LPT Ports (Parallel)RS-232 COM Ports (Serial)
- USB Ports
- IEEE 1394

Stage Device Overview

Motorized Stage control devices are supported from a number of manufacturers. Each product is likely to vary slightly in resolution, speed of travel, repeatability and other characteristics related to moving sometimes heavy mounting hardware small distances accurately.

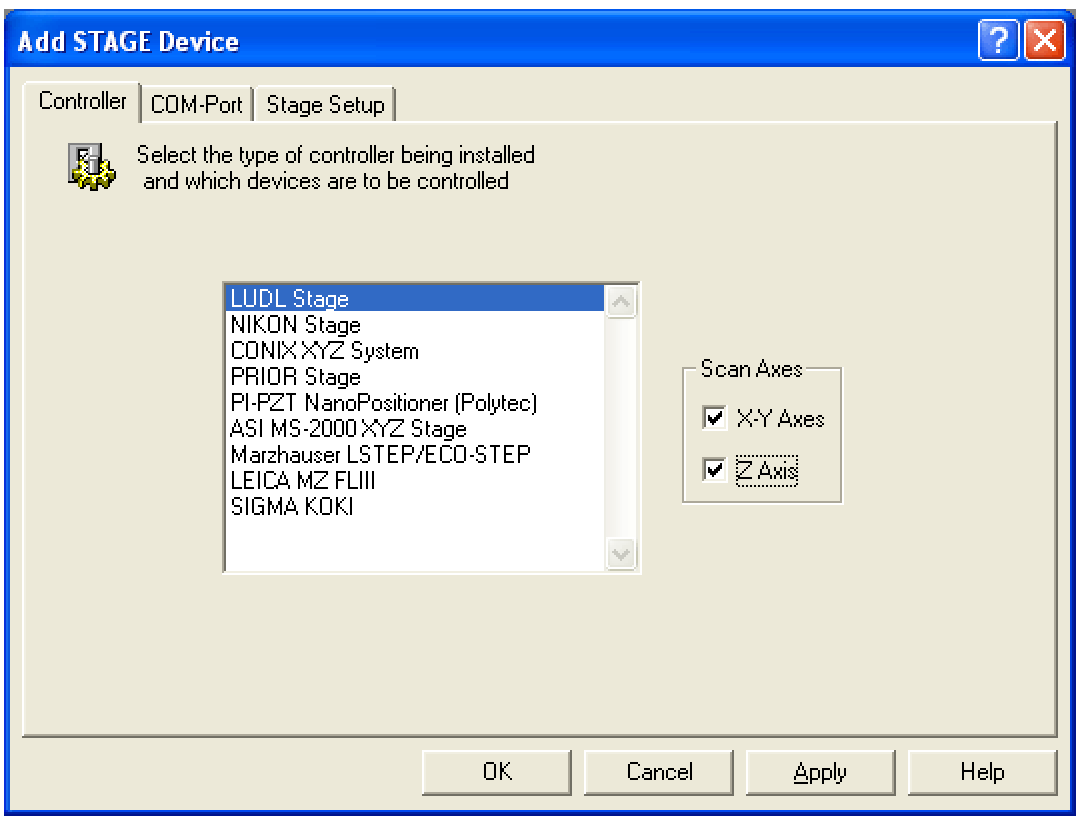
The steps to follow to add a STAGE device are:

1. Select the Stage Device Controller
2. Select the COM Port
3. Enter the Stage Device Controller Properties
4. Calibrate the Stage

Once communication settings have been configured correctly, an Interactive Stage-Control mode can be accessed via the Apply button.

Stage Controller Selection

First select a Controller type, relating to the manufacturer of the device. Select also the axes to control.



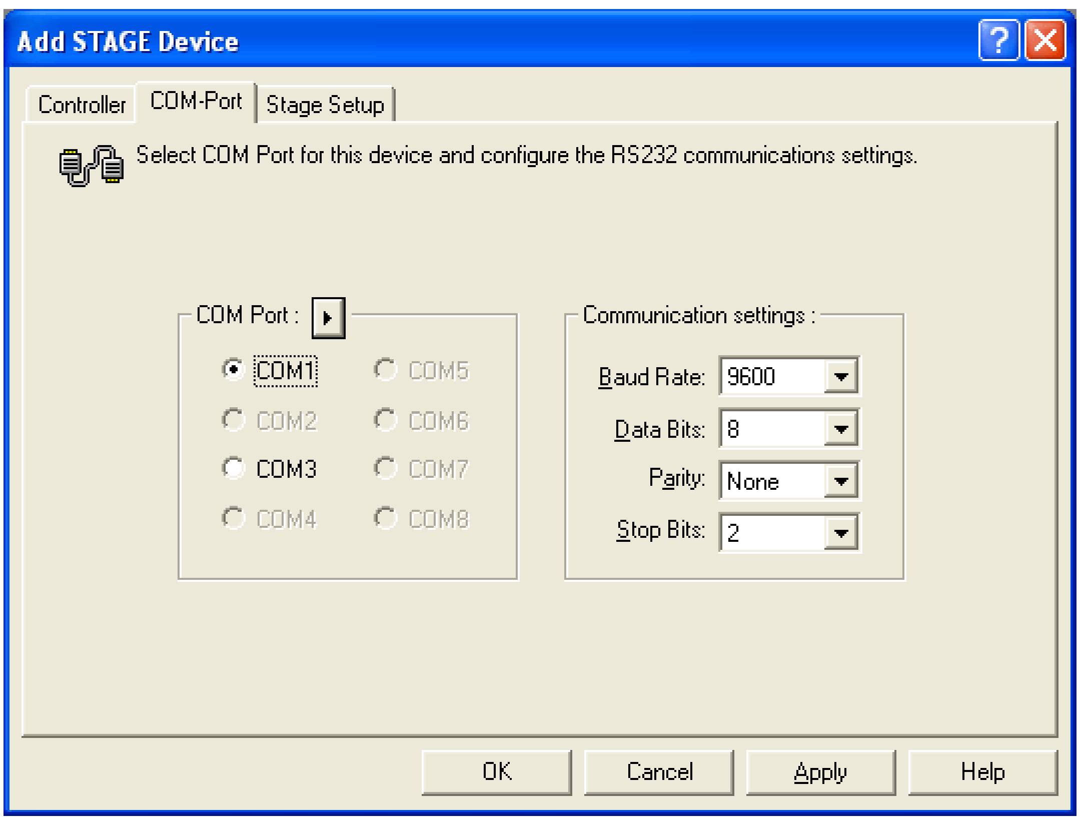
If the type of Controller includes more than one specific device, a drop-down list will appear directly below the Controller type selection box, from which you should specify the specific device you have. For example, choosing a NIKON Stage will show a selection from which either the NIKON Remote FOCUS or BioStation must be specified.

> **Note:** That it is possible to control the X, Y and Z axes independently using more than one controller, possibly from different manufacturers. For example, this capability permits an external XY stage to be used in conjunction with a microscope with a built-in (focus) Z-stage.

> **Note**: To add additional axes control to an installed device, remove the device and re-install it.

Stage COM Port Setup

Select the COM port for RS-232 communications. The Baud rate and other communications parameters must be correctly set to achieve proper control. Check the manufacturer's documentation and specifications first. The default values are likely to be correct, but may not be in all cases.



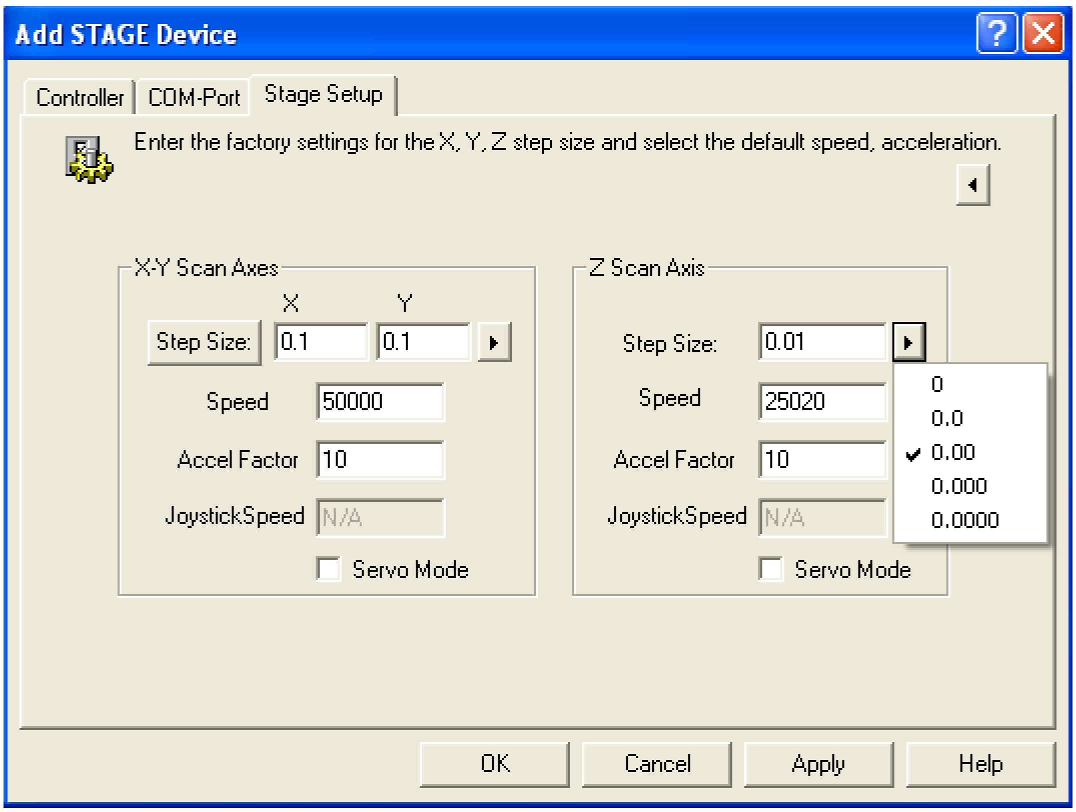
Using the button next to the "COM Port" radio buttons group label will scan for available COM-Ports.

Note: That in some cases, the COM port may be shared with another device (Filter/shutter/Stage/Camera etc) that uses the same controller or may be in use by a different device.

Avoid selecting a COM port to which a modem or other communications device is attached, as this may cause serious system problems when communication is attempted.

If an existing COM port is grayed, it is either already in use by another device, or disabled in the system BIOS.

Stage Controller Properties



For Stage devices with X and Y scan axes the configurable properties include:

- **Step size** in the X and Y directions, in microns. Note that step sizes in X and Y directions may differ.
    - Precision - controls the number of digits of precision that step size is displayed in (up to four decimal places).
- **Speed** – this represents the maximum speed of travel for the device, in units of steps per second.

- **Accel factor** – This defines the acceleration profile of the device, the maximum rate at which it increases in speed. This parameter can limit forces from accelerations, protecting sensitive or loosely attached specimens.
- **Joystick Speed** – If a Joystick device is installed, this gives software control over the sensitivity of the Joystick. This may be useful at high magnification.
- **Servo Mode**

For Stage devices with a Z-scan axes, the Z-axis properties can be configured separately.

Select **Servo Mode** for greater accuracy.

> **Note:** The top-right most button in this screen retrieves the hardware firmware version.

Stage Calibration

It is highly recommended to Calibrate the XY stage to precisely define the step size of the device in the target environment. The manufacturer's calibration may be a nominal estimate.

To Calibrate the Stage use the following steps:

- First make sure that the **Spatial Calibration** of the current selected objective matches exactly with the Current capture device. The calibration is performed in the Calibration Menu of the Capture Dialog .
- Click the **Step Size** button in the Stage Device Properties menu.
- Use the first X,Y(Z) control menu to move the stage so that a recognizable target object is centered in the Green box displayed at the Top Left Corner of the image monitor, and Click Okay. It may also be necessary to make sure that the travel of the stage is exactly parallel to the orientation of the camera.
- Use the second X,Y(Z) control menu to move the stage so that the same object is centered in the Green box displayed at the bottom right Corner of the image monitor, and Click Okay.
- The Stage will now automatically compute the step size and orientation of the capture device, and move the stage back to the original position.

The target object should now be in the Top Left corner box. If this is not the case, check the Spatial Calibration in the capture Menu, make sure the correct Objective is selected and accurately calibrated. Also check that the Capture device is firmly mounted and exactly parallel to the stage travel – even a tiny error in angle will be magnified over many fields and cause poor registration of images. The apparent accuracy with which the target is returned to its original position is dependant on the spatial calibration, objective magnification, and the features of the stage itself.

The step size created should approximate the manufacturer's specifications. If the numbers are negative, this simply means that the Capture device is rotated compared to the XY stage motor directions. If positive numbers are required, either change the controller hardware settings, or rotate the Capture device to correct it. Subsequent scan patterns will be positioned below and to the right of the current image.

Interactive Stage Testing

Once Shutter communication has been configured in the Profile, press **Apply** to verify your device in the Apply Stage Dialog.

The installed Stage's interactive testing dialog will be identical to the Stage Control Tab available on the Capture Dialog. Depending on the Stage Device installed, either the XY Stage Tab, XYZ Stage Tab or the Z Focus Tab will be available.

During Interactive Stage Testing, the Active Camera Device enters Live Mode for immediate feedback on stage positioning.

Filter Device Overview

Motorized Filter / Wavelength control devices are supported from a number of manufacturers. Two types of Filter / Wavelength devices are supported:

- Continuously variable Wavelength Devices
    - Tunable Monochromator
    - Liquid Crystal Tunable Filter (LCTF)
- Multi-position Fixed Wavelength Filter Wheels
    - Single Wheel Device
    - Dual Wheel Device

More than one device can be installed and used at once, allowing simultaneous control of excitation and emission wavelengths. Also by controlling shutter devices and Capture device settings (exposure, gain, etc.) it is possible to completely change the image acquisition illumination environment for successive images.

Combinations of Filter and Shutter positions are configured and selected in the Automatic Filter and Shutter Control settings, accessed from the Sensor Tab of the Capture Dialog.

The steps to add a filter device are:

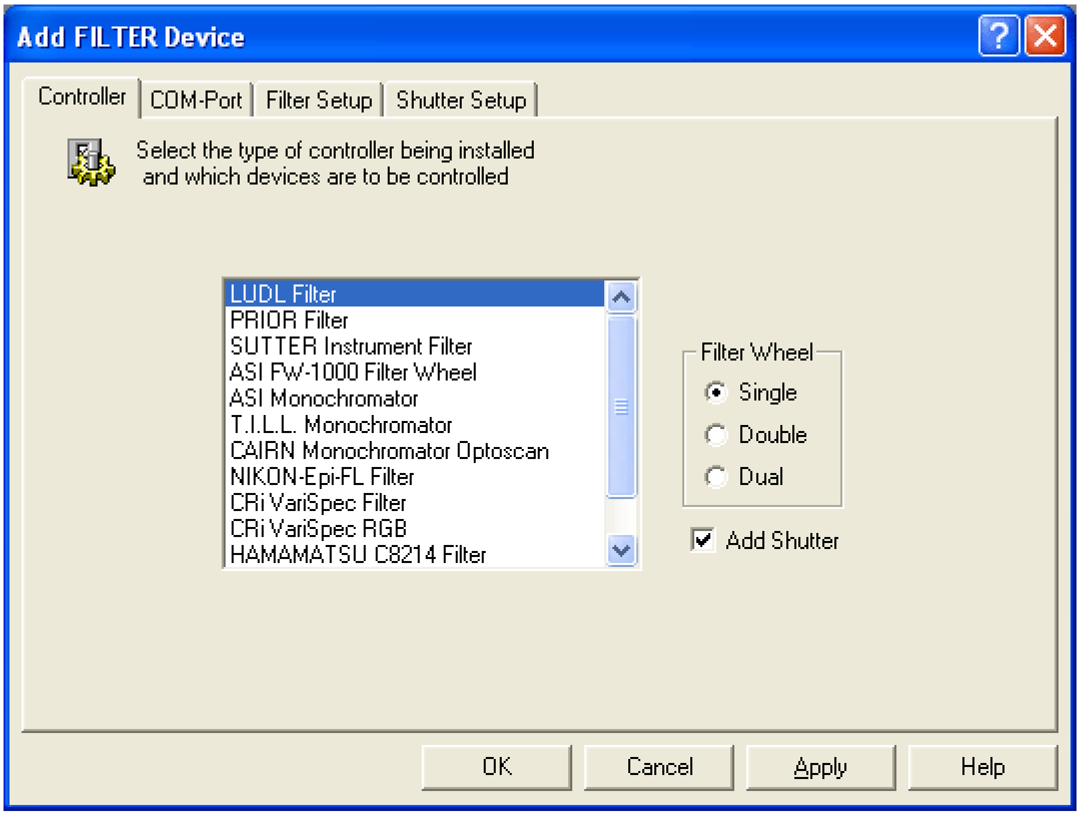
1. Select a Filter Device Controller
2. Select a COM Port
3. Enter the Filter Device Controller Properties

Once communication settings have been configured correctly, an Interactive Filter Setup Verification mode can be accessed via the **Apply** button.

Wavelength Calibration is performed in the Monochromator Tab in the Capture Dialog.

Filter Controller Selection

Use this menu to select the Manufacturer for the **Device Controller** being added. This determines the interface and communications protocol to use to communicate with and control the device.

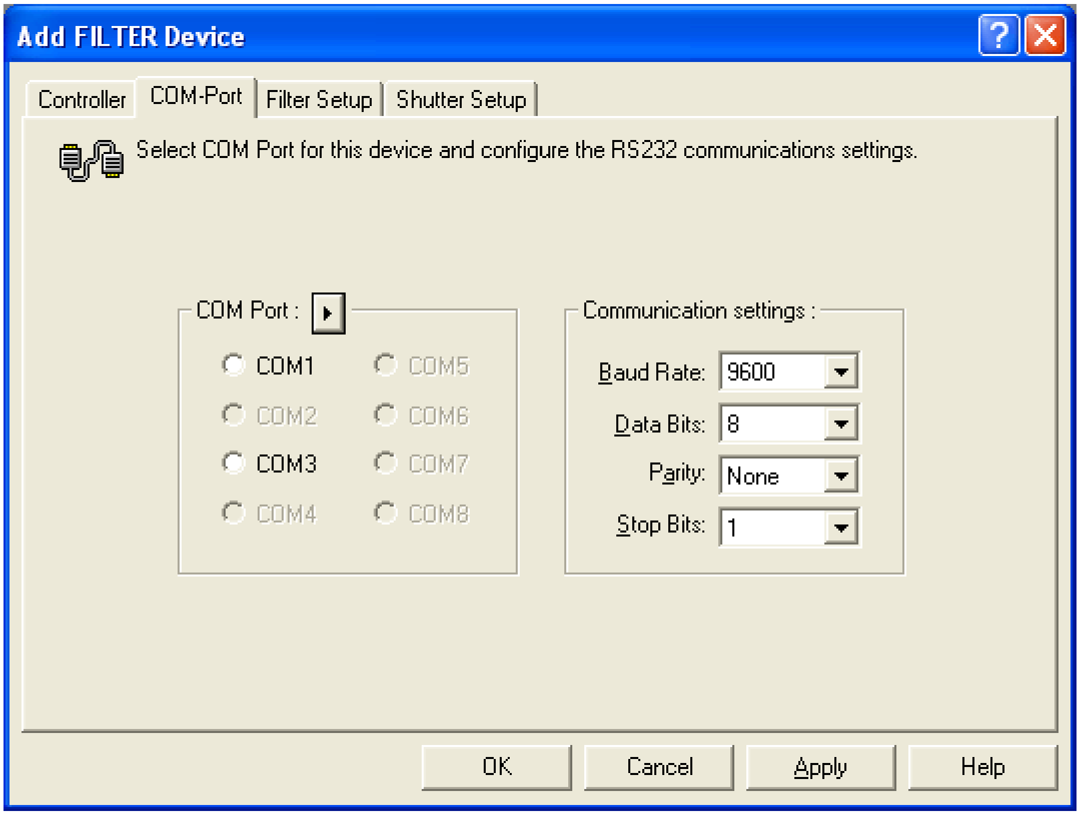


As the filter type is selected, various options will become available to corresponding to hardware options for that device. Configure all settings to match the features on the device you wish to control. Some filter devices include shutters and some allow shutters to be added as an option. A shutter tab will become visible automatically in the former case but appear in the latter case only if the appropriate options are selected (such as "Add Shutter" in the above example).

Specifying the wrong controller type may cause problems when sending commands that are not correctly supported. In such a case the controller and possibly the host computer system may need to be powered off and reset.

Filter COM Port Setup

Select the COM port for RS-232 communications. The Baud rate and other communications parameters must be correctly set to achieve proper control. Check the manufacturer's documentation and specifications first. The default values are likely to be correct, but may not be in all cases.



Using the button next to the "COM Port" radio buttons group label will scan for available COM-Ports.

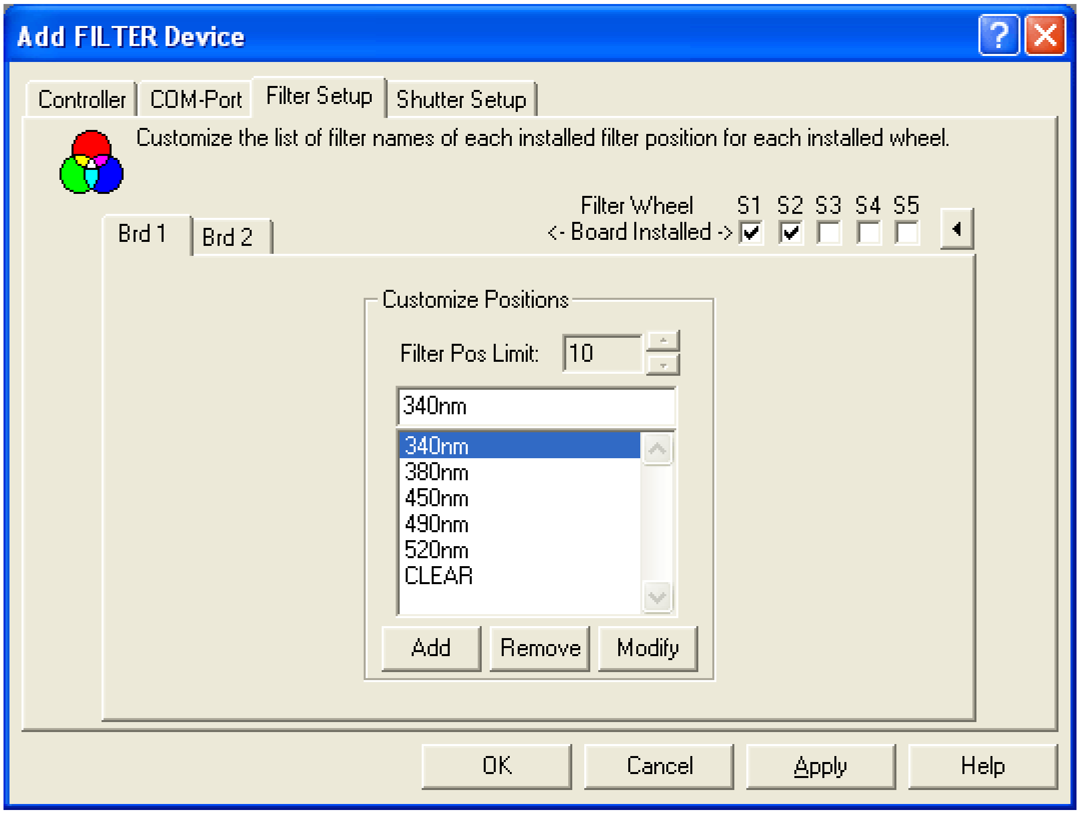
> **Note:** That in some cases, the COM port may be shared with another device (Filter/shutter/Stage/Camera etc) that uses the same controller or may be in use by a different device.

Avoid selecting a COM port to which a modem or other communications device is attached, as this may cause serious system problems when communication is attempted.

If an existing COM port is grayed, it is either already in use by another device, or disabled in the system BIOS.

Filter Controller Properties

The Controller Properties menu allows the physically installed filters to be added to the software control. There may be one or more filter devices listed in this display.



The Filter Pos Limit setting shows the maximum number of filter positions that the controller can address.

In some cases this can be modified to allow more or less filter positions in the list.

Use the **Add**, **Remove** and **Modify** buttons to create a list of filter names in the list. To modify an item, select it and click **Modify** to change the name.

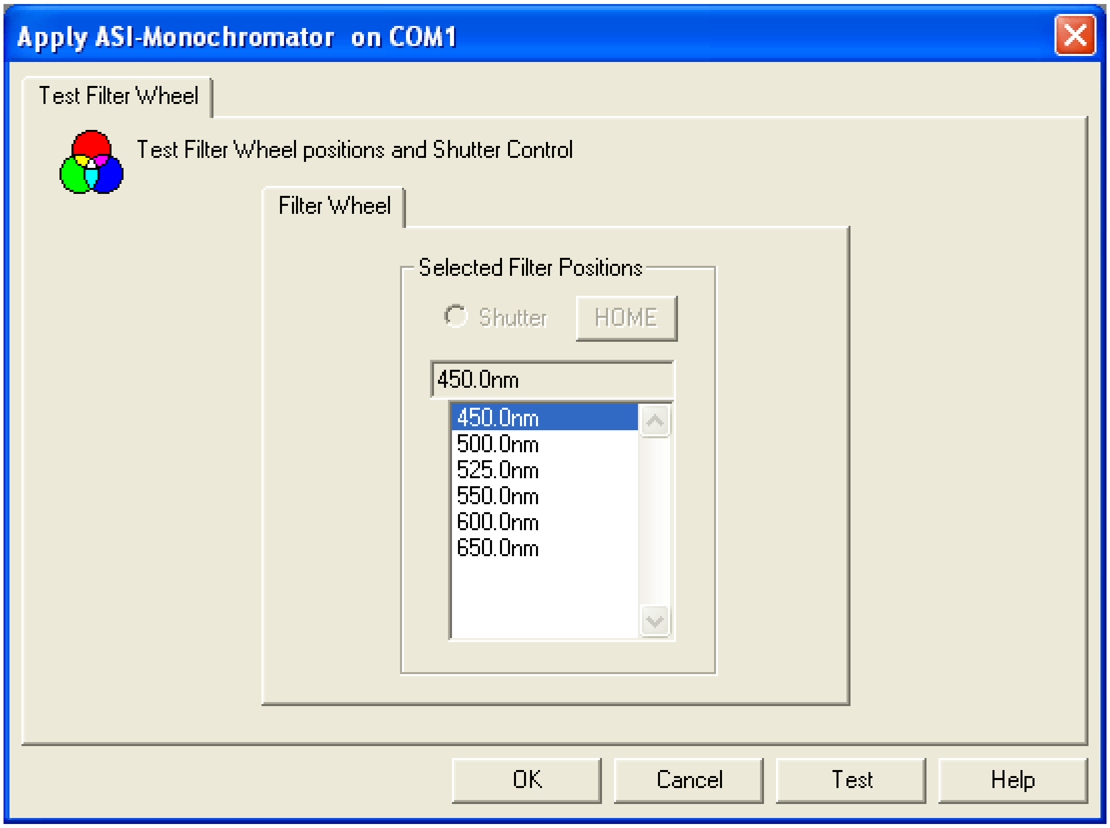
The Apply button can be used to activate the filter positions to verify correct placement of the filters in the device. In the Apply menu, selecting the filter position will activate that position if the hardware is connected and powered properly.

> **Note:** For some devices a single controller can accommodate multiple Filter control cards providing an increased number of devices that can be integrated using one COM Port. For these devices additional items are displayed (as above). Select the Board Addresses manually (**S1,S2** etc..), or use the **Auto** button to detect the Board addresses in use.

Interactive Filter Testing

Once Filter communication has been configured in the Profile, press **Apply** to verify your device in the Apply Dialog.

The **Test** button will activate each filter in the list to allow you to verify that the installed filters match the filter configuration.



Shutter Device Overview

Motorized Shutter control devices are supported allowing synchronized control over the specimen illumination environment.

More than one device can be installed and used at once, allowing simultaneous control of excitation and emission wavelengths. Also by controlling Filter and shutter devices and Capture device settings (exposure, gain, etc.), it is possible to completely change the image acquisition illumination environment for successive images.

Combinations of Filter and Shutter positions are configured and selected in the Automatic Filter and Shutter Control settings, accessed from the Sensor Tab of the Capture Dialog.

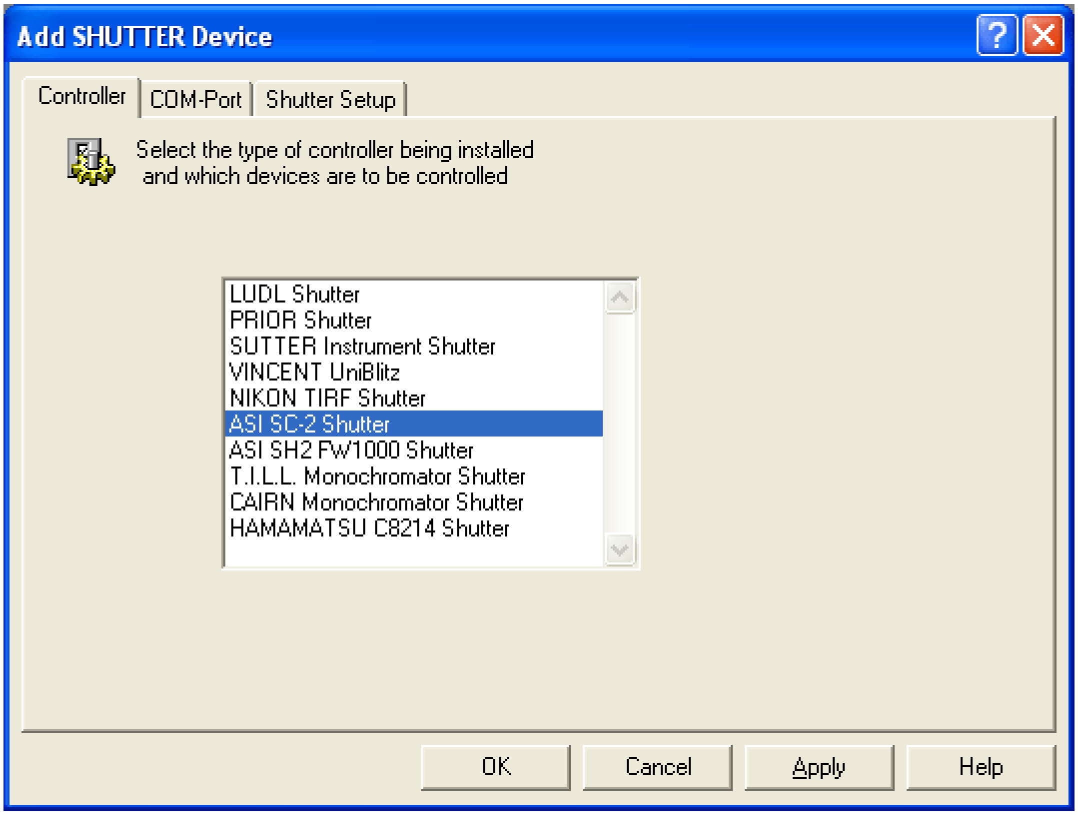
The steps to follow to configure a shutter are:

1. Shutter Device Controller Selection
2. Shutter Device COM Port Setup
3. Shutter Device Controller Properties

Once communication settings have been configured correctly, an Interactive Shutter-Control mode can be accessed via the **Apply** button.

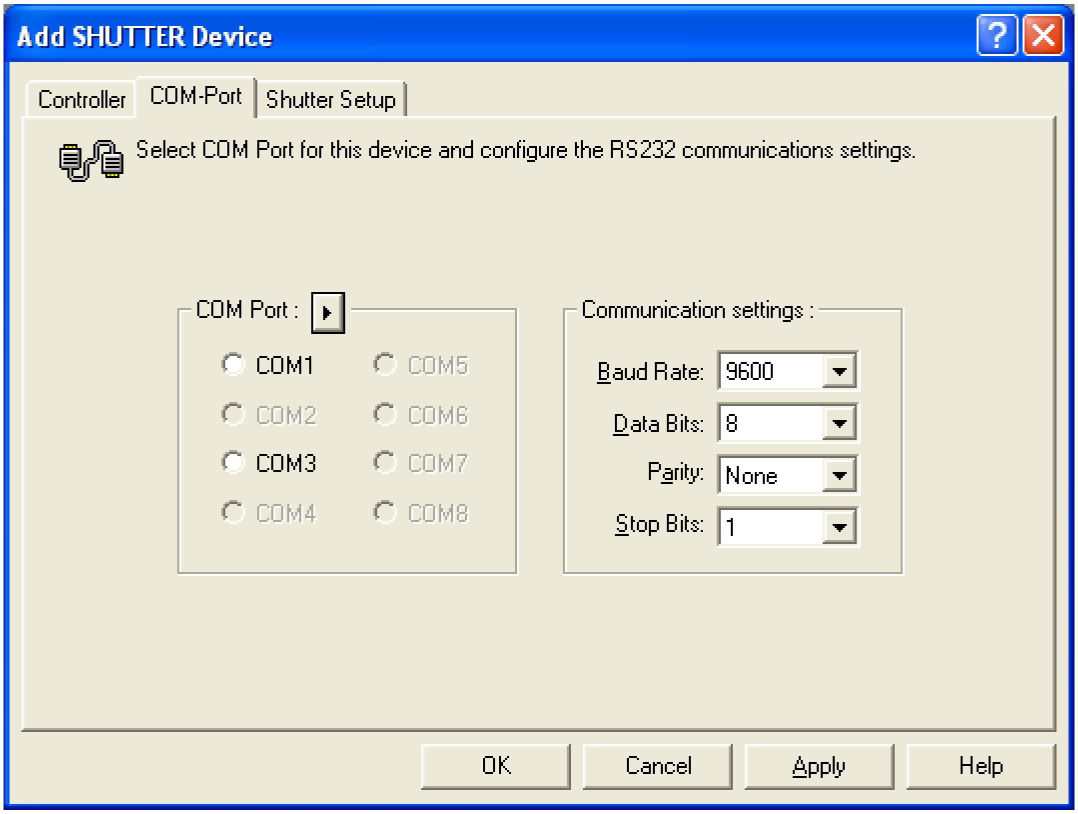
Shutter Controller Selection

Select the Shutter Controller type to add to the Profile.



Shutter COM Port Setup

Select the COM Port for RS-232 communications. The Baud rate and other communications parameters must be correctly set to achieve proper control. Check the manufacturer's documentation and specifications first. The default values are likely to be correct, but may not be in all cases.



Using the button next to the "COM Port" radio buttons group label will scan for available COM Ports.

> **Note:** That in some cases, the COM port may be shared with another device (filter/shutter/stage/camera, etc) that uses the same controller or may be in use by a different device.

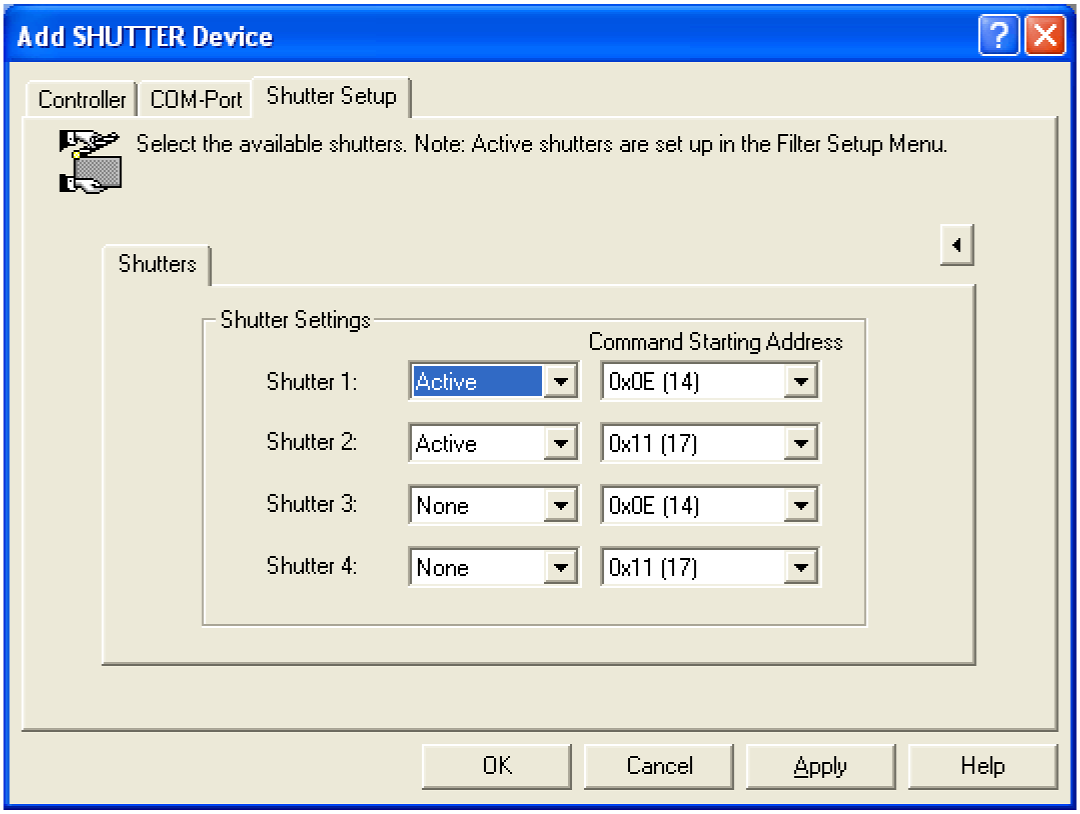
Avoid selecting a COM port to which a modem or other communications device is attached, as this may cause serious system problems when communication is attempted.

If an existing COM port is grayed, it is either already in use by another device, or disabled in the system BIOS.

Shutter Controller Properties

Select the Shutter Settings for each shutter supported by the Controller.

Note: Not all Shutter Controllers can support more than one shutter.



Active – Can be set to Open or Closed in the Filter Setup Menu

Open - Always Fixed in the Open position.

Closed – Always Fixed in the Closed position.

None – No Shutter will be controlled at this address.

In the case of Vincent/Uniblitz and ASI shutters, each shutter has a unique base Hex address for commands. The list of addresses here supports the following styles of controllers:

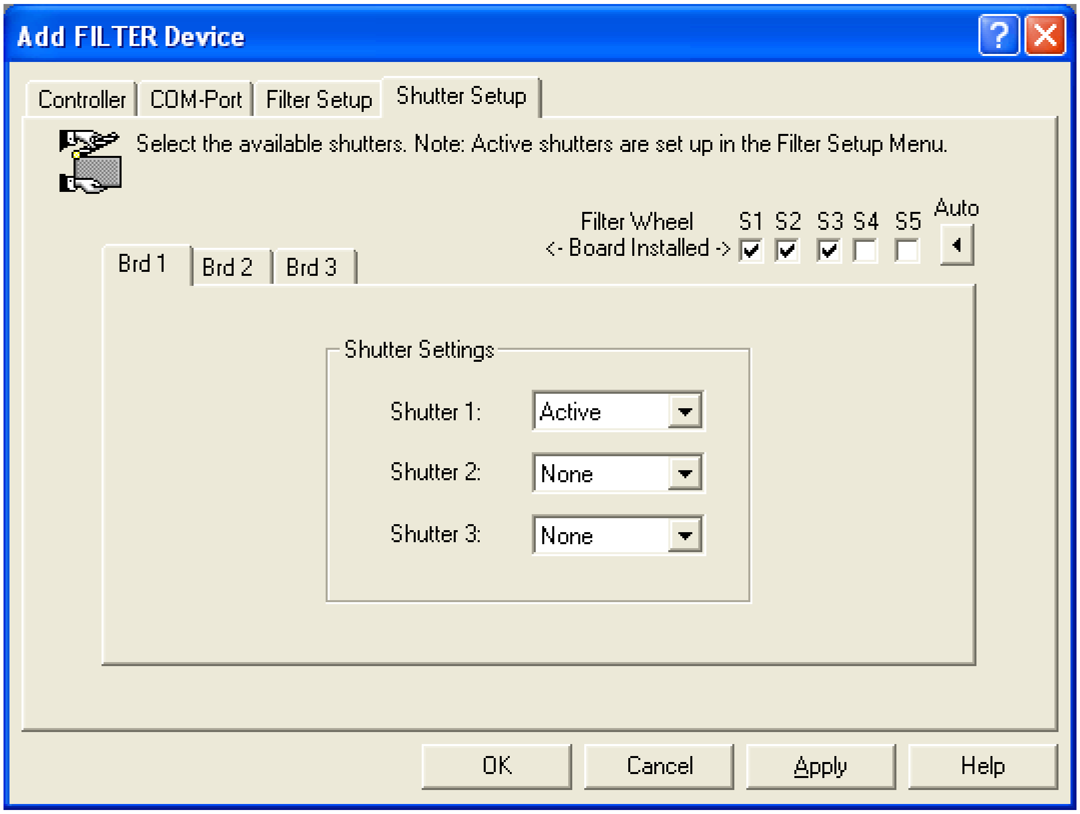
1) A single shutter

2) Daisy chained shutters on the same COM port

3) Multiple shutters on the same controller

In each case, just pick the correct base address to access the shutter.

As always, make sure the Baud Rate is correctly set to match the device.

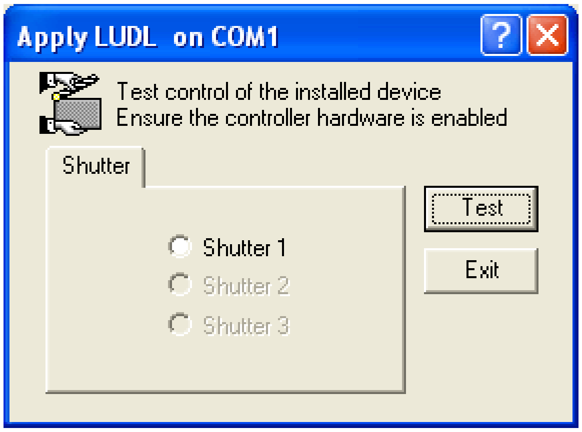


Note: For some devices (e.g. Ludl controllers) a single controller can accommodate multiple Shutter control cards providing an increased number of devices that can be integrated using one COM Port. For these devices additional items are displayed (as above). Select the Board Addresses manually (**S1,S2** etc..), or use the **Auto** button to detect the Board addresses in use.

Interactive Shutter Testing

Once Shutter communication has been configured in the Profile, press **Apply** to verify your device in the Apply Dialog.

Pressing the **Test** button will double- toggle the selected shutter's state to provide a convenient means to verify the shutter's configuration.



Microscope Device Overview

Computer Controlled functionality for microscopes is supported including the following integrated devices:

Stage devices

Z axis control

Filter devices

Transmitted illumination Lamp voltage – continuously variable

Epi-Fluorescence cube movement

Transmitted illumination Neutral Density– continuously variable

Shutter devices

Transmitted illumination Lamp on/off

Epi-Fluorescence shutter

Microscope only devices

Objective Magnification changer

More than one device can be installed and used at once, allowing simultaneous control of excitation and emission wavelengths. Also by controlling Filter and shutter devices and Capture device settings (Exposure, gain etc.,), it is possible to completely change the image acquisition illumination environment for successive images. Combinations of Filter and Shutter positions are configured and selected in the Filter Setup Menu, accessed from each Sensor Tab as described in the Capture Dialog .

The interface to add such devices is consistent.

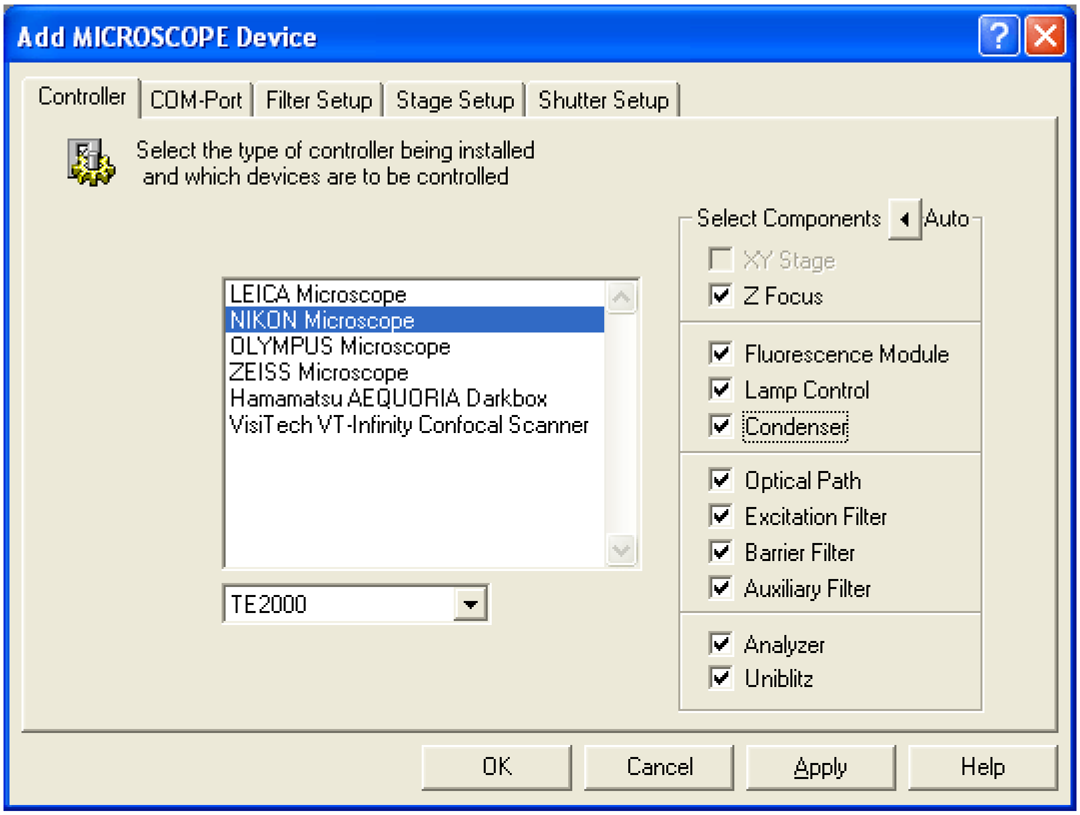
The steps to follow are:

1. Select a Microscope Device Controller
2. Select a COM Port
3. Enter the Microscope Device Controller Properties

Once communication settings have been configured correctly, an Interactive Microscope Configuration mode can be accessed via the Apply button.

Microscope Controller Selection

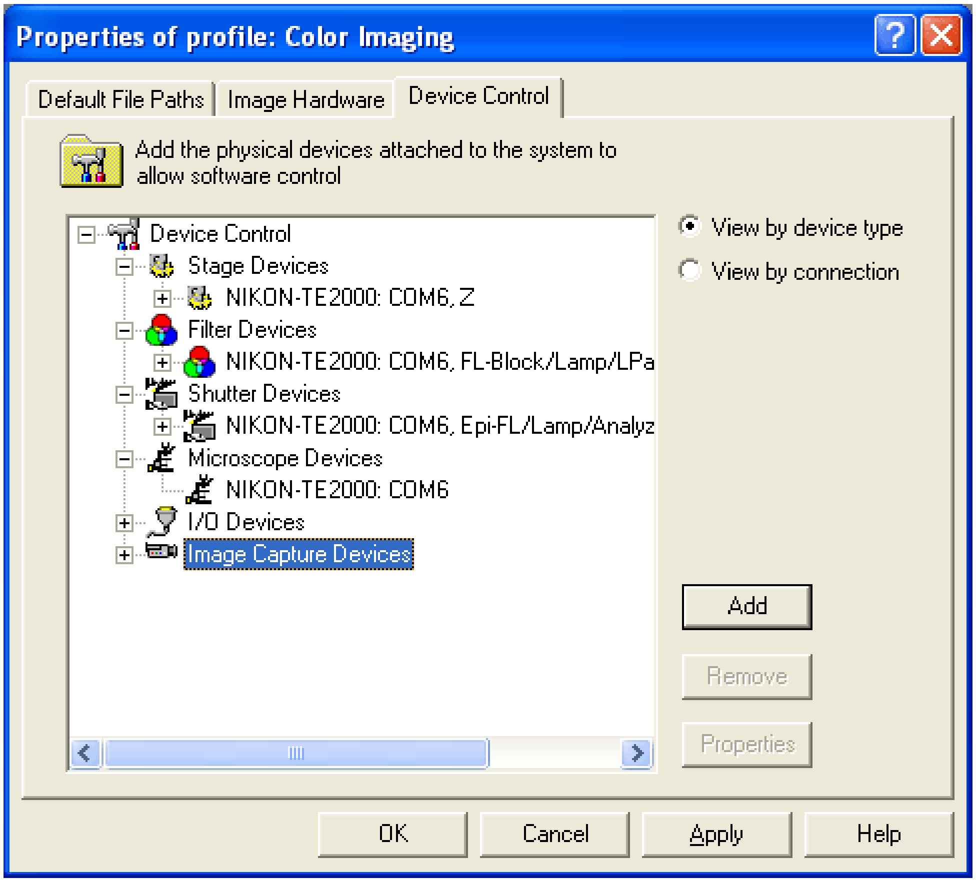
Use this menu to select the Manufacturer for the **Device Controller** being added. This determines the RS-232 communications protocol used to communicate with the device. After choosing the correct major microscope type from the list-box, check off the microscope components which are included in your system.



As the various microscope components are checked and un-checked to indicate which components are present, the Filter Setup, Stage Setup, and Shutter Setup tabs should disappear whenever they have become irrelevant (i.e. the Stage Setup tab would not be visible if Z-focus was not checked).

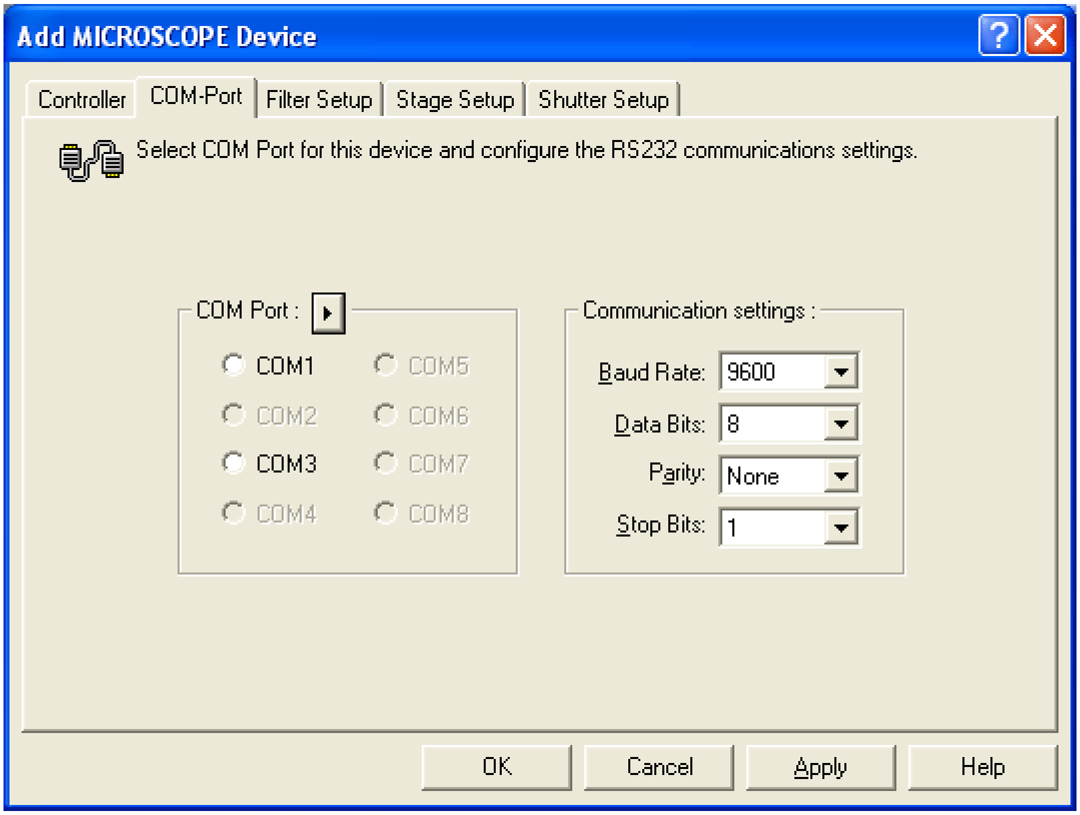
Specifying the wrong controller type may cause problems when sending commands that are not correctly supported. In such a case the controller and possibly the host computer system may need to be powered off and reset.

For convenience, more than one device can be added at the same time. Check all items that are to be computer controlled, and the devices will appear in the Filter, Shutter and XYZ Stage control device lists. e.g.:


Properties of profile: Color Imaging
Default File Paths
Image Hardware
Device Control
Add the physical devices attached to the system to allow software control
Device Control
Stage Devices
NIKON-TE2000: COM6, Z
Filter Devices
NIKON-TE2000: COM6, FL-Block/Lamp/LPa
Shutter Devices
NIKON-TE2000: COM6, Epi-FL/Lamp/Analyz
Microscope Devices
NIKON-TE2000: COM6
I/O Devices
Image Capture Devices
View by device type
View by connection
Add
Remove
Properties
OK
Cancel
Apply
Help

Microscope COM Port Setup

Select the COM port for RS-232 communications. The Baud rate and other communications parameters must be correctly set to achieve proper control. Check the manufacturer's documentation and specifications first. The default values are likely to be correct, but may not be in all cases.



Using the button next to the "COM Port" radio buttons group label will scan for available COM-Ports.

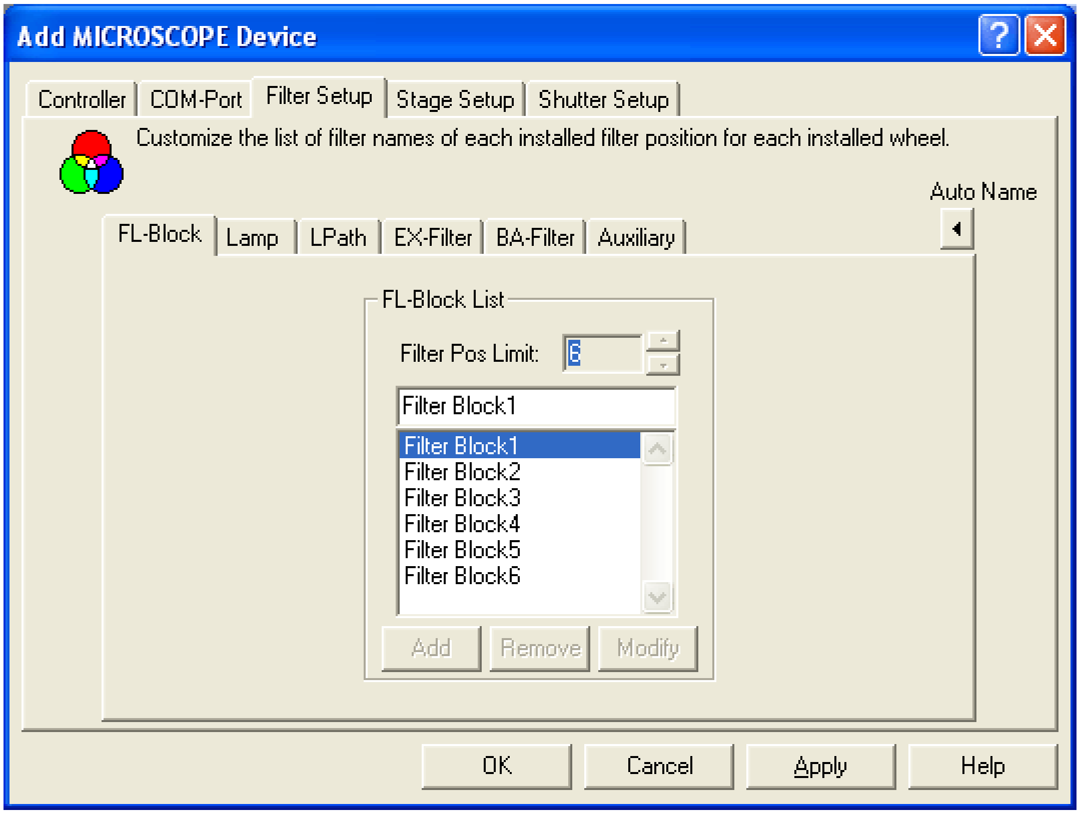
> **Note:** In some cases, the COM port may be shared with another device (filter/shutter/stage/camera, etc.) that uses the same controller or may be in use by a different device.

Avoid selecting a COM port to which a modem or other communications device is attached, as this may cause serious system problems when communication is attempted.

If an existing COM port is grayed, it is either already in use by another device, or disabled in the system BIOS.

Microscope Filter Setup

The Filter Setup page allows the physically installed filters to be configured for software control. There may be one or more filter devices listed in this display, where each can be configured with an interface such as that discussed under Filter Device Controller Properties.

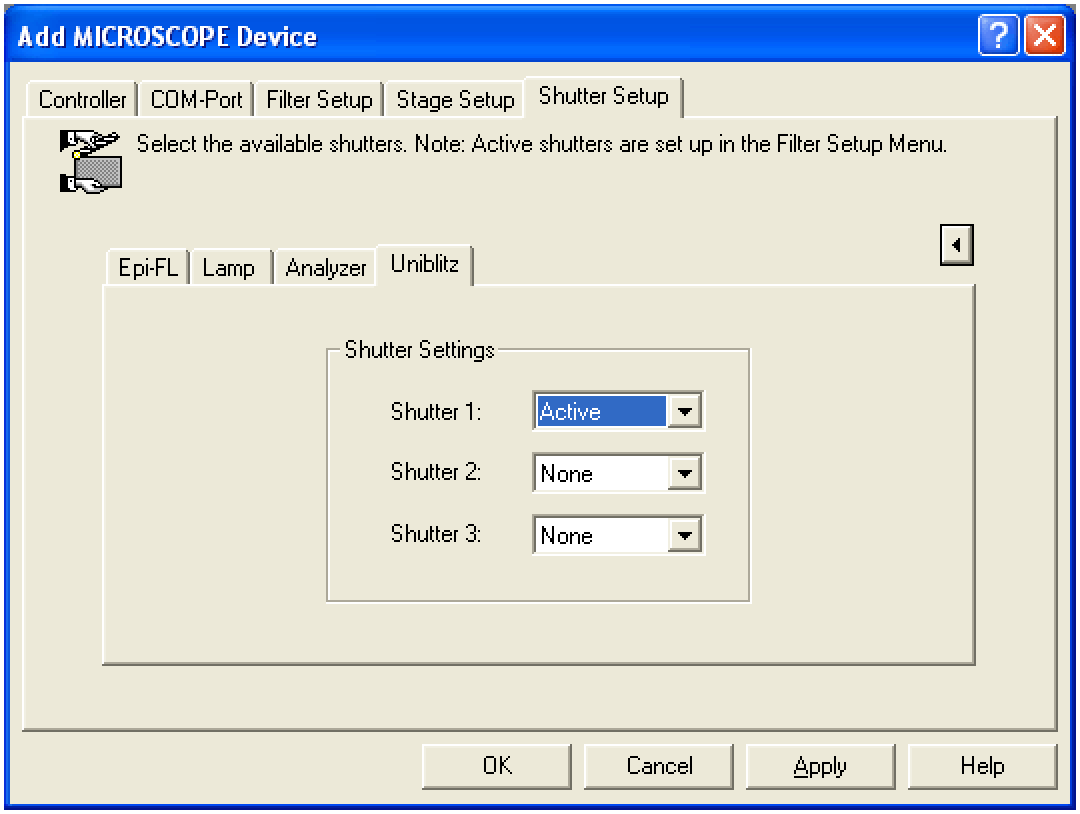


> ***Note:*** *If the microscope being configured does not have filters to control, or if none of these filters were checked on its Controller-selection tab, the Filter Setup tab is not visible.*

Refer to the Microscope Control Manual for details on the operation and compatibility of available wavelength-control devices for specific microscopes.

Microscope Shutter Setup

The Shutter Setup page allows the physically installed stage actuators to be configured for software control. There may be one or more shutter device sub-pages shown on this tab-page, where shutter device can be configured according to the same type of interface discussed under Shutter Device Controller Properties.

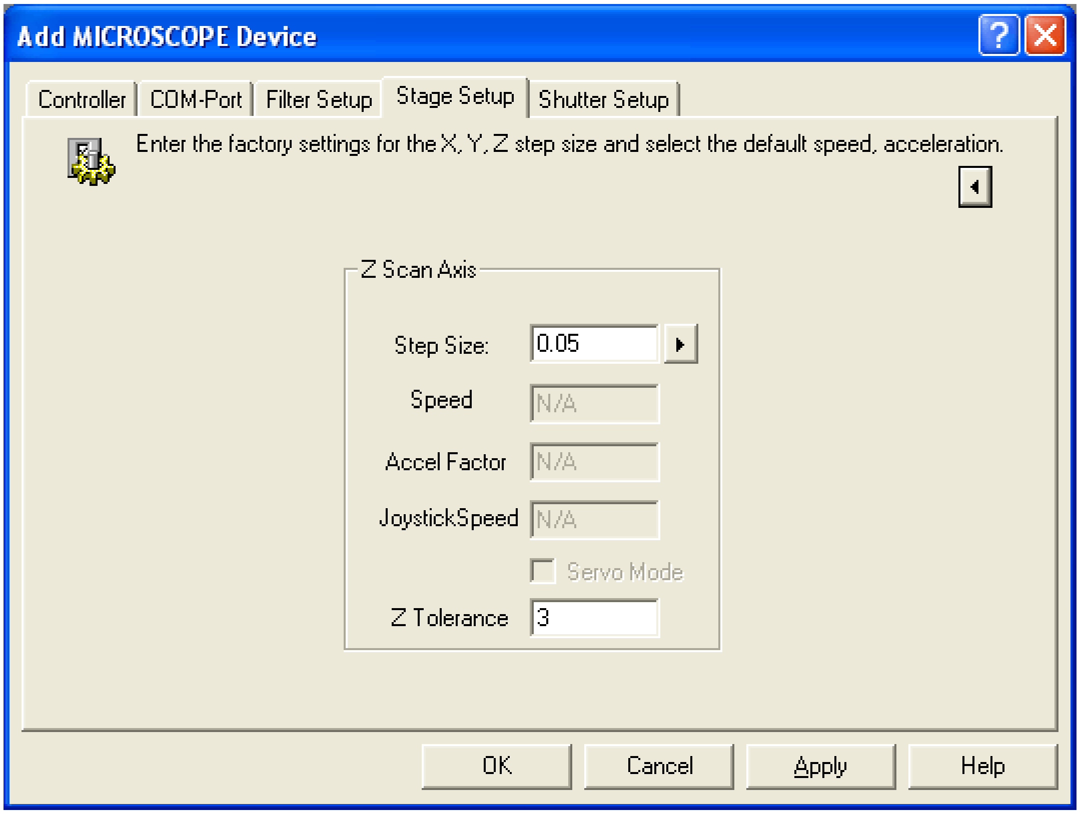


> ***Note:*** *If the microscope you are using does not have any shutters to control, or if none of these options were checked on the Controller selection tab, the Shutter Setup tab should not be visible.*

Refer to the Microscope Control Manual for details on the operation and compatibility of available integrated stage-control devices for specific microscopes.

Microscope Stage Setup

The Stage Setup page allows the physically installed stage actuators to be configured for software control. There may be X-Y, Z or both stage axes listed in this display, where each can be configured with an interface such as that discussed under Stage Device Controller Properties.



> ***Note:*** *If the microscope being configured does not have stage control available, or if this option was not checked on its Controller-selection tab, the Stage Setup tab should not be visible.*

Refer to the Microscope Control Manual for details on the operation and compatibility of available integrated stage-control devices for specific microscopes.

Interactive Microscope Testing

Once I/O communication has been configured in the Profile, press **Apply** to verify your device in the Apply Microscope Dialog.

You may wish to verify your device as a whole or you may wish to verify the individual units. If you **Apply** while on the COM port page, the installed microscope's interactive testing dialog will be identical to the Microscope Tab available on the Capture Dialog. If you **Apply** while on the Stage Page, SimplePCI will perform Interactive Stage Testing. Pressing **Apply** while on the Filter page will initiate Interactive Filter Testing. And, finally, Interactive Shutter Testing is available on the Microscope Shutter Page.

The microscope controls are customized to correspond to features each manufacturer has included in their specific automated microscopes.

For detailed help, consult the microscope's control manual.

I/O Device Overview

The I/O Devices category permits custom control of the general-purpose IO devices.

The serial (COM) port and parallel (printer) ports come standard on most computers, while the other items are general-purpose I/O boards.

Some of the available devices have both digital I/O and analog output pins.

Digital Input pins may be used to capture events

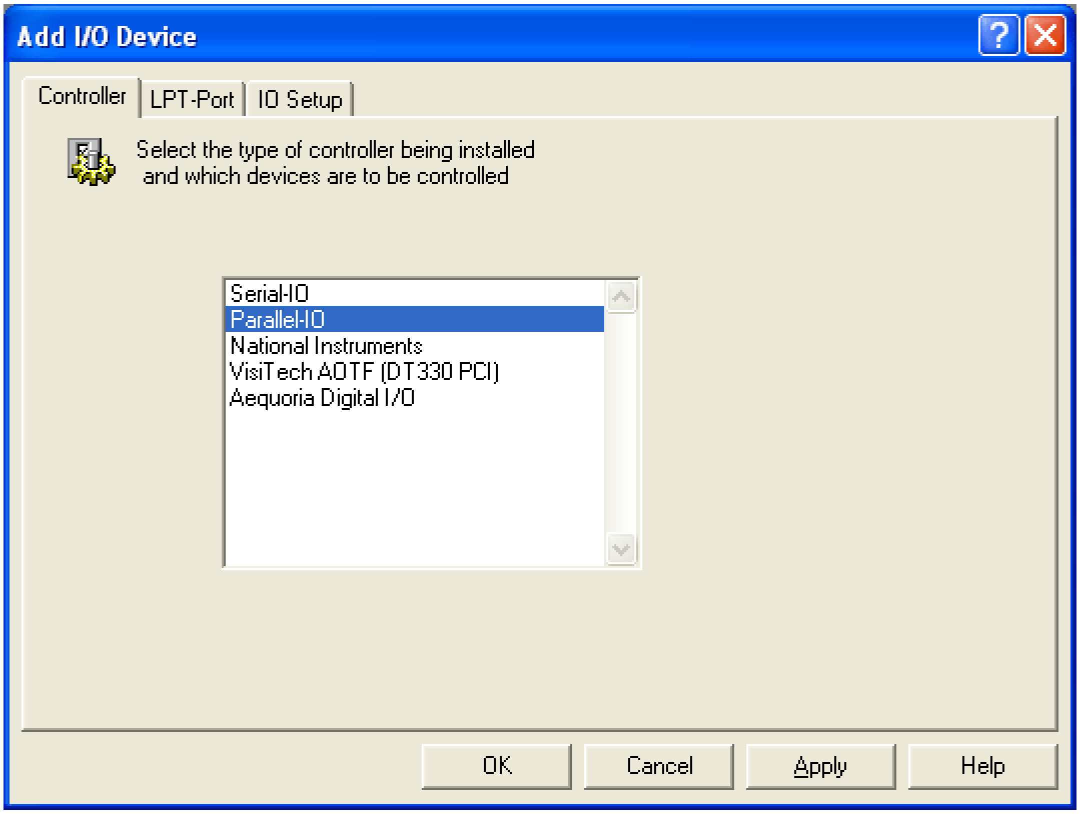
The steps to follow to add a new I/O device are:

1. Select I/O Device Controller
2. Select the Port or Device Type
3. Enter the I/O Device Controller Properties

An Interactive I/O Device Test mode is accessible via the Apply button once communication is established.

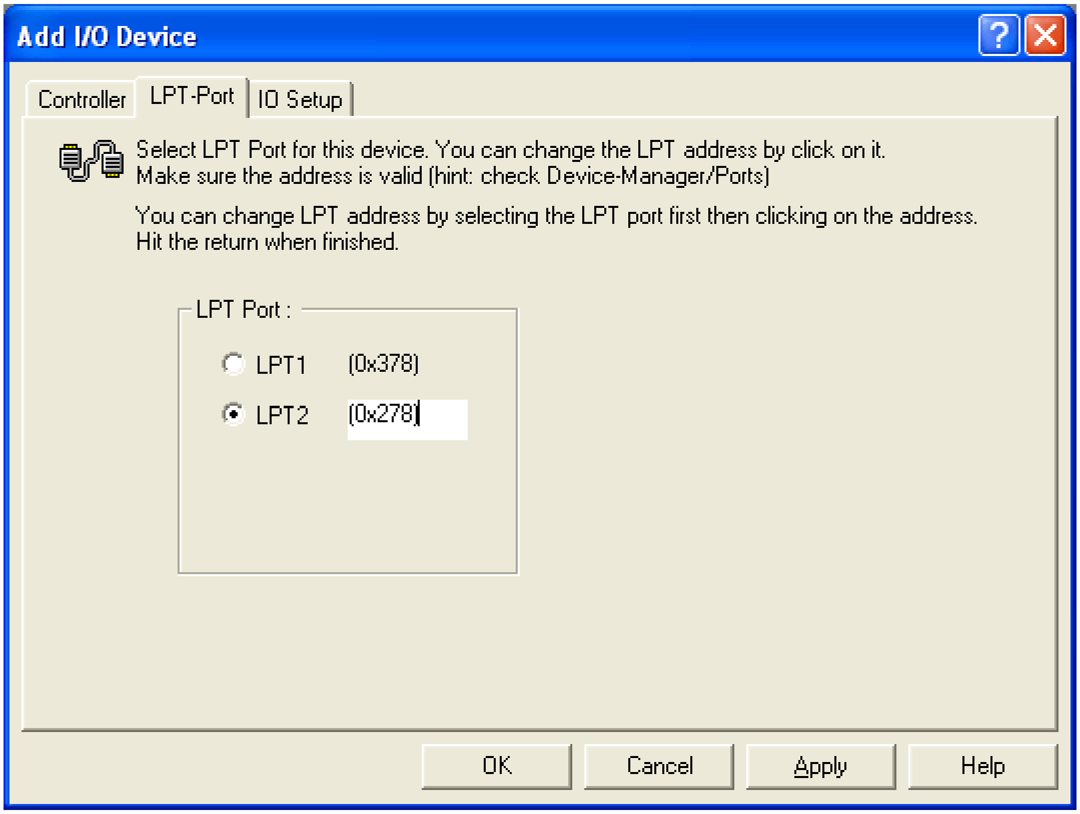
I/O Device Controller Selection

Select the I/O Controller type to add to the Profile.



I/O LPT Port Setup

Select the LPT port for parallel communications. The port address must be correctly set to achieve proper control. The default values are likely to be correct, but may not be in all cases.

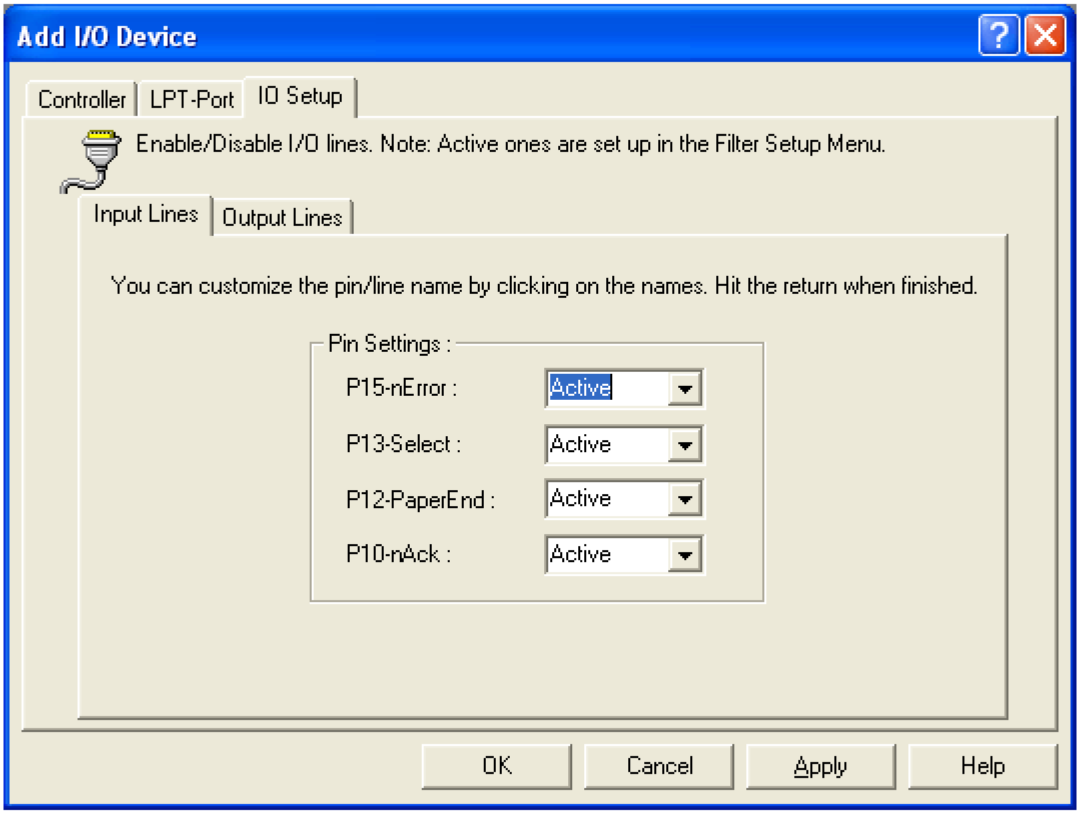


Click in the address field to edit the address.

Note: Your parallel port must be set in SPP mode.

I/O Properties

Depending on the specific device, it may have a number of input lines and a number of output lines.



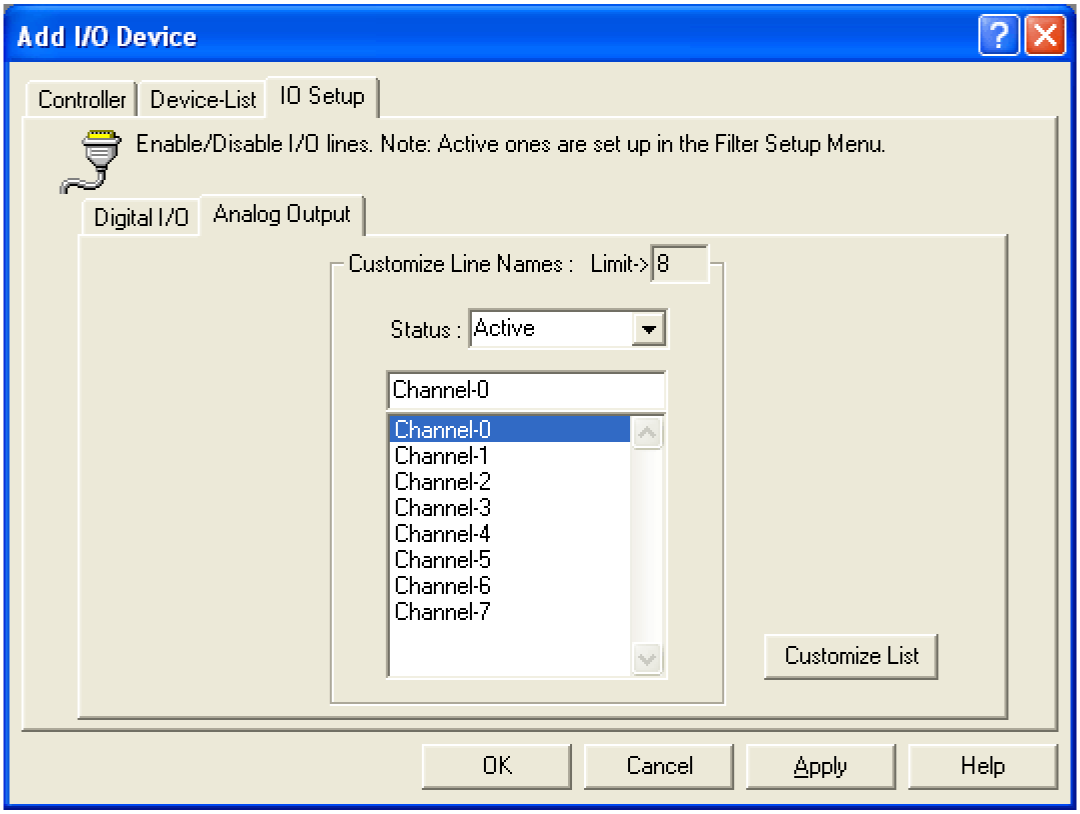
Input lines will typically be able to be configured into one of four possible states:

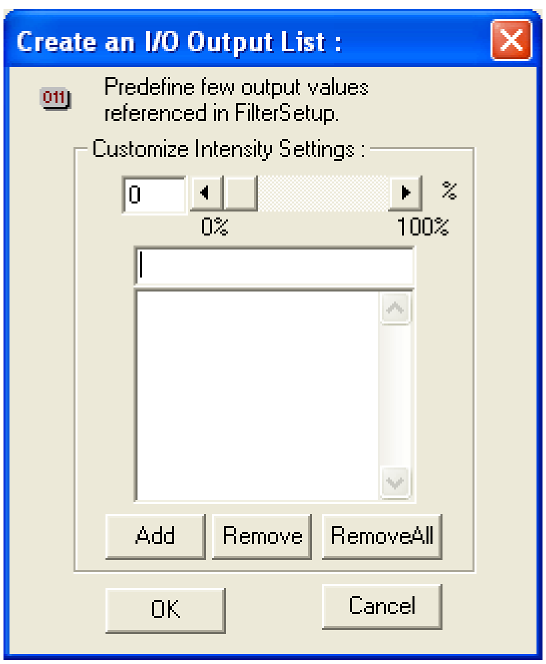
- Disabled - signals which may be present are not read
- Active - senses the signals present when read
- High - always senses a high value when read
- Low - always senses a low value when read

The pin names may be customized, e.g. to reflect the purpose of the signal they are sensing. This can make future interactions within the software involving the device more intuitive.

Digital output lines have a similar interface. In addition, combinations of output values on the available pins can be configured and assigned names which will then be available.

Analog output lines on some I/O cards have additional settings permitting control over the range of output voltages which will be produced.





Add: Add a new custom output level

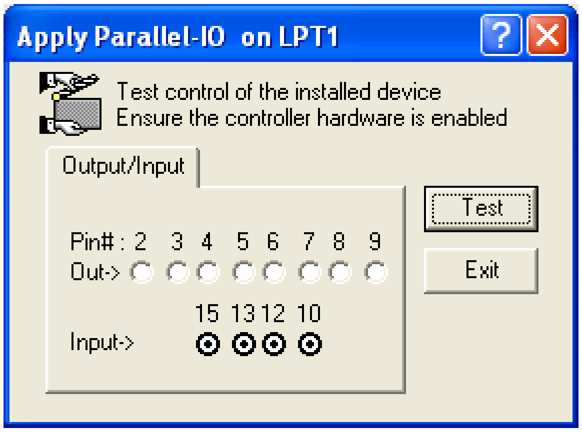
Remove : Remove the high-lighted custom value from the list

Remove All: Clear the custom-value list

Interactive I/O Testing

Once I/O communication has been configured in the Profile, press **Apply** to verify your device in the Apply Dialog.

Pressing the **Test** button will perform a scan of each of the active input pins and a double- toggle of each of the output pin's states to provide a convenient means to verify the I/O device's configuration.

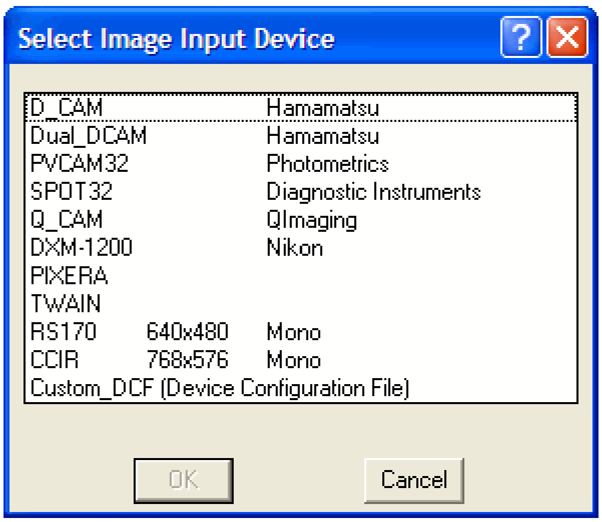


Capture Device Overview

The System Profile supports multiple **Image Capture Devices** installed in the same profile. This provides several useful facilities:

- A single profile can contain multiple different capture devices. Software switching is supported.
- The same capture devices can be configured with different Capture properties (Spatial resolutions and color depth). See the Device Setup Menu in the Capture Dialog.
- Different Profiles can contain Capture devices configured for specific users or applications.

To install an Image Capture Device from the Device Control Tab of the Current Profile, select the Image Capture Device item in the tree and click Add. A list of possible devices will be displayed.



Devices in the list will vary according to the Imaging Hardware capability.

Meteor2 driven:

- **Video Cameras: Meteor2/Std. or Meteor2/MC**
  - RS170; CCIR; NTSC; sVHS; PAL; RGB
- **Digital Cameras: Meteor2/DIG**
  - HC4742 Hamamatsu; DVC
- **Custom DCF files**: Non-standard acquisition using the Imaging Hardware

Driver controlled:

- **DCAM** - Hamamatsu

  All DCAM supported cameras. (1394, Phoenix, Coreco)
- **PVCAM** - Roper Scientific Cameras

  All PVCAM supported cameras (Photometrics and Princeton models)
- **QCAM** - QImaging

  All QCAM supported cameras. (1394)
- **SPOT32** - Diagnostic Instruments

All SpotCam supported cameras (1394, PCI,..)

- **DXM** - Nikon

  The DXM1200 series.
- **Pixera** - Pixera Corporation

  Pixera Penguin (color and monochrome)

TWAIN capture devices:

- all installed TWAIN capture devices

Depending on the device, further input by the user may be necessary.

TWAIN Devices

Supported devices: Any image capture device providing a TWAIN interface.

The TWAIN interface allows a wide variety of image capture devices to be attached and integrated into the system.

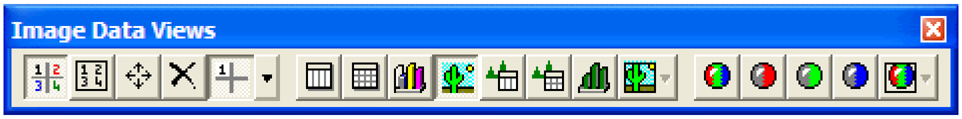
The normal interface for TWAIN devices is found in the **File Menu**. Use **File|Select TWAIN Source** to choose the desired device. **File|TWAIN Acquire** captures an image into an Image Document for further manipulation, printing and archiving.

Further support is enabled by adding a TWAIN device in the Capture Device Setup menu. This allows direct capture of images from TWAIN devices into the Image Display for further processing and analysis.

TWAIN devices are supported using the driver supplied by the manufacturer which may allow acquisition with or without the user interface being activated. Devices allowing acquisition without the user interface can be used for time based acquisition using the Sequence Capture options in the Capture Dialog .

When installing a TWAIN device, test the configuration in the standalone acquisition software provided by the manufacturer before configuring in this software. Please note, TWAIN acquisition is typically slow and should only be used if direct driver support is not available.

Image Data View Summary



The Image Data can be viewed as a normal image or in a variety of graphical and numerical Views selected from the above Image Data View Toolbar (shown in fully expanded mode) including:

- **Quadrant View (Image Document only)**

- **Normal Image View**
- **Table View**
- **Spreadsheet View**
- **Graph View**
  - **2D Profile**
  - **3D Profile**

- **Table Histogram View**
- **Spreadsheet Histogram View**
- **Histogram View**
  - **2D Histogram**
  - **3D Histogram**

- **Image Document Source Color Channel**

Image Display bit-depth drop-down menu

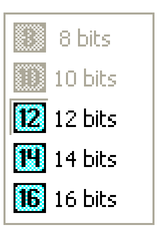
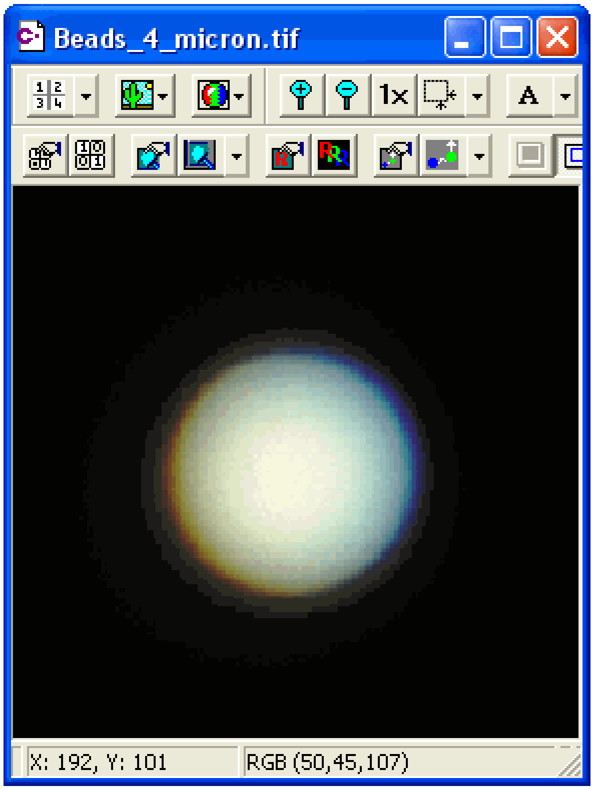


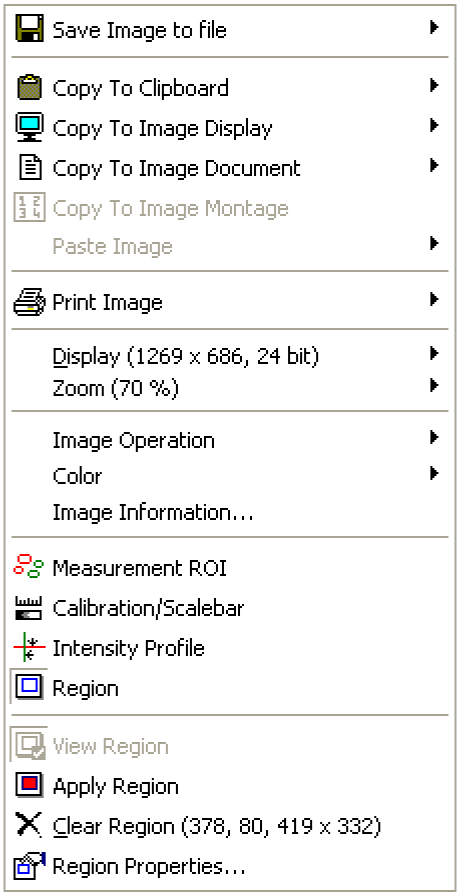
Image files stored using 12-bits per channel can be displayed using expanded bit-depths via the Image Display bit-depth drop-down menu. This does not affect the actual data, but can improve the viewing clarity of spreadsheet and histogram data in the display. It also affects the maximum slider value in the contrast dialog, identify dialog, and qualify dialog. The actual bit-depth of captured data can be controlled in Capture Device Setup.

Image View



The **Image View** provides the normal method of viewing image data as an intensity bitmap, or picture.

A comprehensive right-click context menu is also available for the Image Document providing access to items on the toolbars and menus.



The Image View can display the image data from either an image document or the "Original Image" node of a data document field, with enhancements to display any intricate details in the pixel intensity data more perceptibly to the human eye without affecting the actual data content to be processed by the computer when measurement process is initiated.

How? Operations available on the Image Processing toolbar act on a copy of the Original Image to create a Processed Image upon which measurements are made. Likewise, only a copy of the Processed image is used when items on the Image View toolbar are employed to improve the perceptibility of image details, creating an image called the Display image which is the image visible on the screen (or sent to the printer). This is crucial to remember when looking at measurement results since there can be dramatic differences between the true and displayed pixel intensity values, for example when pseudo-coloring contrast enhancements are utilized.

Image View Layers

Several layers of information display can overlay an Image View, depending on the context and mode it is being used:

- **Annotation Layer**
- **Scalebar Layer**
- **Intensity Profile Layer**
- **ROI Shapes Layer**
- **Region Layer**

Activating Image View Layers

Only one layer can be active at a time. Activating a layer changes the mouse-interaction behavior of the display accordingly.

Activating an image layer can be performed in two ways.

1. When the corresponding toolbar is open, the "Activate <Layer Name>" button, located beside the down-arrow menu anchor for the commands associated with that layer will activate that layer and make it visible.
2. Within the right-clicking context menu of the image view, selecting the appropriate layer name activates that layer.

For example, activating the Region layer causes mouse drags on the image to define a Region of Interest Rectangle. Enabling the Region of interest rectangle will cause all data outside the Region of Interest to be ignored but the mouse image interaction behavior will remain that of the currently active layer.

Likewise, merely toggling the intensity profile layer's visibility on maintains the currently active layer as active (mouse image interactions do not change), but shows or the intensity profile. Activating the Intensity Profile layer will allow mouse drags to move the intensity profile cross-hairs.

Layers must be visible while active, so some layers cannot be made invisible while active; to hide these layers, another layer must first be activated. Layers in the Image Display toolbar (Scalebar, Annotation) can be activated and then de-activated using the same button instead of activating another layer; in which case the active layer will reset to the Region layer.

Note: That while similar in many respects, the Image Display has its own unique properties and behaviors and is not considered a true **Image View**. The Image Display window uses a ROI Shapes toolbar only when defining the capture ROI (which must be configured from within the capture icon's configuration window) and not for defining shapes to use for measurements. For this reason, the Image Display's ROI layer cannot be activated by any other means than through the Capture icon's configuration window's ROI button. Likewise, although the Image Display will display a rectangular Region layer while a Focus Region is being configured, this is provided only through the Focus Setup button in the capture icon's configuration window.

Image Bit Depth

The bit depth of each component refers to the memory size used to store each pixel in the image.

- 8-bit data: An 8-bit pixel requires one byte of data, and can contain 2 to the power 8 different values, from 0 to 255.
- 16-bit data: A 16-bit pixel; requires two bytes of data, and can contain 2 to the power 16 different values, from 0 to 65535.

Both color and monochrome images can contain 8-bit or 16-bit data.

Bit Depth	**Possible Values**	Image Size (640 x 480 resolution)	**Image Size (1344 x 1024 resolution)**
8-bit	256	307,200 bytes (0.3 MB)	1,380,226 bytes (1.3 MB)
16-bit	65,536	614,400 bytes (0.6 MB)	2,762,998 bytes (2.6 MB)
24-bit	16,777,216	921,600 bytes (0.9 MB)	4,131,196 bytes (3.93 MB)
48-bit	4,294,967,296	1,843,200 bytes (1.85 MB)	8,269,058 bytes (7.88 MB)

There are a number of important factors to consider before using data at 16-bit depth per component:

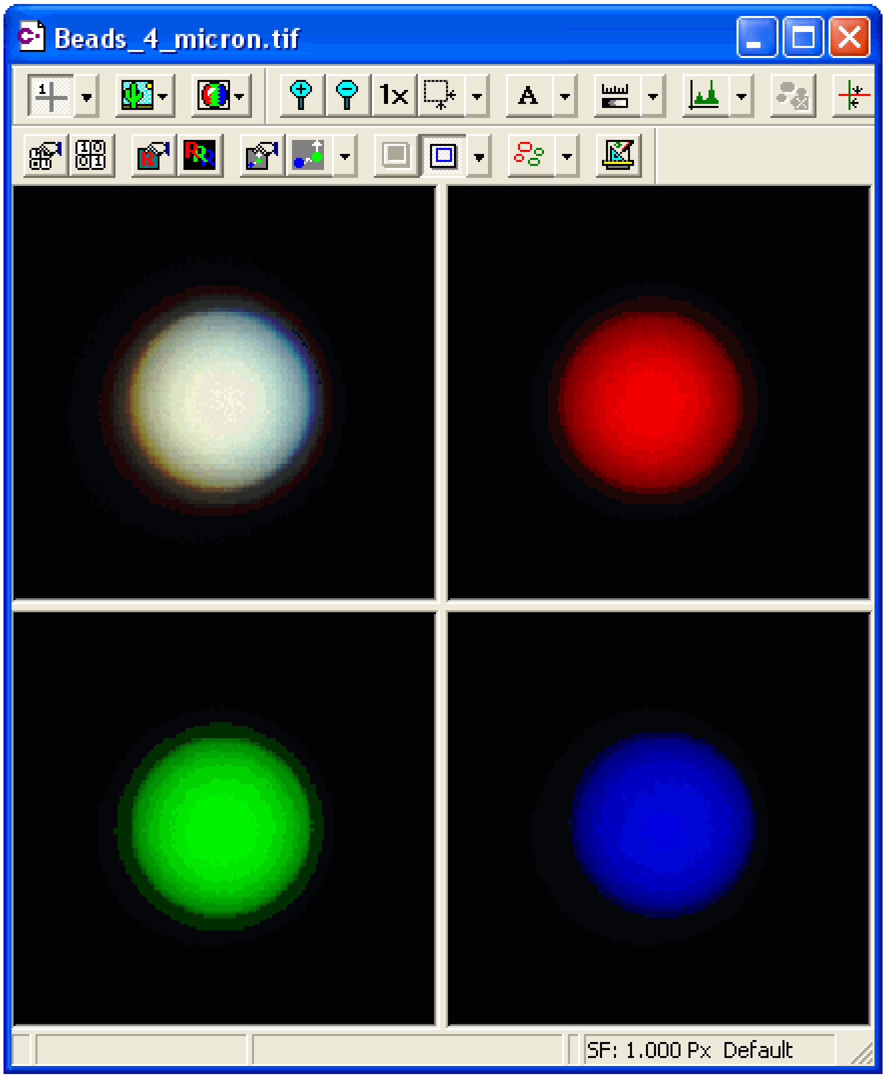
- The data volume of each image component will double.
- Image archiving to disk will require more storage and transfer time.
- Image processing will require more memory and processing time.
- Image display will require more memory and processing time.
- Not all imaging applications can load images with 16-bits per component.
- The data in the additional bits may or may not contain any useful information.

The display of image data is typically limited to a maximum of 24-bits, usually configured as red, green and blue at 8-bits per channel. By convention a zero grey-scale value is displayed as black and the maximum intensity is displayed as white. In the case of color, each color component is displayed from black to its brightest intensity, combining to give over 16 million possible discrete color combinations.

> **Note**: Display resolutions of 32-bits are possible, but do not normally correspond to increased color depth, which is likely to be indistinguishable to the eye. A 32-bit display mode can be used to optimize the transfer speed of pixel data to a graphics display device that uses 4-byte multiples for transferring bursts of data. A non-optimized 24-bit display might transfer pixel data as 3-bytes per pixel in each cycle with one byte of the 32-bit transfer wasted, e.g. RGBX-RGBX-RGBX-RGBX, taking four cycles to transfer four pixels of data. A 32-bit display optimization can transfer the data packed together as RGBR-GBRG-BRGB, transferring four pixels in three cycles.

Contrast and pseudocolor enhancements can be used to highlight portions of the intensity data of interest. Some data reduction is needed to display 16-bit data.

Quadrant View (Image Document Only)



 Use the **Quadrant View** button in the Image Document Data View Toolbar to activate a Split View containing up to four independent image views. Click the Quadrant View button to automatically create a four-pane quadrant view of the image, by default separating the color components using the Display Color Components.

Note: The Quadrant button is not available in the Data Document Image View.

Each view has independent Image Document viewing controls, making it possible to view the Image as an overview at zoomed magnification or with any of the other transforms applied at the same time. As multiple Image Document views are created simultaneously, memory usage for large images can be significant.

The center of the quadrant view can be moved to provide different sized views by clicking and dragging the central quadrant frame.

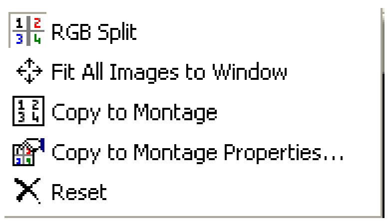
Active Pane

Clicking in each pane selects that pane as current, and displays the toolbars and settings for that pane. Note that the Quadrant button changes to indicate the current pane number and position.

Unpress the Quadrant button to switch between Quadrant view and single-pane view for the currently selected pane.


The drop-down menu from the Quadrant button has further options:

Note: Most drop-down menu items can be promoted to icon buttons and vice-versa (see Toolbar customization for details).



RGB Split

If the RGB Split selection is pressed the quadrant display automatically sets the Display Color Components to All Colors, Red, Green, Blue for the respective quadrants. If it isn't, each quadrant is displayed using All Color Components.

Copy To Montage

The Copy to Montage selection will create a new Image Document containing all four views in a single image as a 2x2 montage. This can then be treated as a single image for further operations, like saving to disk or printing.

Fit All Images to Window

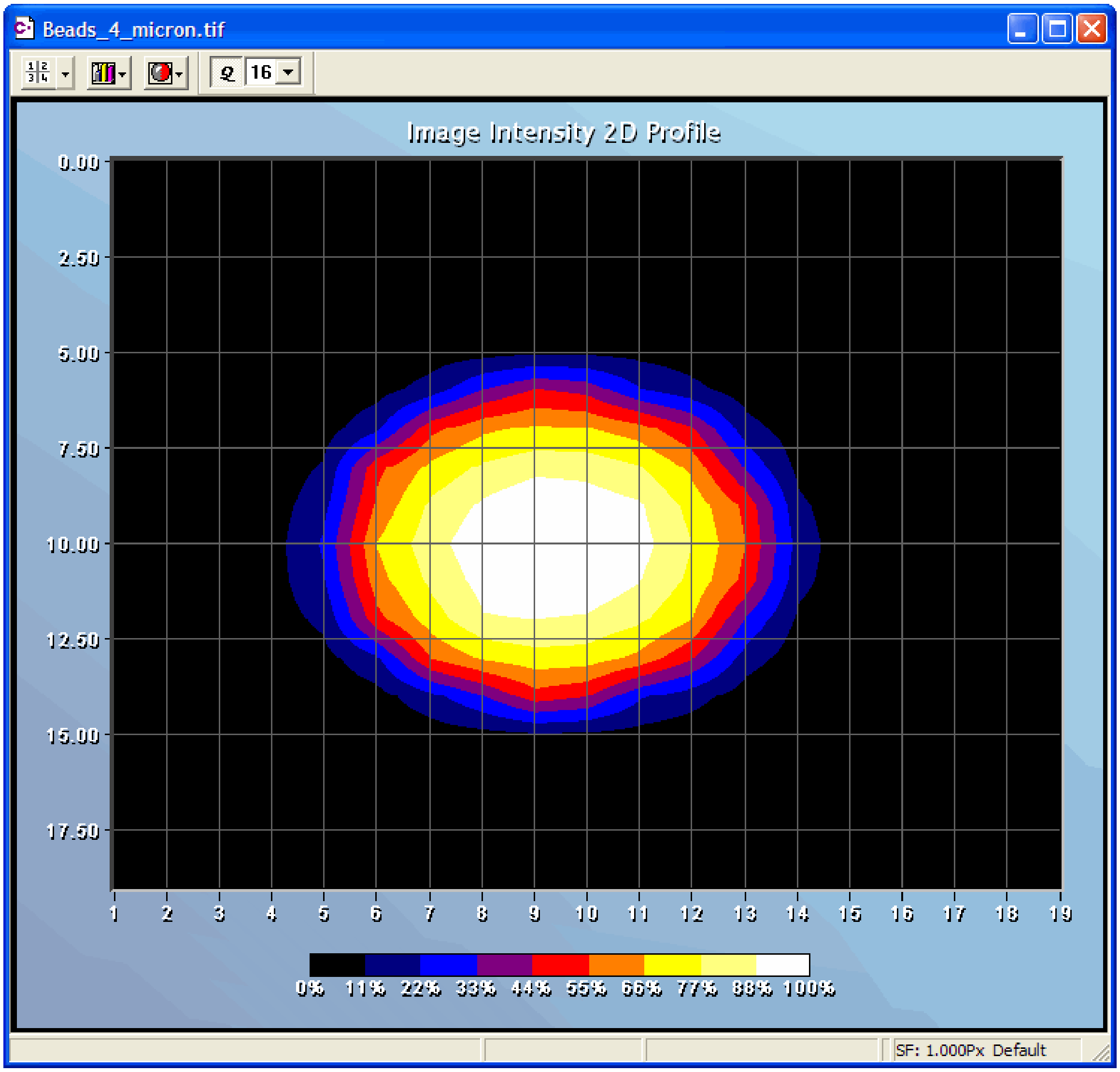
This selection will resize each image pane to fit within the border of its frame (excluding scroll bars). Use this in conjunction with the Fit Window to Image option to create a display like the example with convenient framing of each equal sized pane.

Reset

The Reset option will clear all settings and memory associated with the Quadrants.

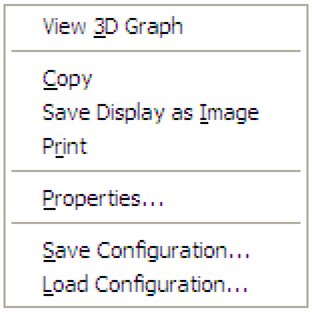
Graph View - 2D Profile

In the Current View drop-menu clicking on **Graph View** will open a **2-Dimensional Profile View** of the image. The graph shows the image pixel positions in the X-Y axes with the pixel intensity data represented by color.



This option is most useful when the Image Data has had the Vertical or Horizontal Squeeze option applied.

Display options can be selected by right-clicking on the image or clicking on Graph in the Main Menu.



The Graph **Right-Click Menu** will display a list of the other controls including:

View 3D Graph: Displays a **3-Dimensional Intensity Profile** of the image.

Copy: Creates a copy of the graph and places it on the clipboard.

Save Display as Image: Saves the Graph as an image and prompts the user to name and choose a location to save the new image.

Print: Sends a copy of the graph to the printer.

Properties: Opens the **Graph Properties** window that allows the user to customize the layout, colors and text of their graph.

Save Configuration: Saves the changes the user made in Graph Properties as a .gxc file for use on other graphs.

Load Configuration: Loads the graph configuration file, replacing the default graph properties with the one saved in the .gxc file.

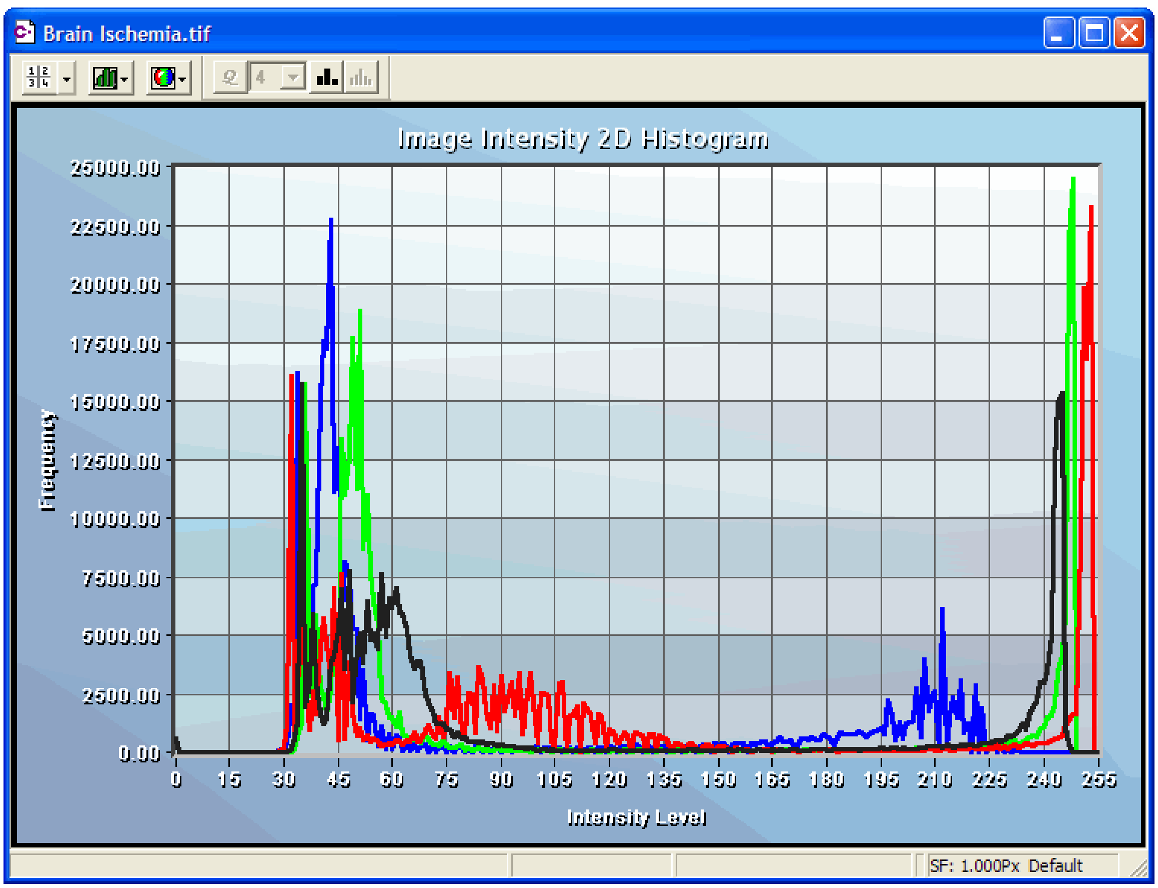
> **Note:** The amount of data in a high-resolution image may be far too great to display with accuracy on the screen, so a Quality Factor is used to decrease the image data before rendering the view. Use this quality factor to increase interaction speed or for a preview, before selecting the final quality factor for the best picture.

2D Histogram View

To see the intensity distribution of an image's pixels graphed in a two-dimensional display, click the **Histogram View**  from the Current View drop-menu. An intensity distribution histogram is plotted which describes how many pixels are contained within the image document within various intensity-level ranges. The bins correspond to intensity (i.e. pixel value) and the width of the bins can be increased or decreased via the "Bin Decrement"  and "Bin Increment"  toolbar buttons.

Histogram bars will change to a line graph if the number of bins is increased until there is one bin for each of the intensity levels available.

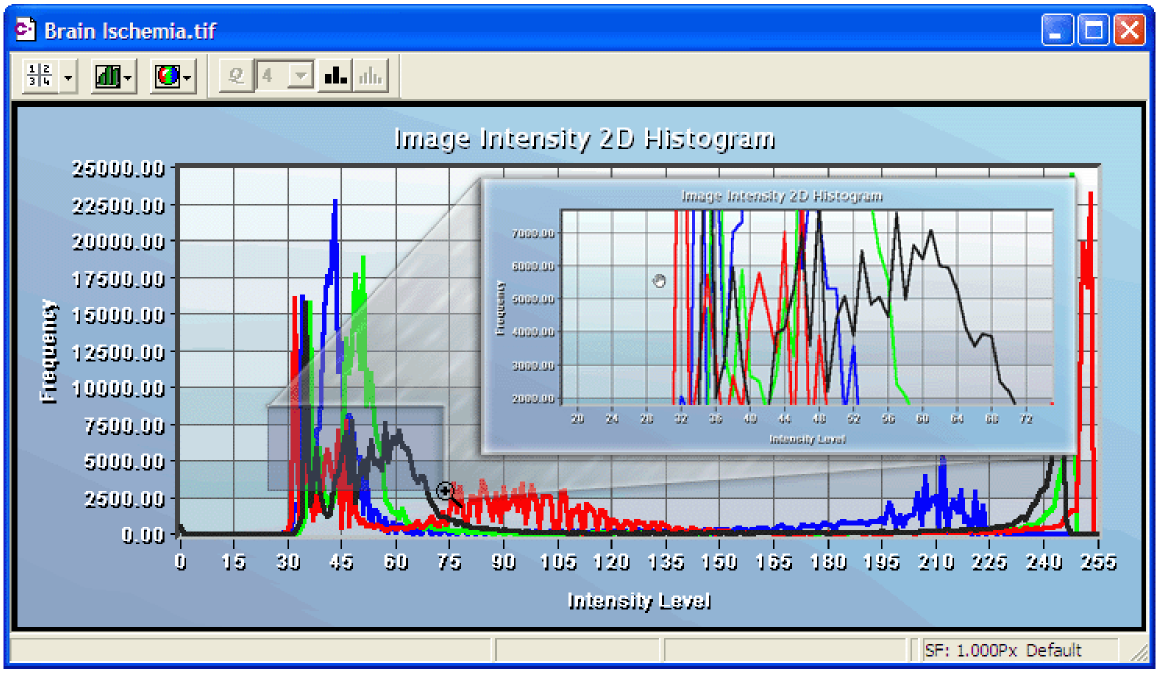
By default, a histogram will be included for each source color channel present in the image and the user is able to customize the graph by right-clicking and selecting Properties. The **Graph Properties** window allows the user to change the layout, colors and text of their graph.



To see the information numerically, either switch to a **Table View** or **Spreadsheet View.**

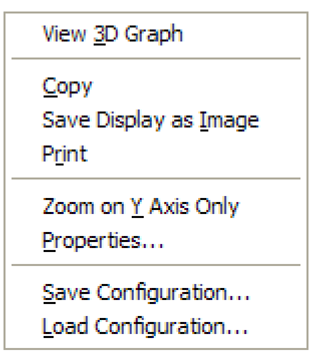
For monochrome images a single component histogram is displayed.

For color images the Source Color Channels button is visible to permit choosing between "All Channels" or a single color component. While the **Source Color Channel** is set to "All Channels", the 2D Histogram view will display a fourth data-set which is the histogram of the equivalent grey-scale image (such as the image which would be displayed after using the SimplePCI menu command "Color>>Grayscale").



To zoom in on a particular area click and hold the left mouse button as you drag a box around the area of interest. Release the button and the graph zooms to the size of the box. Click the left mouse button to return to the normal view. While in a zoomed position the user can pan by dragging the mouse in the direction they wish to view.

The Graph **Right-Click Menu** will display a list of the other controls including:



View 3D Graph: Displays a **3-Dimensional Intensity Profile** of the image.

Copy: Creates a copy of the graph and places it on the clipboard.

Save Display as Image: Saves the Graph as an image and prompts the user to name and choose a location to save the new image.

Print: Sends a copy of the graph to the printer.

Zoom on Y Axis Only: Allows the user to select and expand a range on the Y axis and view it over the entire graph.

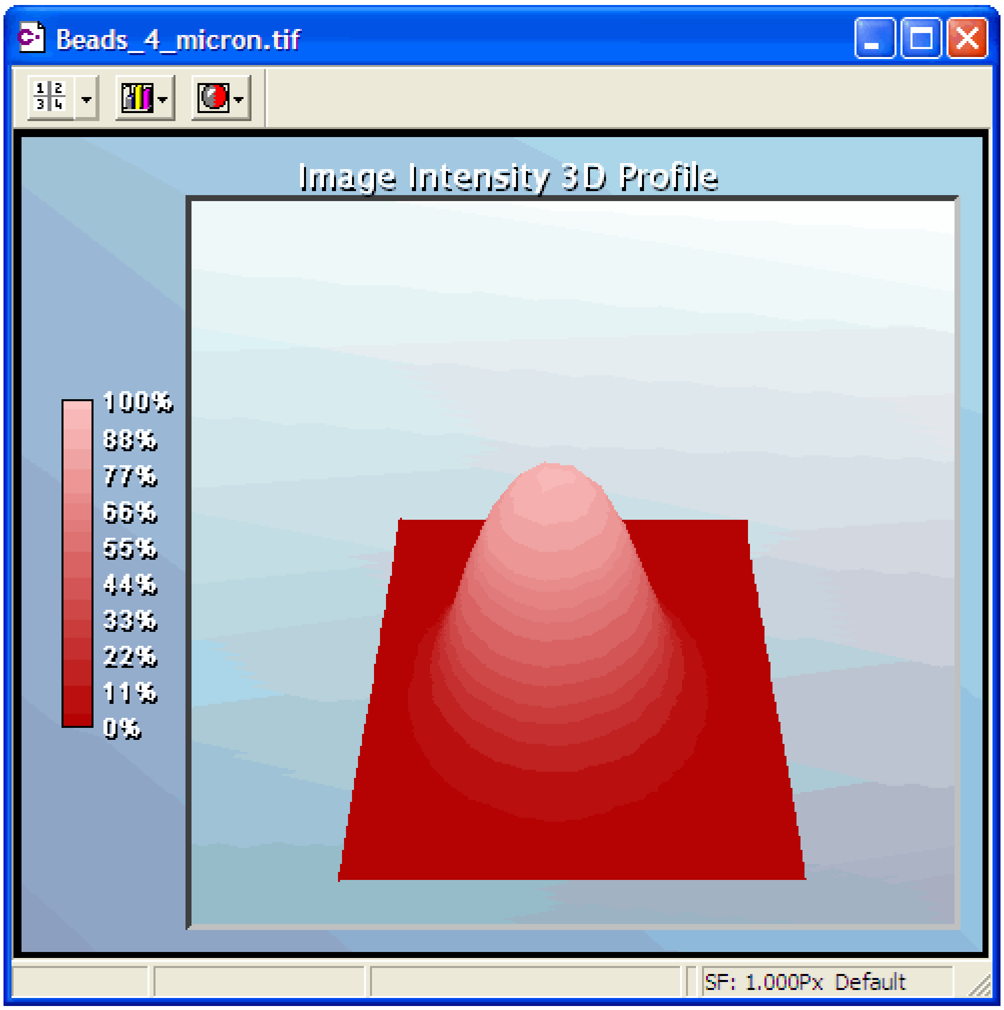
Properties: Opens the **Graph Properties** window that allows the user to customize the layout, colors and text of their graph.

Save Configuration: Saves the changes the user made in Graph Properties as a .gxc file for use on other graphs.

Load Configuration: Loads the graph configuration file, replacing the default graph properties with the one saved in the .gxc file.

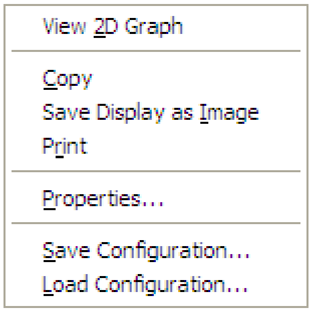
Graph View - 3D Profile

The **3D Profile View** shows the image in a 3-Dimensional Isometric view. The graph shows the image pixel positions in the X-Y axes and the pixel intensity data in the Z-axis.



The display can be rotated in all three planes by click and holding the right mouse button. In order to return the display back to the default view, right-click, select 2D View, then right-click and select 3D View.

Display options can be selected by right-clicking on the image or clicking graph from the Main Menu.



The Graph **Right-Click Menu** will display a list of the other controls including:

View 2D Graph: Displays a **2D Intensity Profile** of the image.

Copy: Creates a copy of the graph and places it on the clipboard.

Save Display as Image: Saves the Graph as an image and prompts the user to name and choose a location to save the new image.

Print: Sends a copy of the graph to the printer.

Properties: Opens the **Graph Properties** window that allows the user to customize the layout, colors and text of their graph.

Save Configuration: Saves the changes the user made in Graph Properties as a .gxc file for use on other graphs.

Load Configuration: Loads the graph configuration file, replacing the default graph properties with the one saved in the .gxc file.

> **Note:** The amount of data in a high-resolution image may be far too great to display with accuracy on the screen, so a Quality Factor is used to decrease the image data before rendering the view. Increasing the quality factor will permit your hardware to improve performance during data interaction. Decrease the quality factor after choosing the optimal viewpoint and other display options to improve the final quality factor for producing the highest quality image output for documenting your experiment.

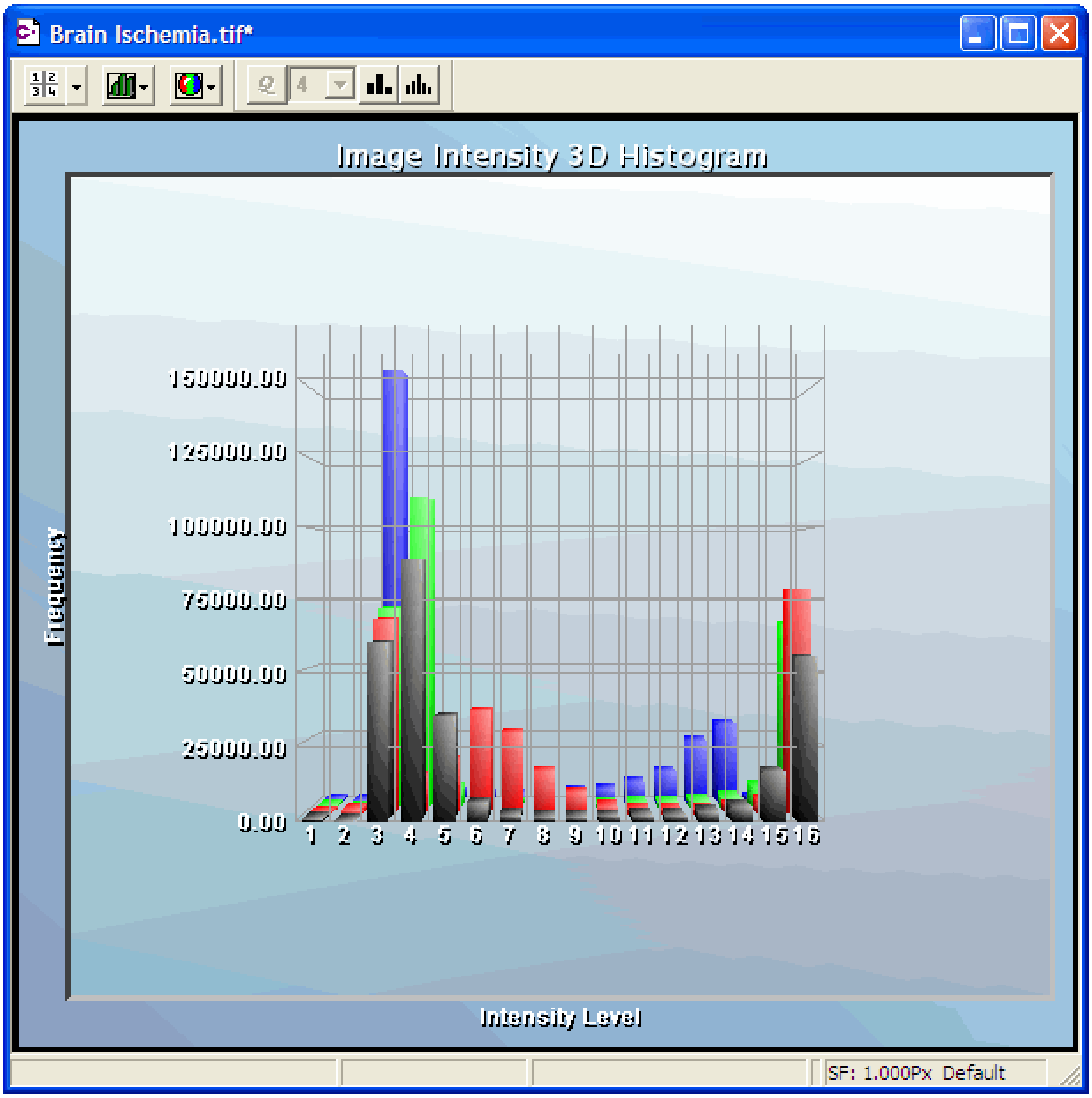
Histogram View - 3D Histogram

To see the intensity distribution of an image's pixels graphed in a three-dimensional display, click on **Histogram View**  from the Current View drop-menu and then right-click and select **View 3D Graph**. An intensity distribution histogram in three-dimensions, with various source-color channels drawn at various depths, will be displayed. As with the 2D Histogram view, the intensity distribution histogram describes how many pixels are contained within the image document within various intensity-level ranges. The bins correspond to intensity (i.e. pixel value) and the width of the bins can be increased or decreased via the "Bin Decrement"  and "Bin Increment"  toolbar buttons.

Histogram bars will change to a line graph if the number of bins is increased until there is one bin for each of the intensity levels available.

By default, a histogram will be included for each source color channel present in the image and the user is able to customize the graph by right-clicking and selecting Properties. The **Graph Properties** window allows the user to change the layout, colors and text of their graph.

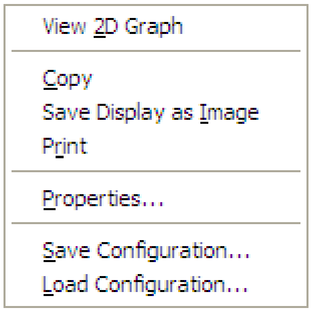
The histogram can be rotated in all three planes by click and holding the right mouse button. In order to return the display back to the default view, right-click, select 2D View, then right-click and select 3D View.



To see the information numerically, either switch to a **Table View** or **Spreadsheet View**.

For monochrome images a single component histogram is displayed.

For color images the Source Color Channels Button is visible to permit choosing between "All Channels" or a single color component. While the **Source Color Channel** is set to "All Channels", the 2D Histogram view will display a fourth data-set which is the histogram of the equivalent greyscale image (such as the image which would be displayed after using the SimplePCI menu command "Color>>Grayscale").



The Graph **Right-Click Menu** will display a list of the other controls including:

View 2D Graph: Displays a **2-Dimensional Intensity Profile** of the image.

Copy: Creates a copy of the graph and places it on the clipboard.

Save Display as Image: Saves the Graph as an image and prompts the user to name and choose a location to save the new image.

Print: Sends a copy of the graph to the printer.

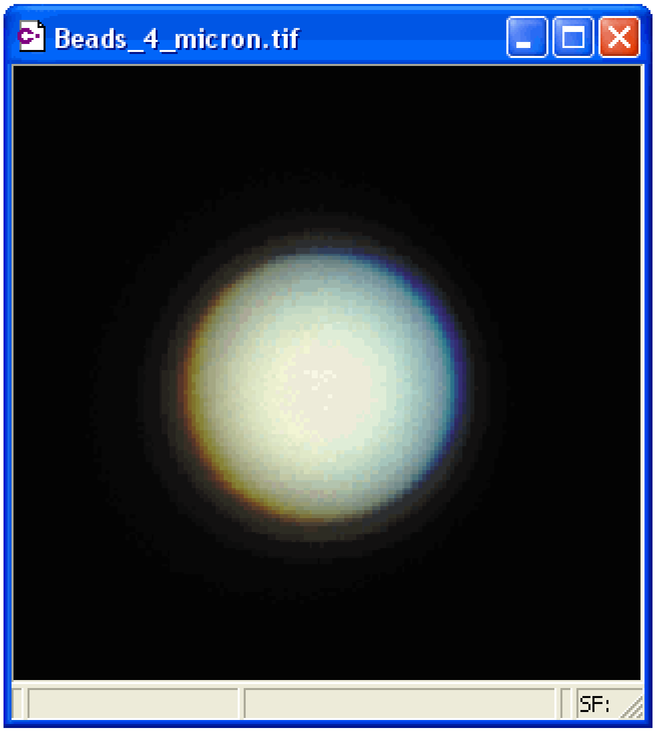
Properties: Opens the **Graph Properties** window that allows the user to customize the layout, colors and text of their graph.

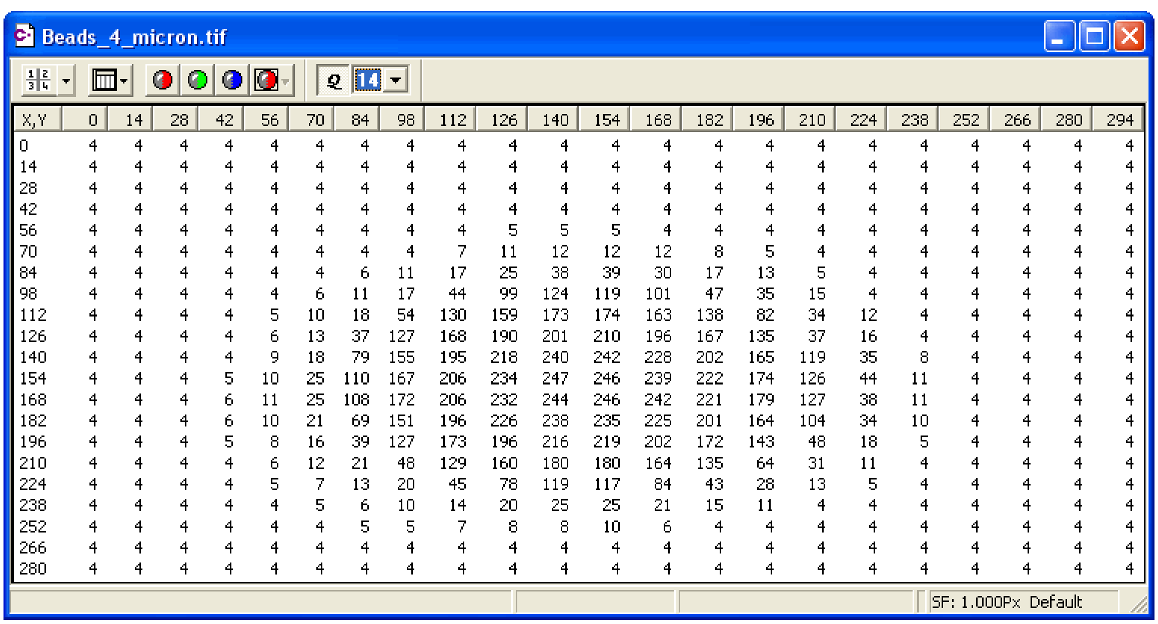
Save Configuration: Saves the changes the user made in Graph Properties as a .gxc file for use on other graphs.

Load Configuration: Loads the graph configuration file, replacing the default graph properties with the one saved in the .gxc file.

Table Profile View

Image Document Data pixel intensity can be viewed in a **Table View** allowing close inspection of image data values.





X,Y	0	14	28	42	56	70	84	98	112	126	140	154	168	182	196	210	224	238	252	266	280	294
0	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
14	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
28	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
42	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
56	4	4	4	4	4	4	4	4	4	5	5	5	4	4	4	4	4	4	4	4	4	4
70	4	4	4	4	4	4	4	4	7	11	12	12	12	8	5	4	4	4	4	4	4	4
84	4	4	4	4	4	4	6	11	17	25	38	39	30	17	13	5	4	4	4	4	4	4
98	4	4	4	4	4	6	11	17	44	99	124	119	101	47	35	15	4	4	4	4	4	4
112	4	4	4	4	5	10	18	54	130	159	173	174	163	138	82	34	12	4	4	4	4	4
126	4	4	4	4	6	13	37	127	168	190	201	210	196	167	135	37	16	4	4	4	4	4
140	4	4	4	4	9	18	79	155	195	218	240	242	228	202	165	119	35	8	4	4	4	4
154	4	4	4	5	10	25	110	167	206	234	247	246	239	222	174	126	44	11	4	4	4	4
168	4	4	4	6	11	25	108	172	206	232	244	246	242	221	179	127	38	11	4	4	4	4
182	4	4	4	6	10	21	69	151	196	226	238	235	225	201	164	104	34	10	4	4	4	4
196	4	4	4	5	8	16	39	127	173	196	216	219	202	172	143	48	18	5	4	4	4	4
210	4	4	4	4	6	12	21	48	129	160	180	180	164	135	64	31	11	4	4	4	4	4
224	4	4	4	4	5	7	13	20	45	78	119	117	84	43	28	13	5	4	4	4	4	4
238	4	4	4	4	4	5	6	10	14	20	25	25	21	15	11	4	4	4	4	4	4	4
252	4	4	4	4	4	4	5	5	7	8	8	10	6	4	4	4	4	4	4	4	4	4
266	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
280	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

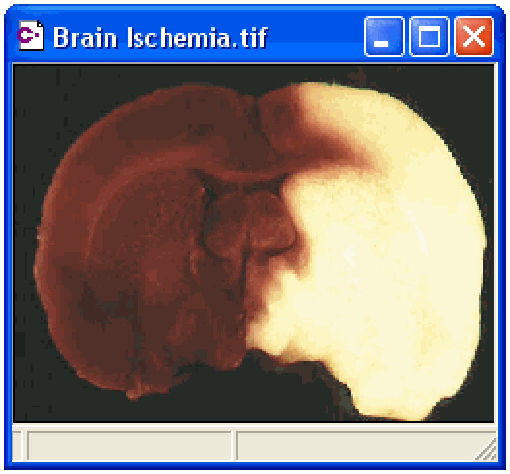
In the Table View,histogram data can be sorted in any column in either increasing or decreasing order by clicking on the column header. It can also be copied to an external spreadsheet, via the

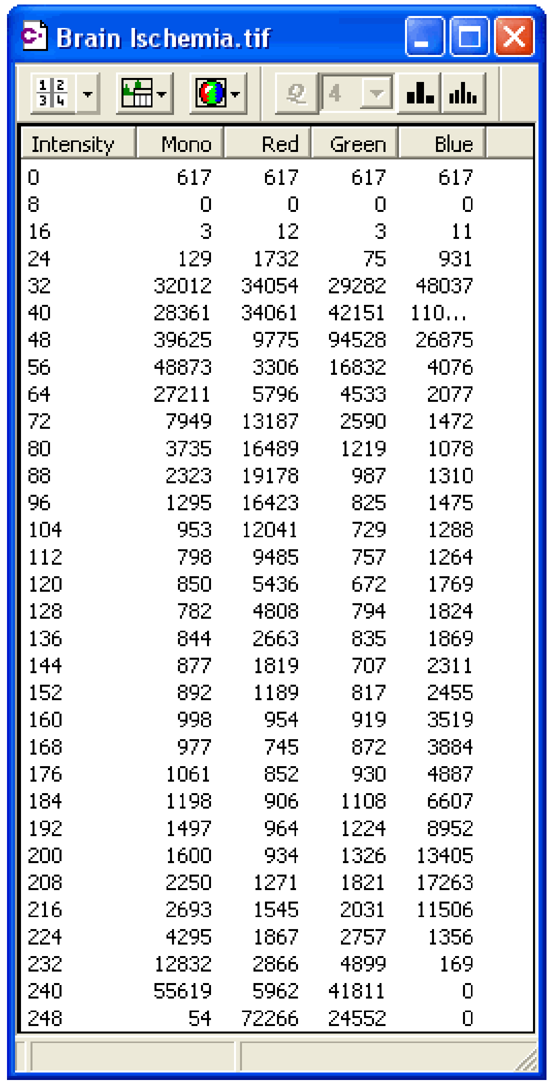
"Edit>>Copy to Spreadsheet" and "Edit>>Copy to Excel" menu items in SimplePCI's main window.

> **Note:** That the amount of data in a high-resolution image may be very large, and viewing this amount of data can be very slow. The **Quality Factor** can be used to decrease the image data volume before rendering the view, or a **Region** can be defined to limit the data to be copied to the spreadsheet for viewing.

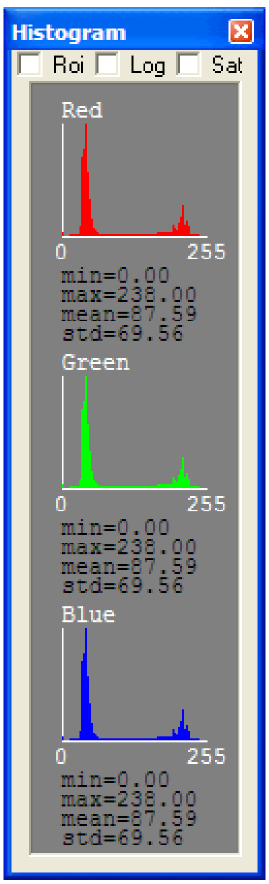
Table Histogram View

Each **Red, Green, Blue Color Channel** (and the equivalent gray-level image's monochrome intensity histogram) is visible in the **Table Histogram View**, which allows numerical inspection of the intensity value distribution.





Intensity	Mono	Red	Green	Blue
0	617	617	617	617
8	0	0	0	0
16	3	12	3	11
24	129	1732	75	931
32	32012	34054	29282	48037
40	28361	34061	42151	110...
48	39625	9775	94528	26875
56	48873	3306	16832	4076
64	27211	5796	4533	2077
72	7949	13187	2590	1472
80	3735	16489	1219	1078
88	2323	19178	987	1310
96	1295	16423	825	1475
104	953	12041	729	1288
112	798	9485	757	1264
120	850	5436	672	1769
128	782	4808	794	1824
136	844	2663	835	1869
144	877	1819	707	2311
152	892	1189	817	2455
160	998	954	919	3519
168	977	745	872	3884
176	1061	852	930	4887
184	1198	906	1108	6607
192	1497	964	1224	8952
200	1600	934	1326	13405
208	2250	1271	1821	17263
216	2693	1545	2031	11506
224	4295	1867	2757	1356
232	12832	2866	4899	169
240	55619	5962	41811	0
248	54	72266	24552	0

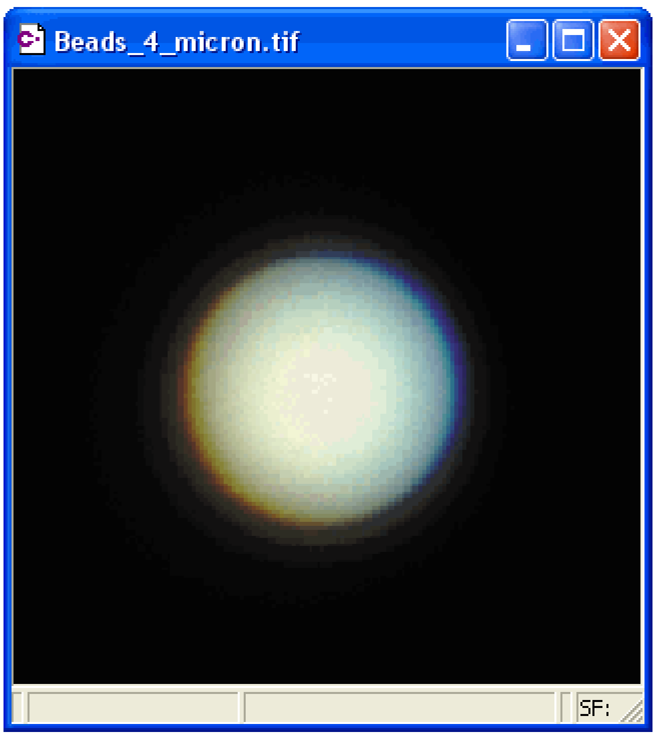


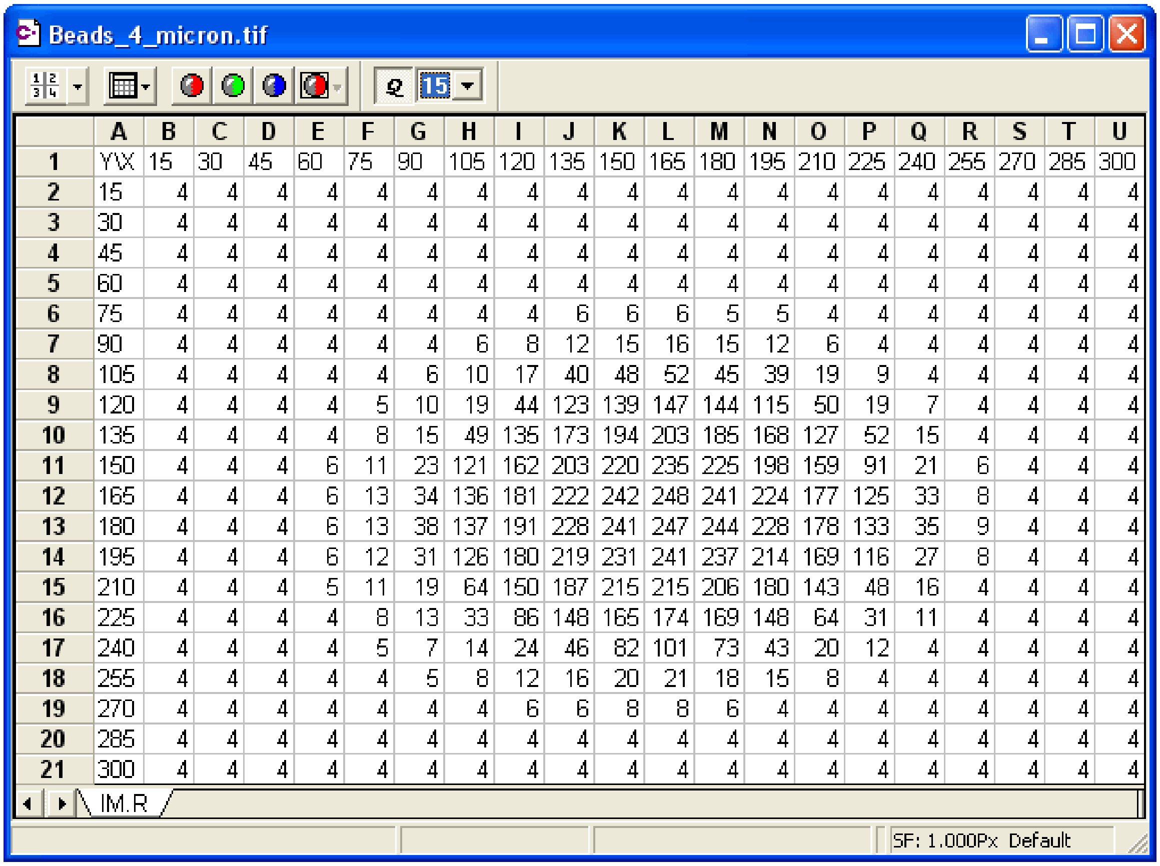
While in this view, the histogram data can be sorted in any column in either increasing or decreasing order by clicking on the column header. It can also be copied to an external spreadsheet, via the "Edit>>Copy to Spreadsheet" and "Edit>>Copy to Excel" menu items in SimplePCI's main window.

Note: That the Increment Bins and Decrement Bins buttons can be used to adjust the sizes of the bins of the histogram which corresponds to using fewer or more rows of data spaced and dropping more or fewer discrete intensity values in each intensity-level bin.

Spreadsheet Profile View

Image Document Data pixel intensity can be viewed in a Spreadsheet form allowing close inspection of image data values. In the **Spreadsheet View**, a column, row or region of the spreadsheet can be copied to the clipboard to paste into a spreadsheet or report application.





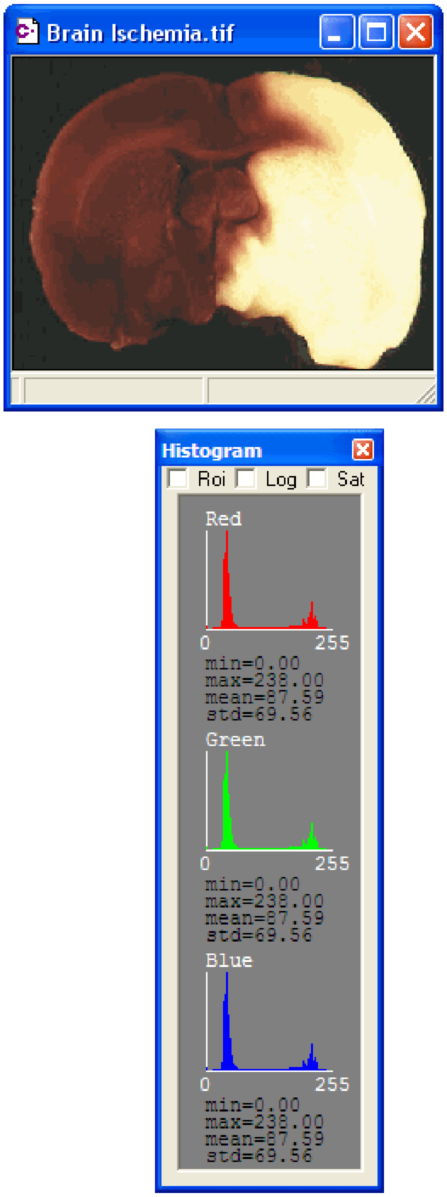
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	Y\X	15	30	45	60	75	90	105	120	135	150	165	180	195	210	225	240	255	270	285	300
2	15	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
3	30	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
4	45	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
5	60	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
6	75	4	4	4	4	4	4	4	4	6	6	6	5	5	4	4	4	4	4	4	4
7	90	4	4	4	4	4	4	6	8	12	15	16	15	12	6	4	4	4	4	4	4
8	105	4	4	4	4	4	6	10	17	40	48	52	45	39	19	9	4	4	4	4	4
9	120	4	4	4	4	5	10	19	44	123	139	147	144	115	50	19	7	4	4	4	4
10	135	4	4	4	4	8	15	49	135	173	194	203	185	168	127	52	15	4	4	4	4
11	150	4	4	4	6	11	23	121	162	203	220	235	225	198	159	91	21	6	4	4	4
12	165	4	4	4	6	13	34	136	181	222	242	248	241	224	177	125	33	8	4	4	4
13	180	4	4	4	6	13	38	137	191	228	241	247	244	228	178	133	35	9	4	4	4
14	195	4	4	4	6	12	31	126	180	219	231	241	237	214	169	116	27	8	4	4	4
15	210	4	4	4	5	11	19	64	150	187	215	215	206	180	143	48	16	4	4	4	4
16	225	4	4	4	4	8	13	33	86	148	165	174	169	148	64	31	11	4	4	4	4
17	240	4	4	4	4	5	7	14	24	46	82	101	73	43	20	12	4	4	4	4	4
18	255	4	4	4	4	4	5	8	12	16	20	21	18	15	8	4	4	4	4	4	4
19	270	4	4	4	4	4	4	4	6	6	8	8	6	4	4	4	4	4	4	4	4
20	285	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
21	300	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

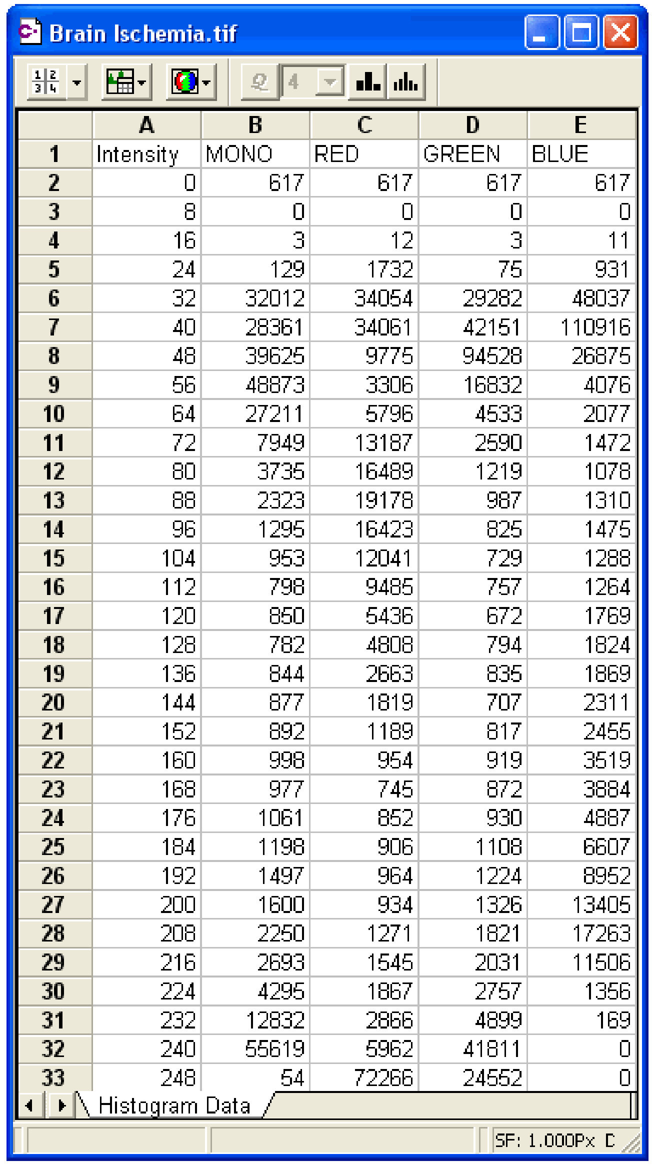
While in this view, data can be copied to an external spreadsheet, via the "Edit>>Copy to Spreadsheet" and "Edit>>Copy to Excel" menu items in SimplePCI's main window.

Note: That the amount of data in a high-resolution image may be far too great to move to a spreadsheet application, and viewing this large amount of data can be very slow. The **Quality Factor** can be used to decrease the image data volume before rendering the view, or a Region can be defined to limit the data to be copied to the spreadsheet for viewing.

Spreadsheet Histogram View

Each **Red, Green, Blue Color Channel** (and the equivalent monochrome intensity histogram) is visible in the **Spreadsheet Histogram View**, which allows numerical inspection of the intensity value distribution.





	A	B	C	D	E
1	Intensity	MONO	RED	GREEN	BLUE
2	0	617	617	617	617
3	8	0	0	0	0
4	16	3	12	3	11
5	24	129	1732	75	931
6	32	32012	34054	29282	48037
7	40	28361	34061	42151	110916
8	48	39625	9775	94528	26875
9	56	48873	3306	16832	4076
10	64	27211	5796	4533	2077
11	72	7949	13187	2590	1472
12	80	3735	16489	1219	1078
13	88	2323	19178	987	1310
14	96	1295	16423	825	1475
15	104	953	12041	729	1288
16	112	798	9485	757	1264
17	120	850	5436	672	1769
18	128	782	4808	794	1824
19	136	844	2663	835	1869
20	144	877	1819	707	2311
21	152	892	1189	817	2455
22	160	998	954	919	3519
23	168	977	745	872	3884
24	176	1061	852	930	4887
25	184	1198	906	1108	6607
26	192	1497	964	1224	8952
27	200	1600	934	1326	13405
28	208	2250	1271	1821	17263
29	216	2693	1545	2031	11506
30	224	4295	1867	2757	1356
31	232	12832	2866	4899	169
32	240	55619	5962	41811	0
33	248	54	72266	24552	0

While in this view, data can be copied to an external spreadsheet, via the "Edit>>Copy to Spreadsheet" and "Edit>>Excel" menu items in SimplePCI's main window.

> **Note**: That the Increment Bins and Decrement Bins buttons can be used to adjust the sizes of the bins of the histogram which corresponds to using fewer or more rows of data spaced and dropping more or fewer discrete intensity values in each intensity-level bin.

Image Document Display Options

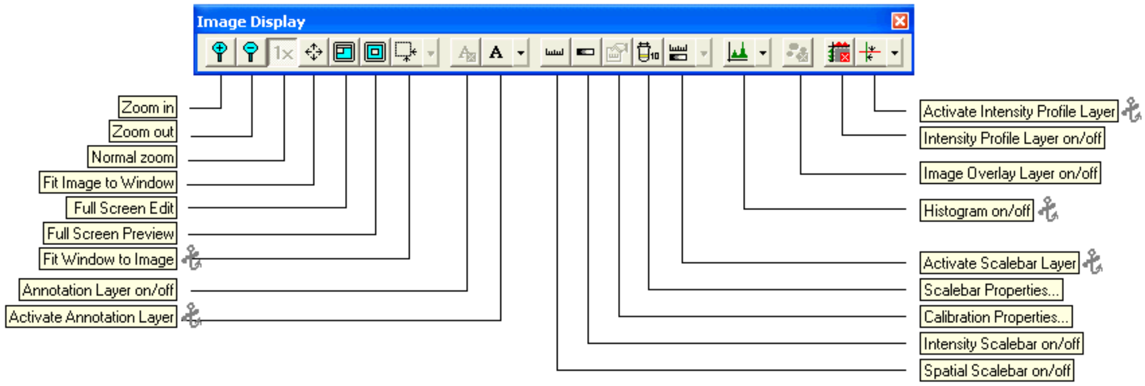


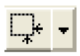
Image Document Display Options are selected from the Image Display Toolbar shown above..

The Image Document Display Options control the display properties of the image, including:

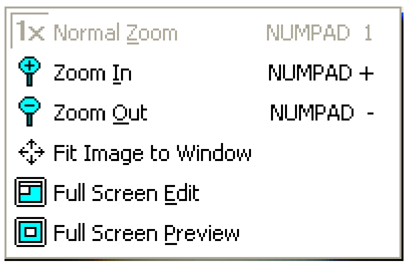
- **Image Document Display Zoom**
- **Annotation Layer**
- **Scalebar Layer**
  - **Intensity Scalebar**
  - **Spatial Scalebar**
- **Dockable Histogram Window**
- **Overlay Layer**
- **Intensity Profile Layer**

Image Zoom / Window Control

The zoom anchor toolbar button in the **Image Display Toolbar** manages several zoom-related commands which will appear in either a drop-down menu, or toolbar icon buttons, depending on how your toolbar is currently configured. If all these zoom commands are currently configured as menu items, the zoom portion of your Image Display toolbar drop down from the anchor icon



and the menu will open as follows:



If all zoom tools are configured as toolbar icon buttons, the left-most portion of the Image Display toolbar will look like this:

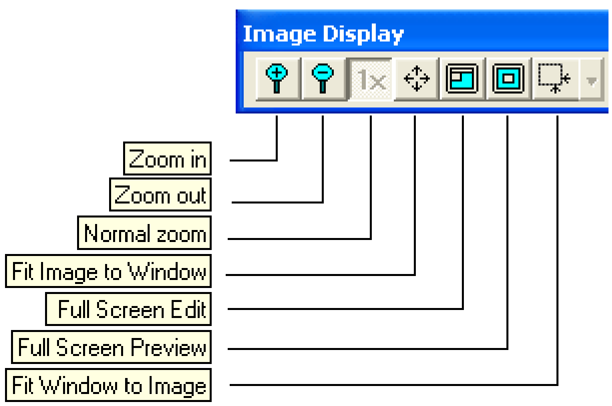


Image display controls for zooming the Image Document View are available in the Image Display Toolbar. The original image data is not modified. This is provided for a visual display zoom only.

Zoom In

Click the button (or press the numerical keypad "+" key) to increase the zoom factor.

Zoom Out

Click the button (or press the numerical keypad "-" key) to decrease the zoom factor.

1x Normal Zoom

Click the button (or press the numeric keypad "1" key) to view the image in its original size (1x magnification) with every pixel un-modified. This is especially important for images where there are fine lines that may not be visible due to sub-sampling when a low zoom factor is used.

Fit Image to Window

This option expands the image to fill the current Image Document window size. The window may be resized to match the aspect ratio of the image.

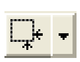
Full Screen Edit

This option will expand the frame of the image to fill the screen, providing the main menu and toolbars along the top edge of the screen while maximizing the view of the image. Image options can be applied in this mode. Use the Close Full Screen floating menu choice, or the Escape key, to return to a normal view.

Full Screen Preview

This option will expand the frame of the image to fill the screen. The main menu and toolbars will be removed also. Image options cannot be applied in this mode, but zooming is still functional using the short cut keys listed above. Use the Close Full Screen floating menu choice, or the Escape key, to return to a normal view.

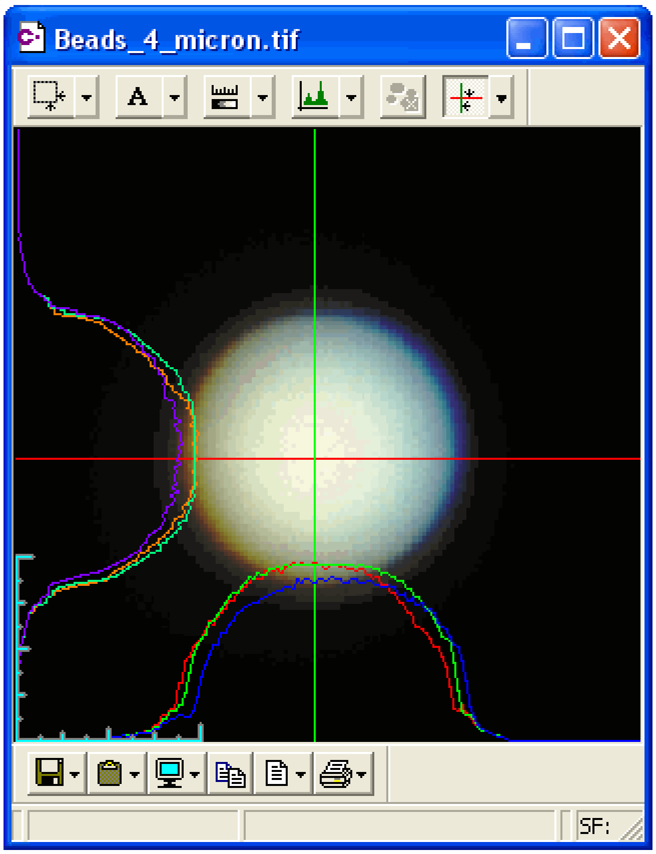
Fit Window To Image



The Fit to Window button in the Image Document Toolbar will zoom the current image to fit the image Document Frame so that the full image is visible, taking into account the screen size, the image size and aspect ratio. It may apply a zoom factor to re-scale the image display for this purpose, but the original image data will not be modified.

Intensity Profile Layer

The **Intensity Profile Layer** displays either a sole gray-scale intensity profile or a set of per-channel intensity profiles, along the bottom and left edges of an image. As with the annotation, region of interest and scale-bar layers, this intensity profile layer is only a visual overlay. As such, the only way to save, print, or copy it is to use the associated "Display Image + All Layers" command while the layer is made visible.

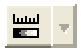


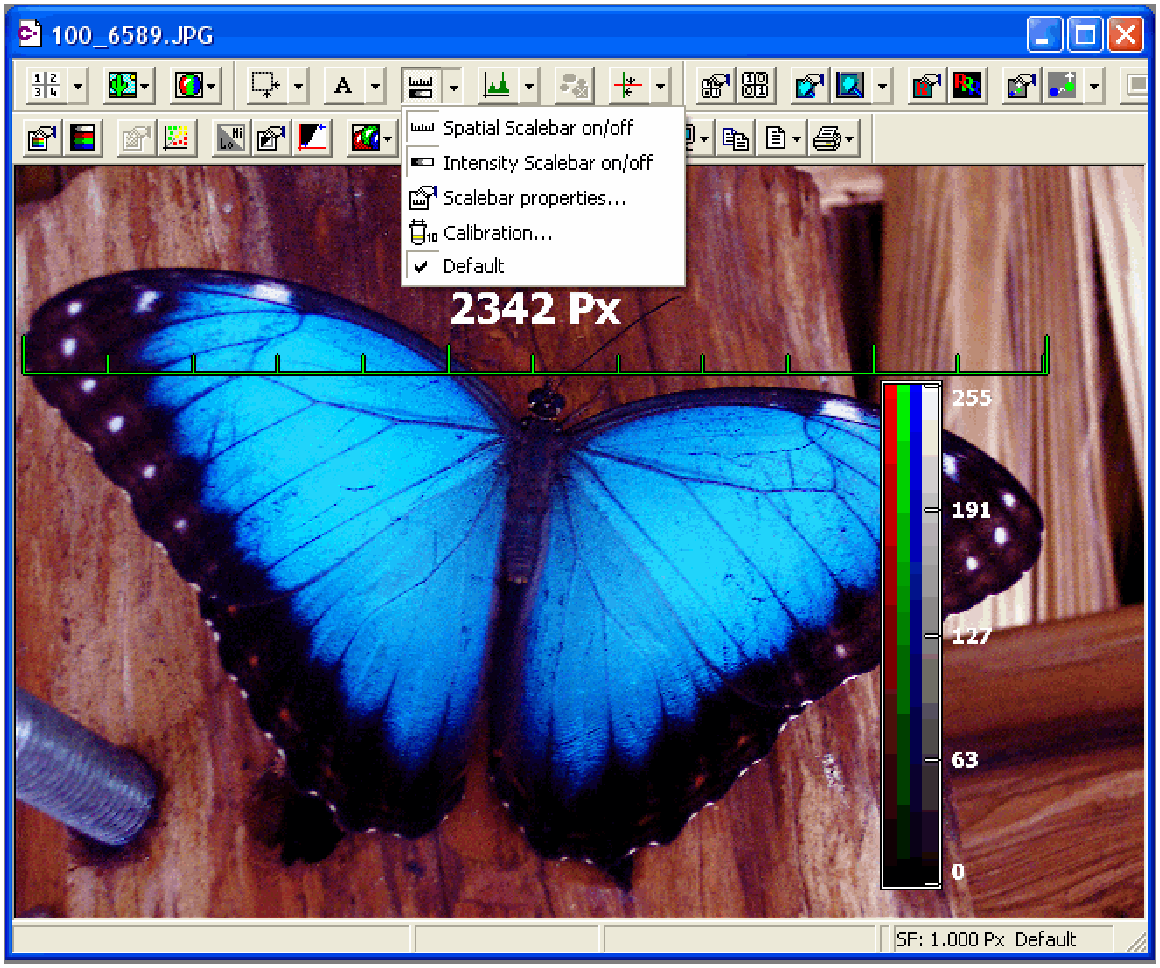
When the anchor icon for the intensity profile layer, **Activate Intensity Profile Layer** is switched on, the mouse cursor will be able to drag the red and green cross-hairs (which initially appear near the top-left corner of the image) throughout the image to specify which image intensity profiles should be plotted.

If the image may be thought of as three-dimensional (with pixel X-Y location plotted on the screen surface and intensity data coming out of the screen rather than rendered in a non-spatial 'brightness dimension'), then the cross-hairs could correspond to knives, slicing down through the image and display the resulting cross-sectional view of intensity vs. X (and intensity vs. Y) plots along those slice locations.

This view mode can be particularly useful during alignment of color registers.

Image Scalebar Layer

The Image Scalebar button  in the Display Toolbar allows a Spatial or Intensity scalebar to be placed on the image for visual comparison. The Scalebars are drawn in the Image Scalebar Layer, and can be displayed or hidden independently from other image layers.



The Image Scalebar can be applied in the following cases:

- **Image Document**
- **[Image Display]**
- **Data Document (Image View)**
- **[Image Montage]**

To display an Image Scalebar, select the Image Document Scalebar button to activate the Scalebar Layer for the Spatial scale-bar to be selected as visible.

The **Spatial Scalebar** shows a calibrated graticule with a title showing the Scalebar size and units. The size, color, font, and number of graticules can be configured from the Spatial Scalebar properties tab-page of the Scalebar Properties dialog.

The **Intensity Scalebar** shows a graduated intensity wedge with annotations showing the intensity range. In a color image, it can optionally provide parallel bands showing the intensity ranges of the color bands. This option is available from the Intensity Scalebar tab-page of the **Scalebar Properties** dialog.

To move the Scalebars, make sure the Scalebar Layer is active. Notice that the cursor changes to a four-way arrow when over the scalebars. In this mode the Scalebars can be dragged to a new location.

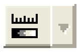
To re-size of the Scalebars, first select the Scalebar by clicking on it – the scalebar then shows in highlighted mode - then click and drag on the side or corner handles to resize it. To precisely size a scale-bar, specify the desired size in its properties dialog.

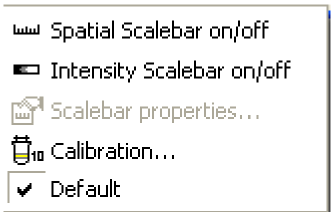
To hide the Scalebar, toggle the option in the Image Document Scalebar Menu.

To modify the size and shape of the Scalebars, select the Scalebar shape by clicking on it, then click and drag the corner or side handles. The Image Scalebar can also be modified by using the Image Region Properties dialog, activated from the Image Scalebar menu, or by right clicking on the Image Scalebar.

Click the arrow button for the Image Document Scalebar Layer Menu for further options.

Image Scalebar Menu

 The drop-down menu from the Image Scalebar button has further options:



Spatial Scalebar on/off

Toggle the Spatial Scalebar layer display on or off.

> **Note**: If the scalebar is toggled off while the Scalebar Layer is active, the Region Layer will be activated

Intensity Scalebar on/off

Toggle the Intensity Scalebar layer display on or off.

> **Note***:* If the scalebar is toggled off while the Scalebar Layer is active, the Region Layer will be activated

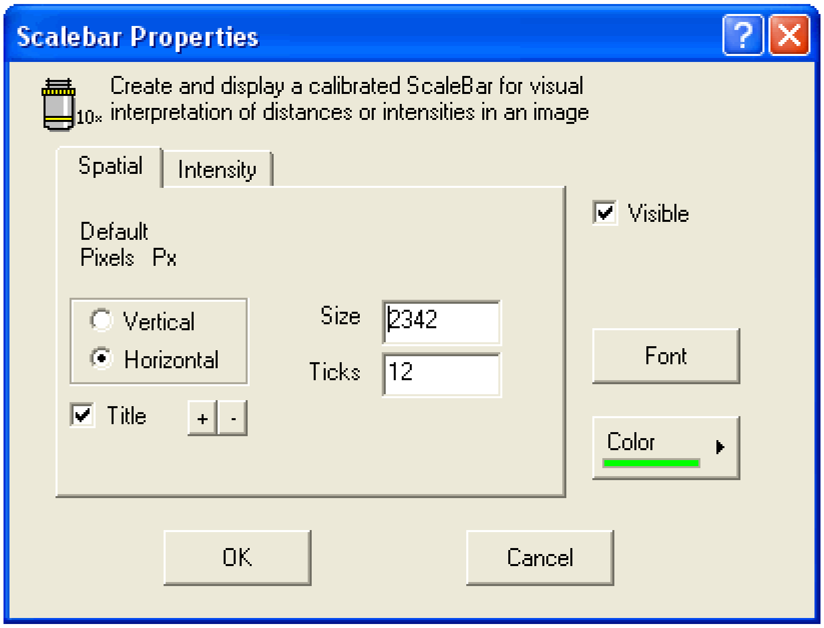
Scalebar Properties…

> Spatial Scalebar properties...
>
> Intensity Scalebar properties...

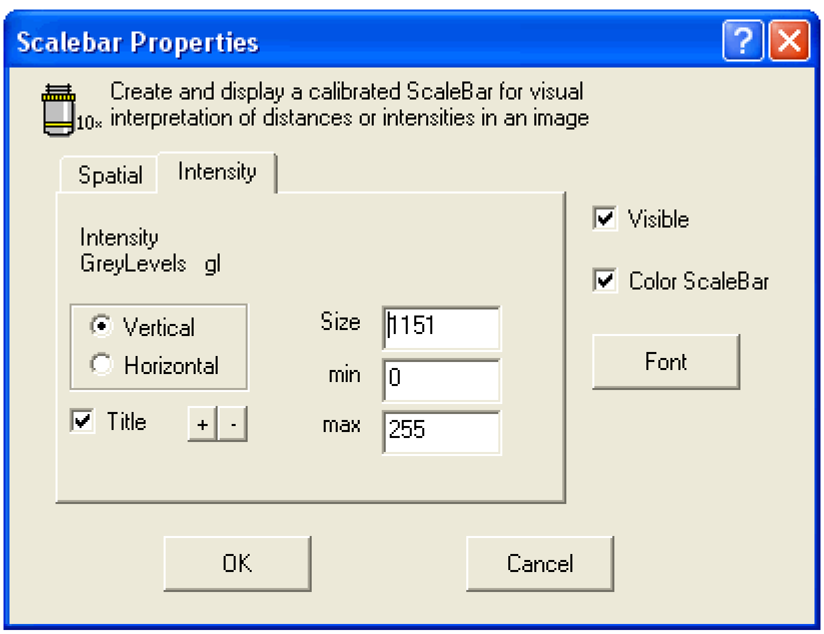
Calibration…

This dialog shows the current Scalebar properties, including size and location, and allows the values to be numerically entered or modified.

Spatial Scalebar Properties

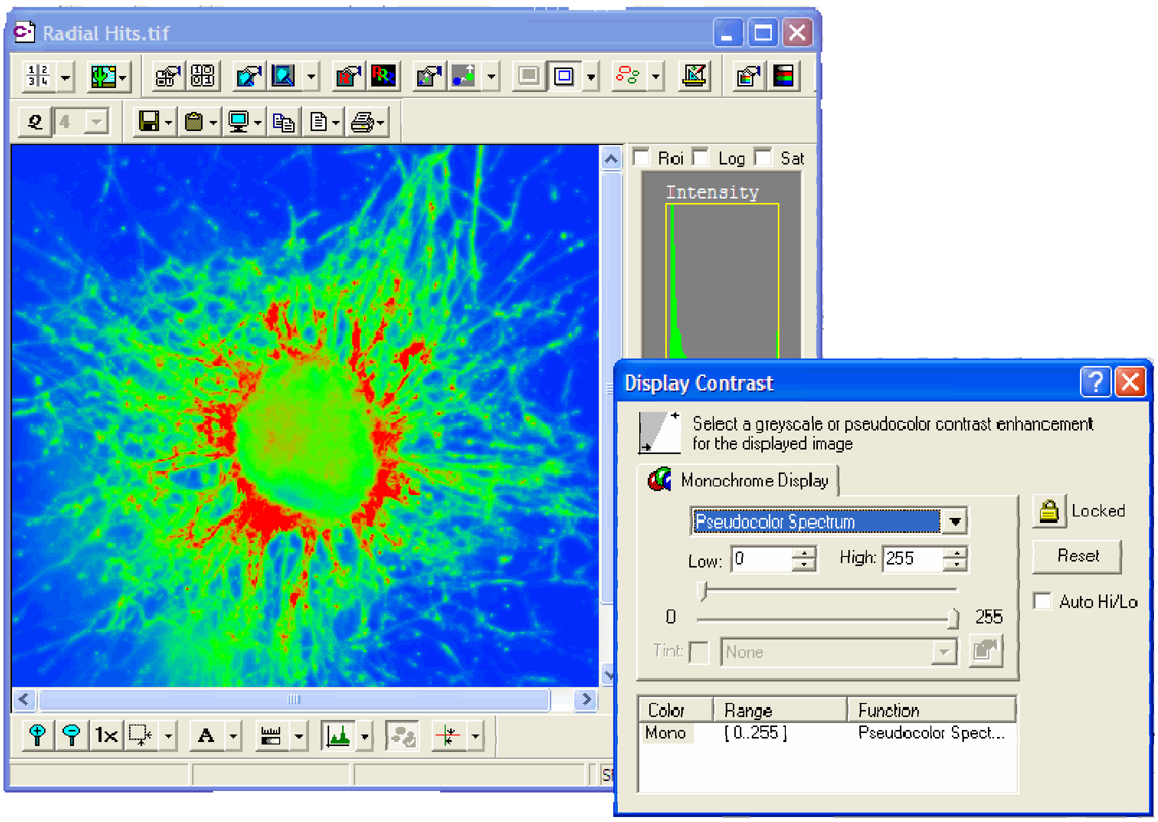


Intensity Scalebar Properties



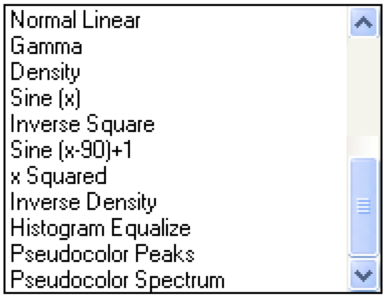
Contrast

The Contrast and coloring of any Image view (i.e. an image document, an "Original Image" field in a data document", the Image Display window) can be modified using the Display Contrast Dialog. The output image controls can be adjusted between various monochrome and pseudo-color enhancements. These can be used to draw attention to specific aspects of the image that would otherwise be indistinct. Display Contrast adjustments are non-destructive and do not modify the image intensities for measurement purposes.



For color images, various contrast stretches or pseudocolor Look Up Table enhancements may be made on each channel separately (Unlocked mode) or simultaneously on all channels (Locked mode).

Use the drop-down list to select the transform to be applied to the selected component. A list of monochrome and pseudocolor Contrast Transforms is available.



The Intensity Range of the Contrast Transform can be compressed, making the image appear to have more contrast, emphasizing values between these limits. Both slider and spin controls make it possible to interactively change the range while viewing the result on the Image Display. The values can be entered manually if known. For 16-bit images, the intensity scaling for the Display Contrast is from 0 to 65535 instead of 0 to 255, per channel. The full dynamic range of the original image is maintained, and the Display Contrast range allows flexibility in selecting which portion of the information to enhance for visual interpretation or threshold detection.

The **Auto** button will compute the minimum and maximum Intensity in the image and apply the Contrast Transform between these two values for each component.

Use the **Locked** checkbox to synchronize the three-color component together (Locked) or to allow separate adjustment of both the Contrast Transform function and the range of values. This can be used to create a custom pseudo-color look up table for a monochrome image, or to create a color balance effect for RGB images.

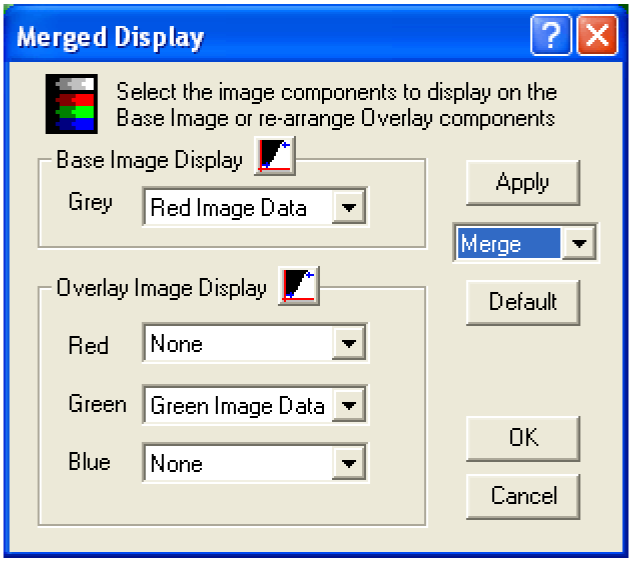
The **Default** button will reset all settings to the default values, using a Normal Linear transform and the full intensity range.

After choosing contrast settings, quickly review the change on the image that those settings have caused by using the **Apply Contrast** button to quickly toggle the contrast enhancement on/off.

Note: Intensity profile data is not affected by the contrast enhancement. The contrast enhancement function is only an image display tool rather than an image processing operation and operates only on the display data. If it is desirable to work on the contrast-enhanced image, copy the **Display Image** to an image document.

Merge

Three **Merge** operations are available: **Merge**, **A/B Ratio** and **Blend**.



A typical application of **Merge** is to show multi-channel fluorescence on a background image from a normal or enhanced transmitted light source.

The Menu allows selection of the color components and images to be used for the **Base Image** and **Overlay Image**. These options allow an image to be created which displays one or more of its color components overlaid on another image, or on one of its components shown in grey-scale mode.

The Base Image is shown as the background image. A monochrome or color image can be used, or a Red, Green or Blue color component from the initial image. In the case where a single color component is used, this image is treated as a monochrome image.

In a three-color image acquisition, it may be that one component of the data is grey-scale, from transmitted illumination, showing detail of the external shape and size of a cell, and the other components are of fluorescing areas of labeled structures within the cell, showing no context of the cell border.

Example:

The Merged display can be used in Merge mode as follows:

1. Select the color component to be used as the grey-scale background
   Base Image = Red Component

2. Select the components to be overlaid in each color channel

   Overlay Image Display

   Red = None
   Green = Green Image Data

   Blue = None

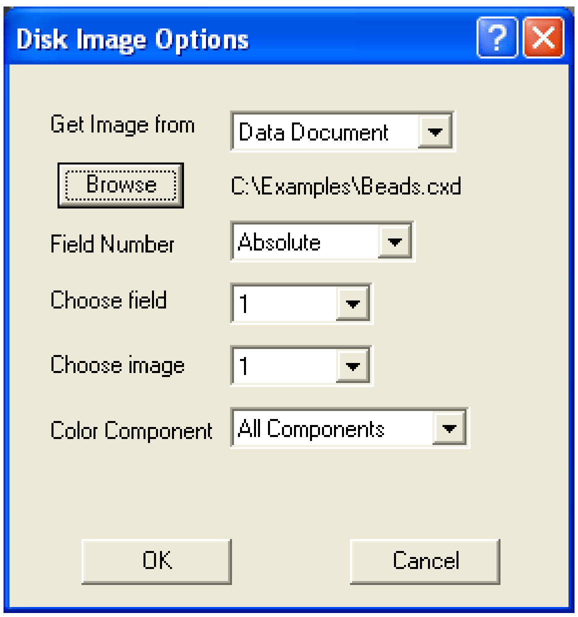
3. Click **Apply**

Observe that the result shows the Red Data from the Original Image displayed as a grey background behind the Green highlights from the fluorescence channel.

Additional **Contrast Enhancement** of the result can make some details more apparent.

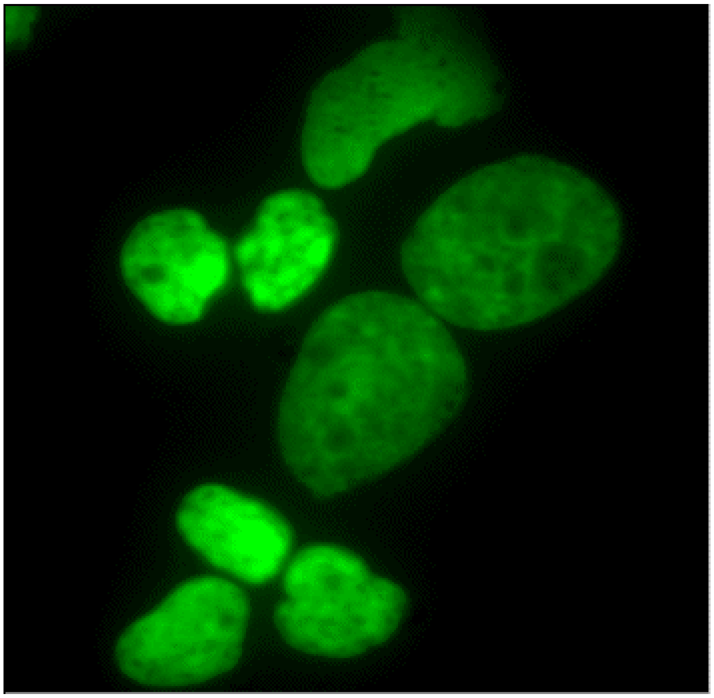
The Base Image can also be external, so if an image has been captured of the transmitted illumination sample and stored in a grey image, three subsequent channels can be captured in a new three-color image and the Base Image can be selected from a Disk file.

Note: This also applies in the Data Document, making it possible to play a sequence of three-color images with a specific (time 0) image used as the base. Alternatively, if a new background image is captured each time, then the Base Image can be updated for every frame in the sequence.



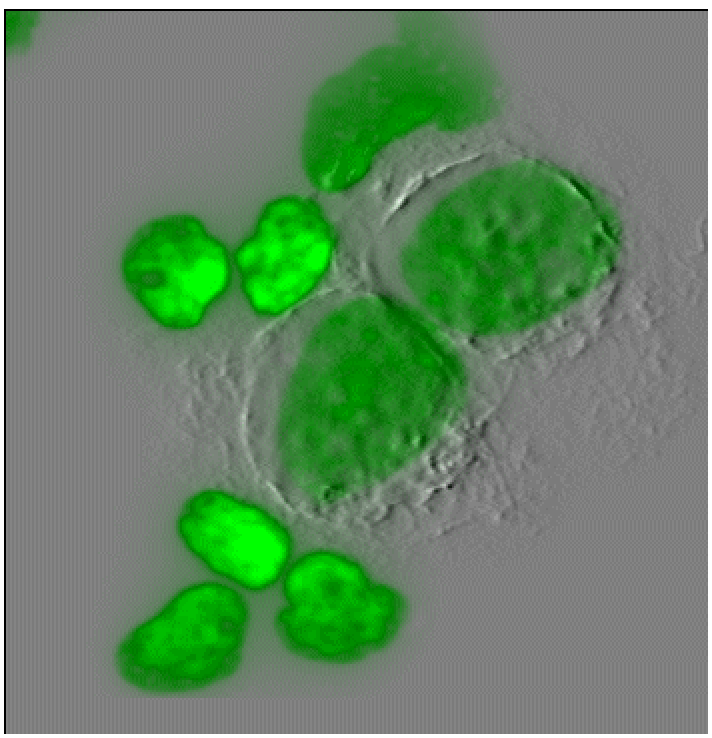
Merged Display Example

Flourescence Image for Overlay

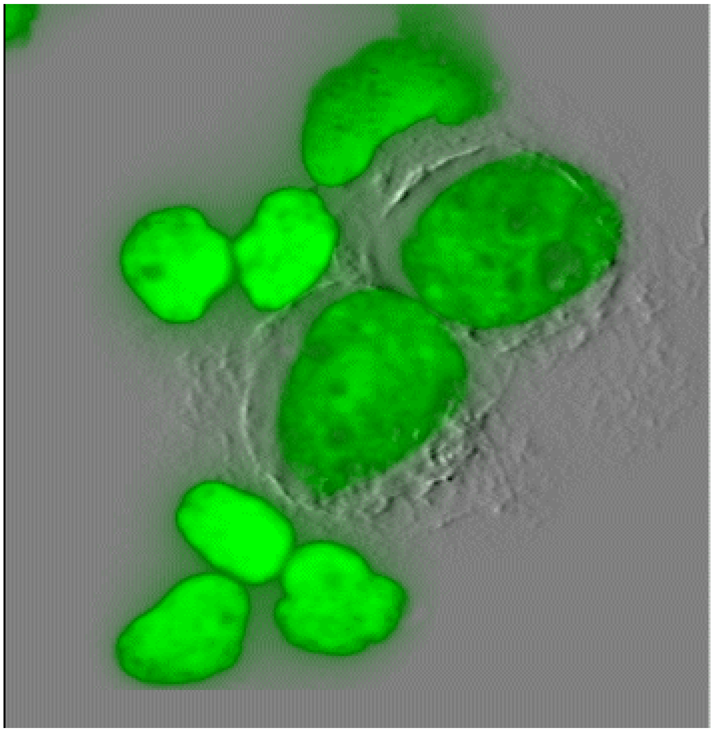


The original 24-bit color image with green fluorescence.

Result



Result + Contrast Enhancement



The original 24-bit color image has a grey-scale image displayed in the Base Image, showing the locations of the green fluorescence activity.

The Green image has been Contrast Enhanced to exaggerate the green fluorescence.

Blending

Up to 10 images can be blended into one image. Each image can be individually contrasted and or tinted.

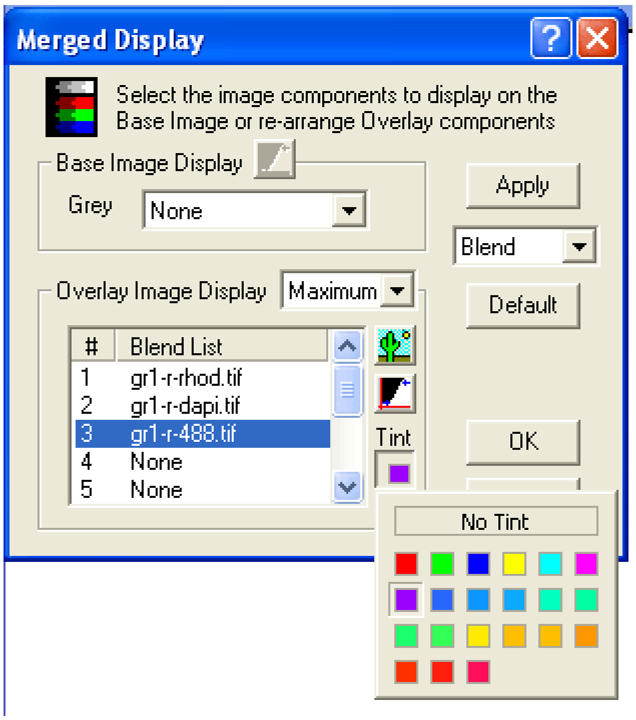
Click on the blend list to select your images. Open images should appear in the list.

Click on the **Tint** button to choose a Quick Image Tint.

Click on the **Contrast** Icon for more sophisticated contrast methods.

Click on the **Image** Icon to easily view the selected image blend component.

Press **Apply** to view your blended result.



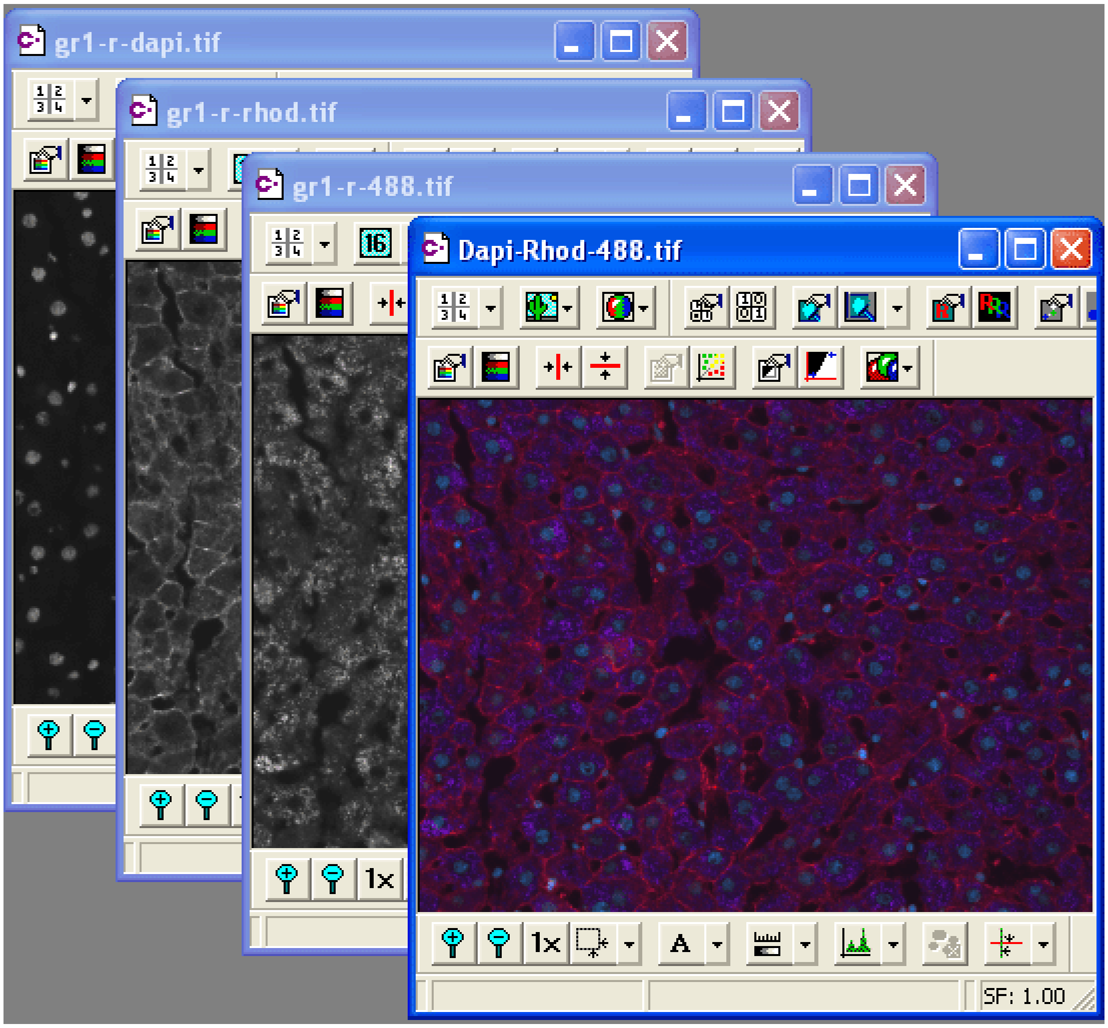
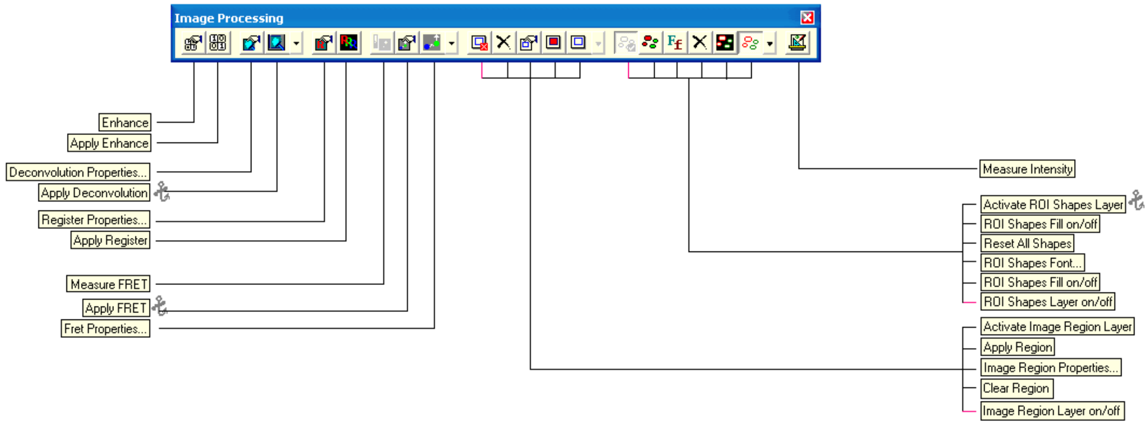
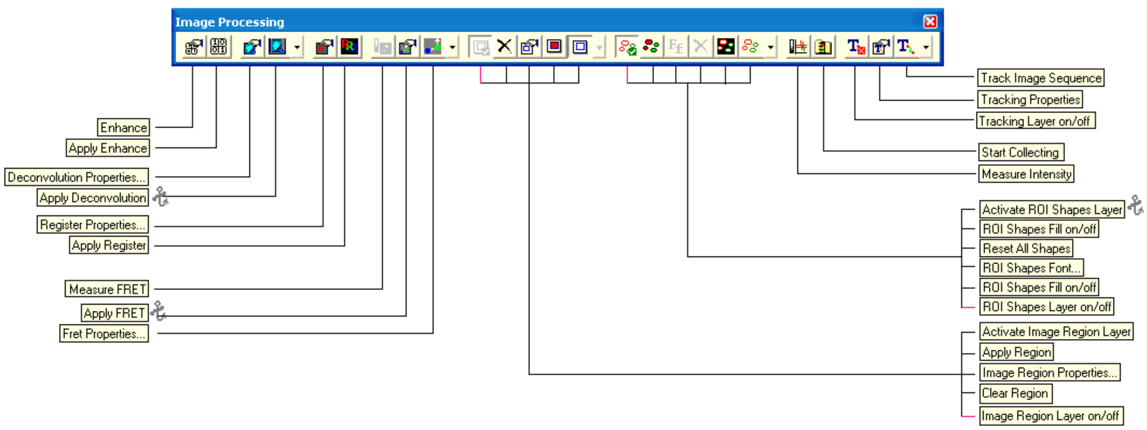

gr1-r-dapi.tif
gr1-r-rhod.tif
gr1-r-488.tif
Dapi-Rhod-488.tif
1x
SF: 1.00

Image Processing Toolbar

- **Image Processing toolbar- Image documents (shown fully expanded)**



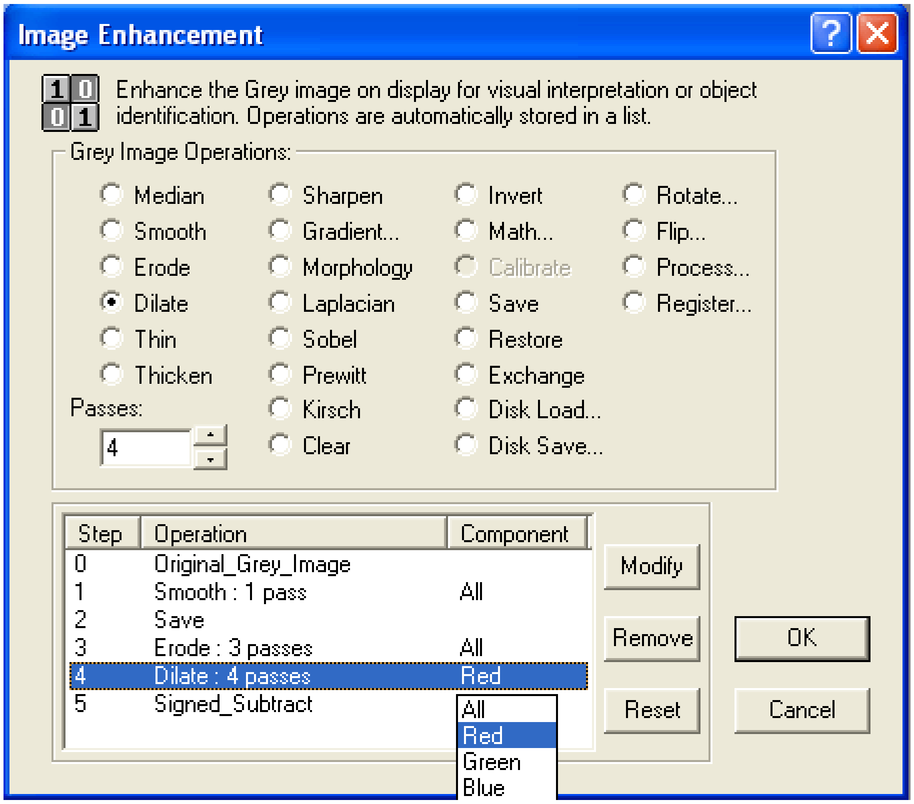
- **Image Processing toolbar - Data documents (shown fully expanded with IPA and MTA modules activated)**



Note: The Batch FRET Icon is not shown in the either of the Image Processing Toolbars.

Enhance

The Enhance Dialog allows a combination of image enhancement operations to be applied sequentially to an image.



To select an operation for the current image on display, click on the radio button checkbox of the operation. The operation selected will be applied to the current image on display.

Each time an operation is applied to the current image, the operation is added to the List. Selecting an operation from the list will jump to the appropriate place and allow editing of the operation sequence.

Some Enhance operations can be re-applied repetitively to strengthen their effect. These operations are located in the left hand column in the Enhance Dialog. At the bottom of the column is a counter labeled "passes". This **Pass Counter** shows the number of times that the currently selected operation has been applied. To specify the number of passes click on the operation multiple times, use the spin control arrows or type the desired value. The maximum number of passes allowed per Enhance operation is 255. Notice that the number of passes applied counts as only one Enhance operation.

For color images, may operations can be applied per component.

The second column of options in the Enhance Dialog are image processing operations that are not normally applied repetitively, although this is possible by re-selecting the same option more than once.

The latter two columns of Enhance options are for controlling the storage of a second image in memory, for arithmetic operations between the stored and display images, and for loading and saving disk images.

Enhance Operations:

See Image Enhancements.

List Operations:

To **Modify** an operation in the list, select the item and click Modify. A dialog will pop up listing other possible operations for selection. The new imaging operation will be applied and replace the original operation in the list.

To **Remove** an item from the list, select the item and click Remove. The selected item will disappear from the list and the image will be processed to represent the previous item in the list.

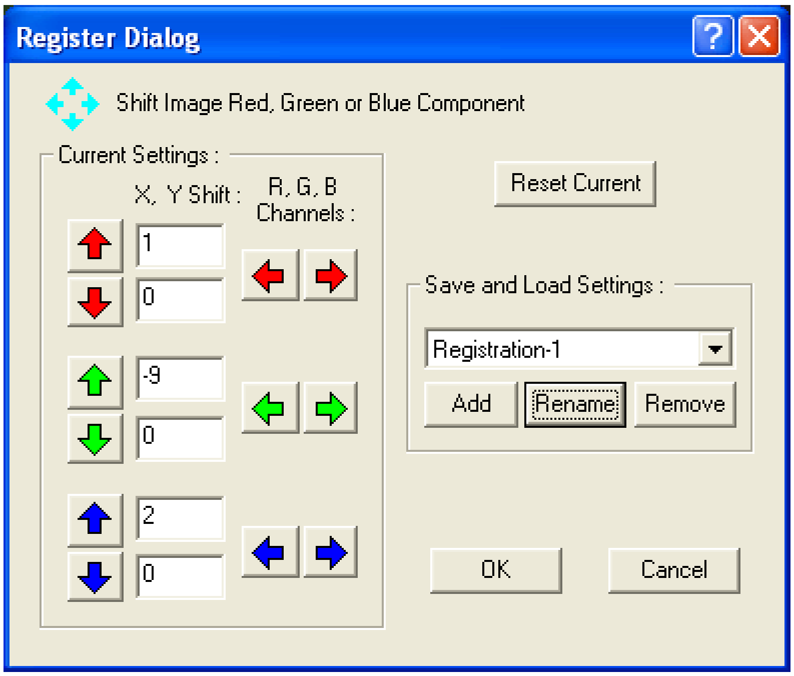
Click **Reset List** to remove all items from the list.

Note: the top most item in the list represents the original image and cannot be modified or removed.

Registration

Color images may exhibit a misregistration between image components caused by vibration or movement between sequential image captures, or by misalignment of optical components.

Image Registration allows image components to be shifted on a per pixel basis in X or Y to accurately register the components in the image. The shift is applied directly to the view. Note that the Image Profile View can be applied as a 2D graph in a splitter view to show this dynamically.



Use the arrow keys to move the image component by single pixels, or enter the shift numerically for larger values.

Reset Current: Reset values to defaults.

Save and Load Settings: You can name and save registrations setting to be used across multiple documents. Once saved, these settings will be remembered the next time you run ***SimplePCI***.

1. Fill in your X and Y shift values.
2. Click **Add**.
3. Enter a name for your settings.
4. Click **OK**.

Click **Rename**, to change the name of the selected settings. You will be able to **Add** multiple sets of Registration settings and switch between them by changing your selection in the drop-down list. These settings will exist until **Removed**.

> **Note:** The images below show an image with the Image Profile Intensity Layer (found on the Image View toolbar) activated

Image before registration correction

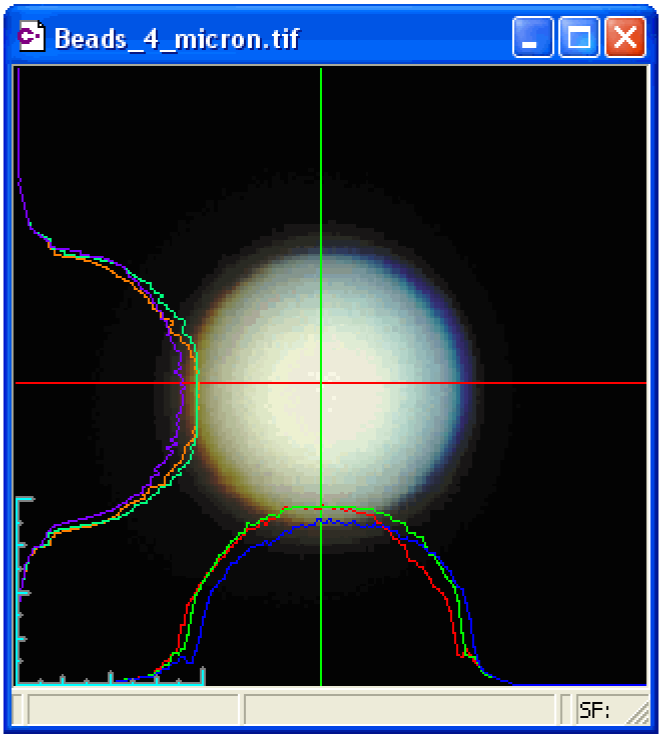


Image After registration correction

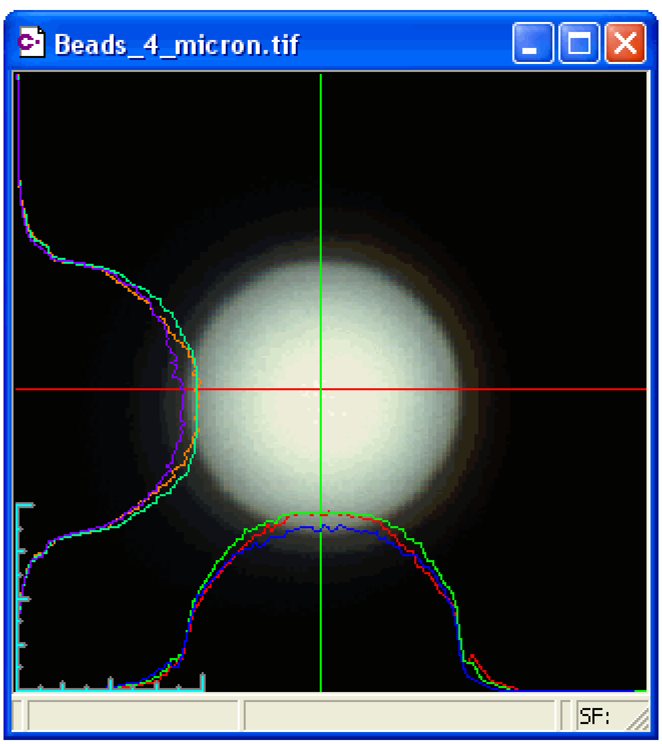
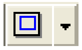


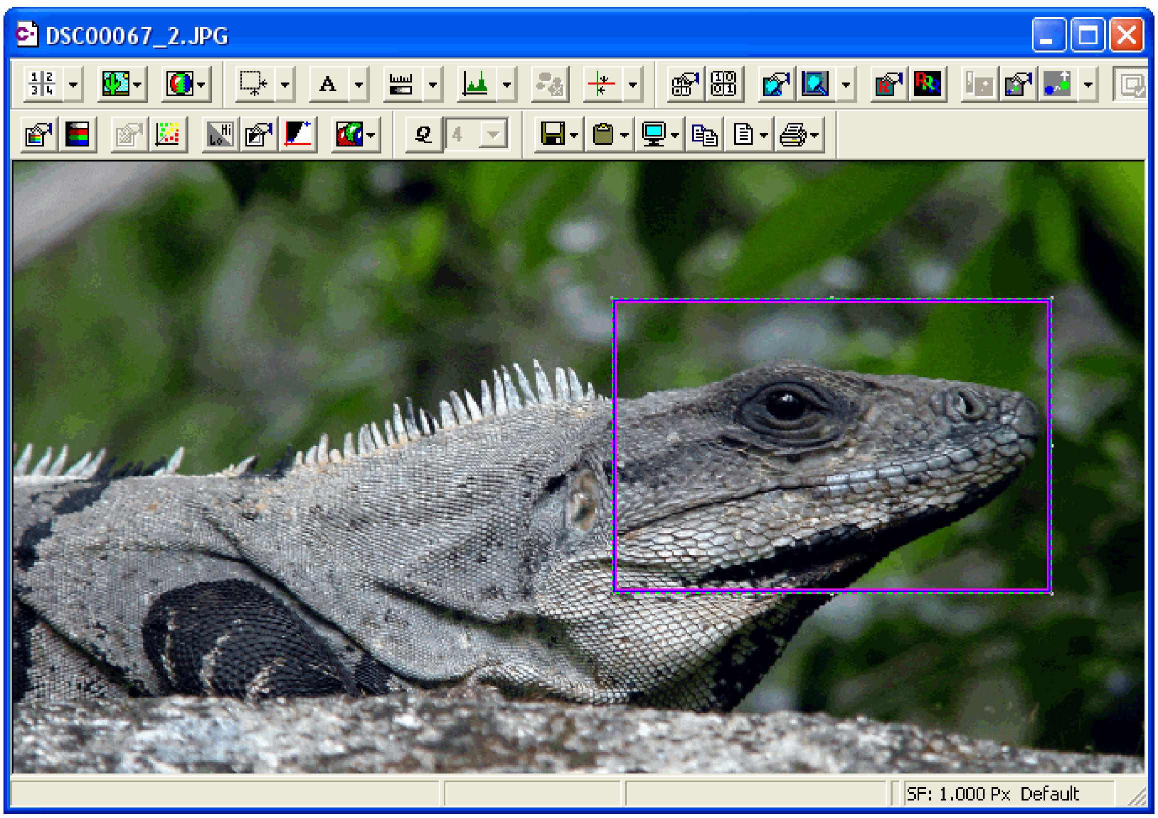
Image Region

 The **Region** button in the Image Processing Toolbar allows a rectangular portion of the image to be identified. The Image Region is created in the Image Region Layer, and can be displayed or hidden independently from other image layers.

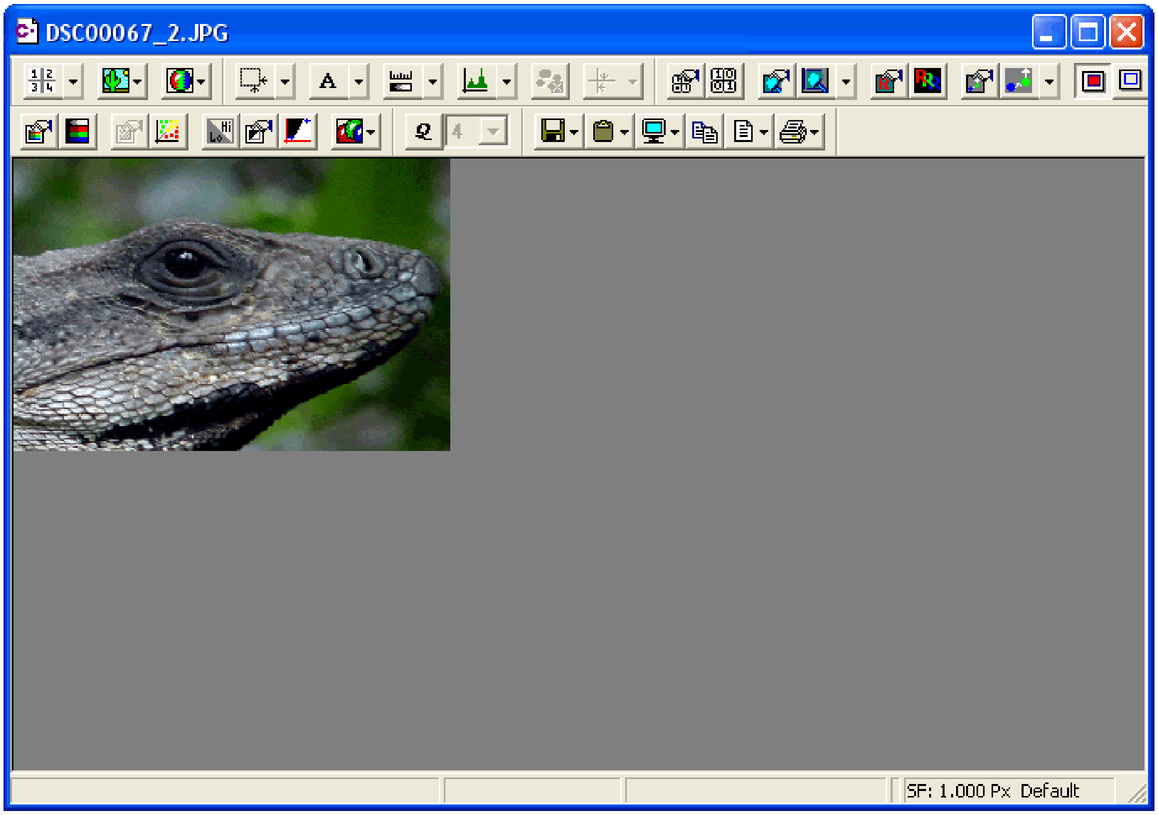
The Image Region can be applied for the following operations:

- **Display**
- **Copy to Windows Clipboard**
- **Export to an image file**
- **Printing**
- **Image Montage operations**
- **Processing operations**

To create an Image Region, select the Image Region button and click and drag a rectangular region in the image enclosing the area of interest.



To apply the Image Region, press the Apply Image Region button  in the Image Region menu.

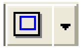


Click on the Region to select it, highlighting the Region shape. Move the selected Region by dragging it with the cursor.

To modify the size and shape of the Image Region, select the Region shape by clicking on it, then click and drag the corner or side handles. The Image Region can also be modified by using the Image Region Properties dialog, activated from the Image Region menu, or by right clicking on the Image Region.

Click the arrow button for the **Image Region Layer drop-down menu** for further options.

Image Region Menu

 The drop-down menu from the **Image Region** button has further options:

View Region

Toggles the Image Region layer display on or off.

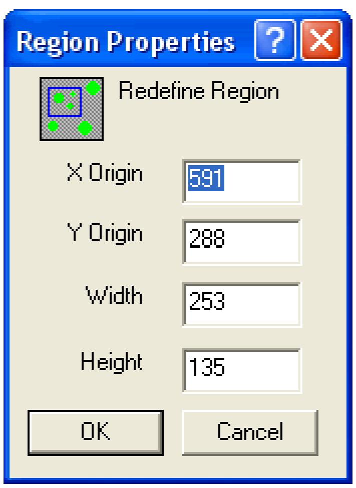
> **Note:** This layer cannot be toggled off while it is the Active Layer, activate another layer to enable this option.

Reset Region

The Reset Region option removes the Image Region. If the region is selected in the image, the Delete key will also delete the Region.

Region Properties…

This dialog shows the current Image Region size and location, and allows the values to be numerically entered or modified.



Deconvolution Overview

Deconvolution is an advanced image processing procedure (enabled when the DNN module is activated) which can correct blurring effects when supplied information about the imaging system used to acquire an image.

No Neighbor / Nearest Neighbor - use a deblurring deconvolution

- **No Neighbor** - acts on a single image.
- **Nearest Neighbor** - applies only to a data document containing a Z-scan

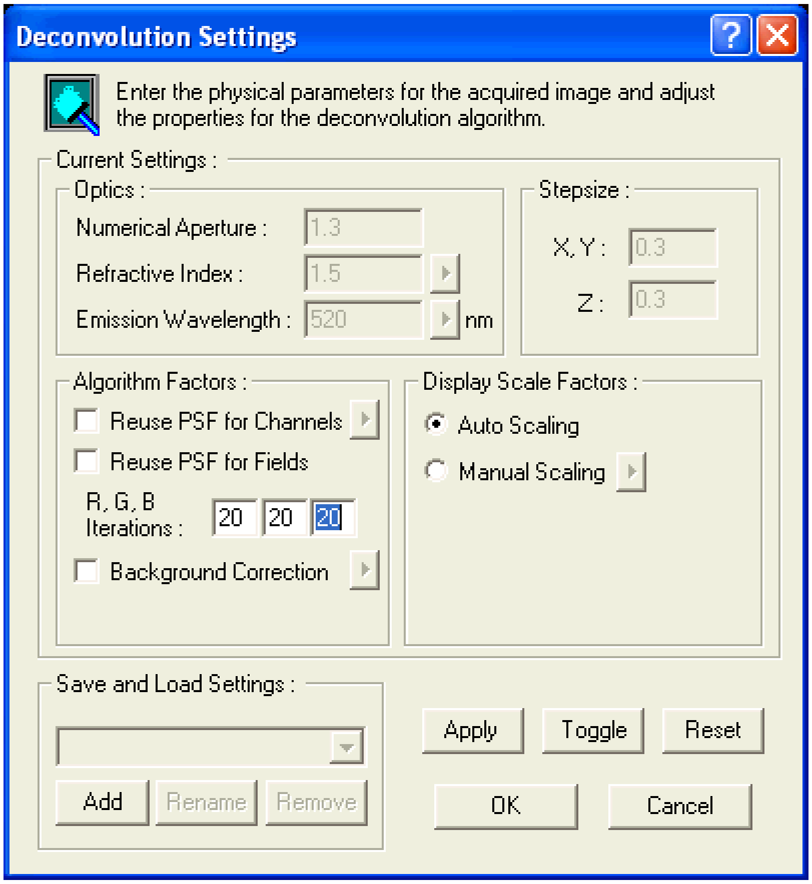
  The nearest neighbor uses the image above and the image below each image in the Z-stack to determine the point-spread function to use in performing the deconvolution.

2D Blind - uses an iterative/restorative algorithm.

The algorithm iteratively determines the point-spread function in the image, processes the image with that point-spread function, and repeats.

While applying this algorithm, the point-spread function will appear in the top-left corner of the image as an indicator of the calculations progress.

2D Blind



Re-use the PSF for Channels: Speed up the de-convolution by re-using the PSF (point-spread function) calculated for the selected color channel for de-convolving the other channels

Re-use the PSF for fields: Speed up the de-convolution process by assuming the PSF does not change from one field to the next (i.e. that the same aberrations are present). Only applies to data documents with multiple fields

RGB Iterations: Control how many iterations each color channel should iterate through while computing its PSF

Background Correction: Check this box to set the minimum intensity near zero, therefore making the background darker

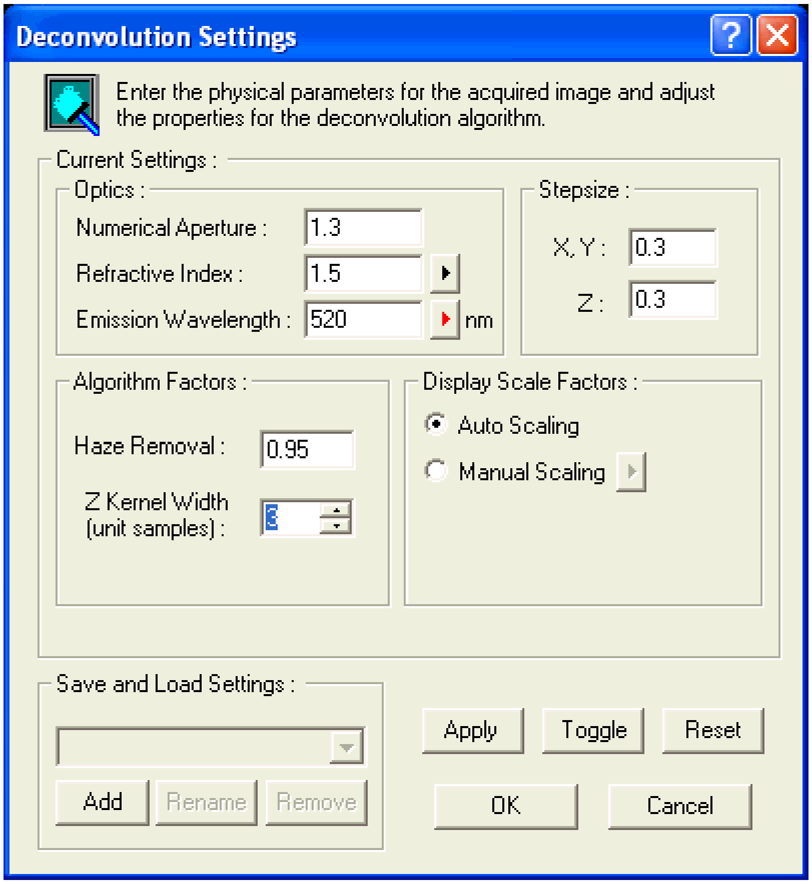
Autoscaling: Check this option to automatically detect the minimum and maximum intensity values in each frame and scale the de-convolved output accordingly

Apply: Execute the current settings to the image. Useful to get a pre-view of the image without closing the settings window.

Toggle: Toggle between the de-convolved and original images, to better see the de-convolution effects

Reset: Reset all controls in the de-convolution settings window to default values

No Neighbor / Nearest Neighbor

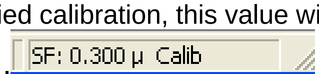


Numerical Aperture: Enter the NA of the objective lens used to acquire the image (generally written on the lens)

Refractive Index: Enter the refractive index of the sample media. You can select from the pull-down menu according to the type of sample media used.

Emission Wavelength: Enter the emission wavelength of the fluorochrome used to label the sample. For example, 520 nm for FITC, 566 nm for Cy3, 461 nm for DAPI, 666 for Cy5, etc.

Stepsize:

- X,Y: Enter the pixels size (in microns/per pixel i.e. the distance between where one pixel begins and where the next pixel begins) . This can be obtained from a calibrated stage. If you have already applied calibration, this value will be visible in your status bar, as the example shown here: . 

- Z: Enter the Z-increment between slices, in microns, using the same units as for the X,Y pixel size.

Algorithm factors:

- **Haze Removal**: Controls the amount of de-blurring performed on the image. The higher the value, the more de-blurring is performed. Experiment with different values until satisfied with the result.
- **Z-Kernel Width**: Determines the number of slices above and below the focal plane considered in the calculation of the out-of-focus PSF (point-spread function). Possible values are from 1 to 5.

Display Scale factors:

- **Auto-scaling**: Check to automatically detect the minimum and maximum intensity values of each frame and scale the de-convolution data accordingly
- **Manual scaling**: Check to use the minimum and maximum intensity value of the current frame for the entire image sequence. This option will give a consistent scale value, although it may not be the best because of the arbitration of each field.

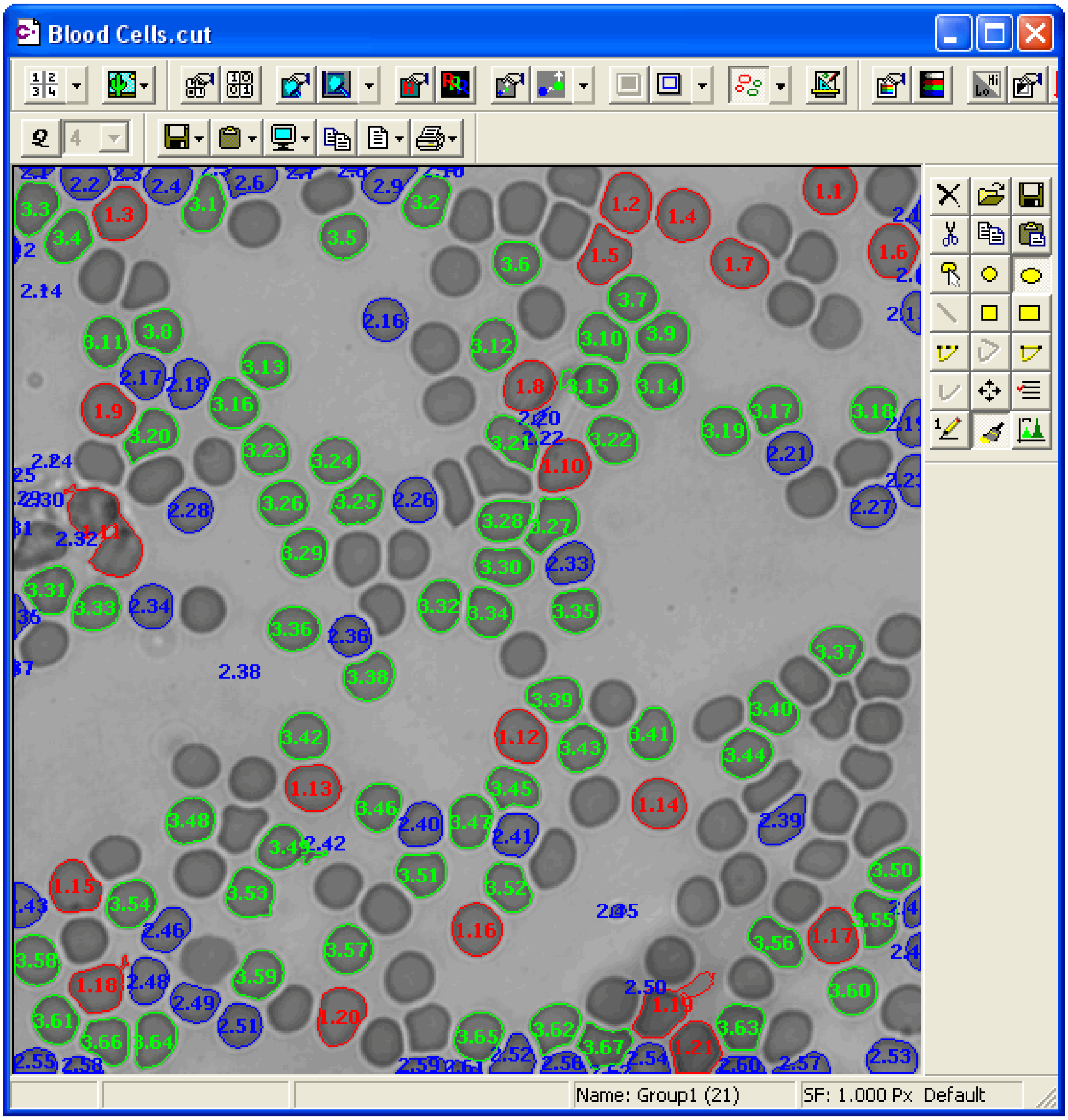
Apply: Execute the current settings to the image. Useful to get a pre-view of the image without closing the settings window.

Toggle: Toggle between the de-convolved and original images, to better see the de-convolution effects

Reset: Reset all controls in the de-convolution settings window to default values

Image ROI Shapes Layer

The **ROI Shapes Layer** allows the creation and modification of a series of shapes defining regions of interest (ROIs) in an Image Document. The ROI's can then be used to isolate specific parts of an image for measurement purposes, e.g. Intensity histograms, scattergrams, etc.



Shapes

Regardless of whether each shape was created manually or via the automation-assisted identification techniques, shapes can be copied, pasted, cloned, moved, saved to disk, loaded from disk, recolored, labeled with numbers, or deleted. Closed filled shapes can be converted to outline-only shapes, and outline-only shapes can be converted to filled shapes.

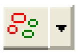
Understanding the Difference Between Filled Shapes and Outline Shapes

Filled Shapes and Outline Shapes yield very different results during image measurement. For example, measuring the area of an *outlined* circle will yield a result many times smaller than the area measurement of the same *filled* circle because only the area pixels in the boundary would be considered.

Shape groups

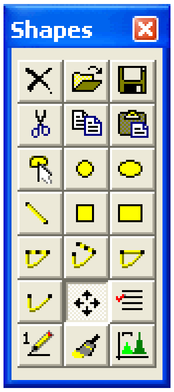
In the example above, three groups of shapes have been defined. Each group is displayed in its own color with shape numbering enabled. When measurements are made on this image, the results for each shape group will be organized in separate measurement class nodes within each field node of the data document.

Activating the ROI Shapes Layer

 This Layer is activated using the button (shown at left). When this layer is active, the Image Document has an additional toolbar, the ROI Shapes Toolbar for controlling the creation, and management of ROI shapes.

Clicking the ROI Shapes down-arrow menu offers options for labeling groups of shapes.

ROI Shapes toolbar



Reset Shapes

Reset All	Deletes all groups. Names, colors and fonts are reset to the c
Remove Current Group	Deletes the current group.
Delete Shapes in Current Group	Empties the current group but it remains the current group.
Delete All Shapes in All Groups	Empties all groups of shapes but retains their custom names

The difference between reset all and delete all shapes in all groups is that delete all shapes in all groups does not delete the group definitions.

Shape Editing

Open Shapes File	Prompts to open a ROI Shapes (*.roi) file. If the dimensions of the ROI definition shape layer loaded exceeds the dimensions of the image, the action will abort with an error message.

Save ROI Shapes list	Prompts to specify a filename to save to. If more than group is defined, prompts for whether to save all groups or only the currently active group.
Cut Shape	Cuts selected shapes to document-specific shape buffer
Copy Shape	Copies selected shapes to document's shape buffer
Paste Shape	Pastes selected shapes to document's shape buffer. *Note* at this time, cut and paste be used to transfer ROI shapes between documents; if the two documents are compatible sizes, save shapes to file and load shapes from file can accomplish the transfer.
Clone Shape	While active, if a single shape is selected, every click of the mouse copies and pastes the selected shape at the click location. If multiple shapes are selected the tool places a small circle.

Creating Shapes

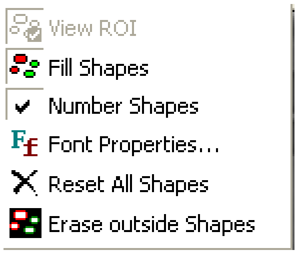
Circle Shape	Click and drag places and resizes a new circle shape (filled or un-filled depending on the Filled/Outline button state)
Ellipse Shape	Click and drag places and resizes a new ellipse shape (filled or un-filled depending on the Filled/Outline button state)
Straight Line Shape	Click and drag places and resizes a new line shape (disabled while Filled/Outline button is set to Filled)
Square Shape	Click and drag to place and re-size a new square shape (filled or un-filled depending on the Filled/Outline button

	state)
Rectangle Shape	Click and drag to place and re-size a new rectangular shape (filled or un-filled depending on the Filled/Outline button state)

Select Line Thickness using the button shown below

ROI Shapes Menu

The **ROI Shapes** menu (available from the ROI Shapes menu anchor in the Image Processing toolbar or at the bottom of the image view's right-click context menu (when ROI Shapes layer is active) has further options:



Activate ROI Shapes Layer

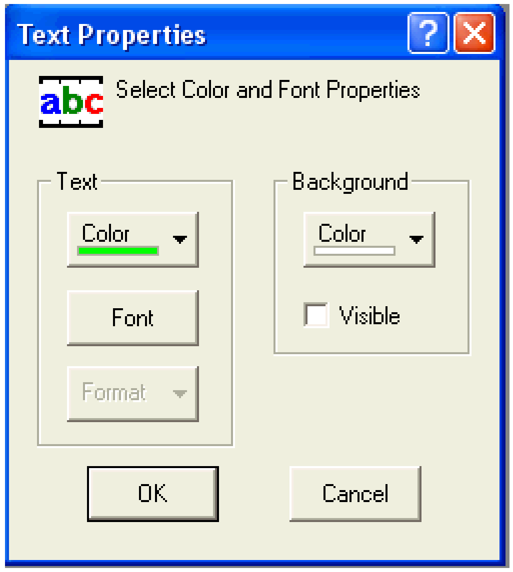
Clicking on the left-hand portion of makes the ROI Shapes layer the active layer; clicking on the down arrow to its right opens the menu of available ROI Shapes layer commands (except which have been promoted to toolbar icons).

View ROI
Toggles the ROI shapes layer display on or off. Note this layer cannot be toggled off while it is the Active Layer – activate another layer to enable this command.

Fill Shapes (on/off)
Toggle the ROI shapes layer display to show the shapes as filled or as outlines only.

Number Shapes (on/off)
Toggle the ROI shapes layer display to show the shapes with Group and Shape numbers on or off.

Font Properties…
This option allows the Font, style, color and size used to display ROI shapes numbers to be modified using the Font Properties dialog. The **Text Properties** are common to all shapes in a group



Reset all Shapes
This option will immediately delete all shapes defined in all groups.

Erase outside Shapes
When this option is selected, the area in the image outside the shapes is draw as intensity 0, normally black, to focus attention on the image areas inside the ROI's.

Image Document Measurements

While the AIC and IPA modules greatly enhance and ease the automated measurement capabilities of SimplePCI in most applications, performing manual measurements with SimplePCI is always an option and can be used in cases not amenable to automated approaches. An example would be using educated guesswork to make estimated measurements of the size of objects which are partially obscured by other objects.

The key steps involved are:

Define which pixels are part of each object to measure using the ROI Shapes layer Shapes toolbar. This is accomplished manually or with the aid of binary identification, modification, and qualification tools at the user's discretion. To measure more than one type of object and keep those measurements separate, add and use various Shape Groups via the Group List management button on the ROI Shapes toolbar. Each shape group's measurements will be written into a separate measurement class node within the field nodes generated into the measurement results data document.

Manual identification:

Manually draw filled shapes to cover all pixels within each shape boundary.

Draw shape outlines to define the shapes as perimeter-only shapes.

Automation-assisted identification: (initiated using the ROI Shapes toolbar's Create Shapes from Overlay button to open the Identify dialog):

Include and exclude various pixels from the initial shape definition by using the monochrome/RGB intensity or HLS thresholding techniques documented for Identify. When the result is accepted, a prompt for whether or not to use "**Advanced Detection**" appears. If affirmed, subsequent Modify and Qualify dialogs can be used to refine the resulting shape list further before completing the automatic shapes identification.

Manual adjustments:

Regardless of whether each shape was created manually or via the automation-assisted identification techniques, shapes can be copied, pasted, cloned, moved, saved to disk, loaded from disk, re-colored, labelled with numbers, or deleted. Closed filled shapes can be converted to outline-only shapes, and outline-only shapes can be converted to filled shapes.

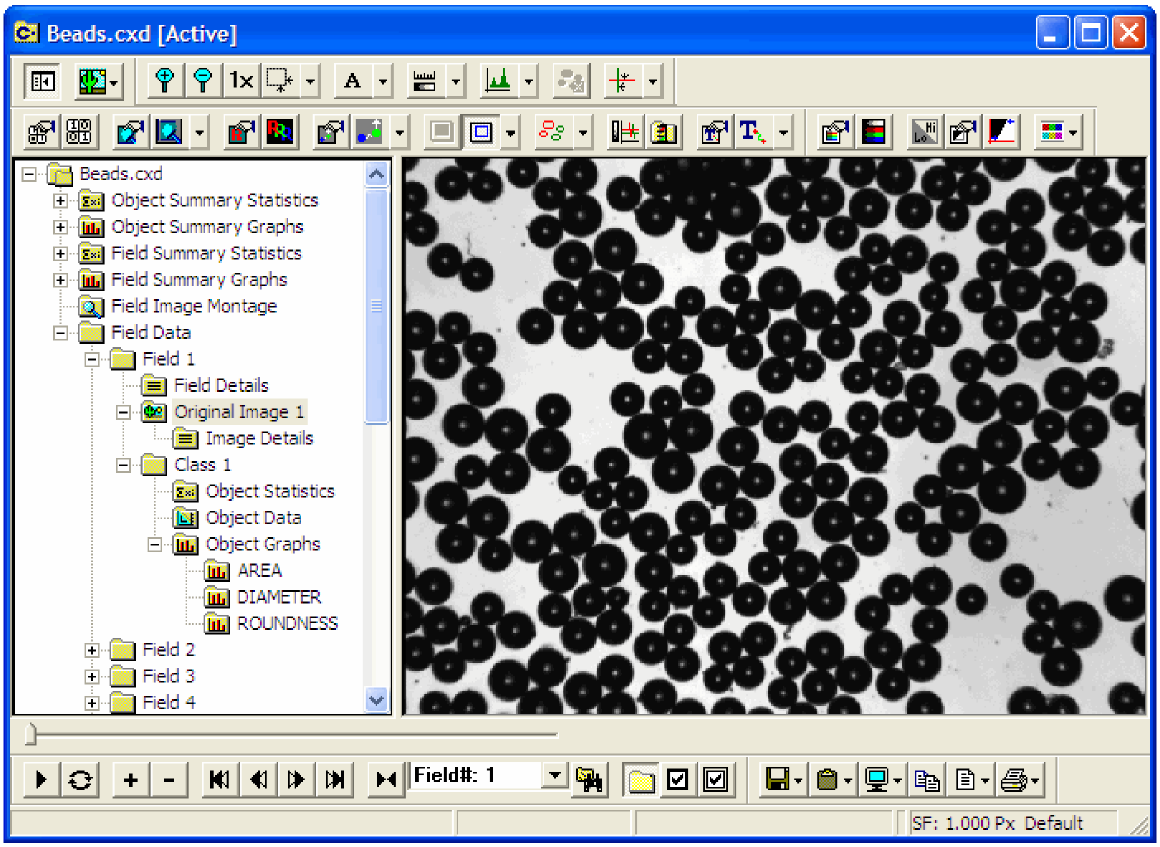
Note: It is crucial to understand the ramifications of filled vs. outline shapes on image measurements.

Choose from the over 150 available pre-defined measurements by clicking the **Measure Image** button in the **Image Processing toolbar** to open the **Select Measurements** dialog. Customize new measurements through its **Define** button.

Generate Measurements by using the **Select Measurements** dialog's **Measure** button. The results can be appended to the current data document, replace the current data document's fields, or be written to a new data document.

Data Document Overview

Data Documents are disk-based files, which can contain a variety of data types, including images, object and field measurements and statistics. The Data Document frame contains a Tree View in the left pane detailing the contents of the file, and a right pane containing a view of the currently selected item. The selected item can be displayed in one or more ways, and can be extracted from the Data Document file for copying to the clipboard, printing or exporting to other file types.



The Data Document format provides a convenient mechanism for collecting many different data types associated with a project and storing them together in a convenient format. Such files may be used for archival purposes or for easily transferring data between workstations or colleagues. Data stored in a Data Document can be manipulated to create further visual and analytical displays after the data has been acquired, such as Image Montages, or customized graphs and spreadsheets.

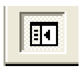
The main aspects of Data Documents are:

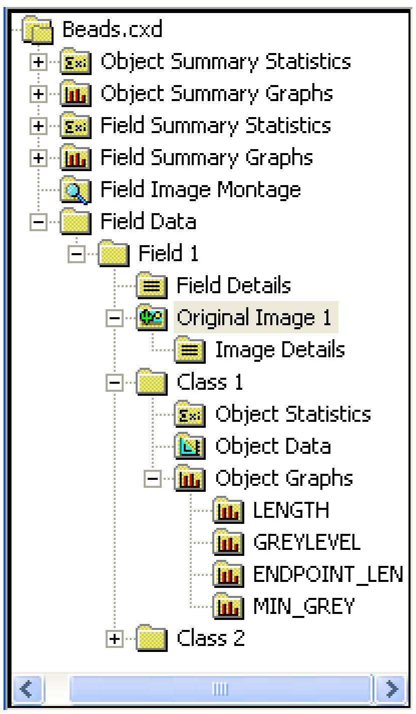
- **Data Document Tree**
- **Data Document Data Types**
- **Data Document Views**
- **Data Documents Toolbars**

The Data Document Tree

The Data Document Tree View contains a number of nodes, each of which may contain either data or other nodes. This structure maintains the relationships of data as it is collected in a database like format, allowing the data to be reconstructed and reviewed after it has been saved. This can be a significant benefit over saving data in a spreadsheet format, as once data is saved to an unstructured file it can be very difficult to reconstruct for useful analysis.

The behavior of the Tree nodes follows the Windows conventions. To expand a node, click on the + sign, to contract a node, click on the – sign. To select an item, click on the name of the item to display its data in the View pane.



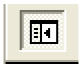


The uppermost (**Root**) node shows the name of the Data Document file. This node displays a text view where capture settings are automatically stored.

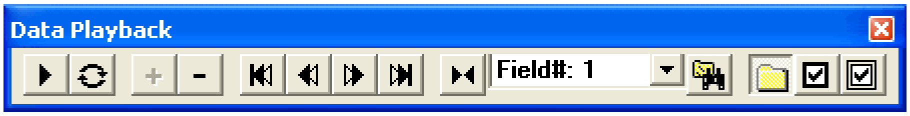
Nodes under the Root Node are Summary nodes for Measurement Statistics and Graphs, and the detailed data containing Field and Object Measurement Data for each Field in the Data Document.

Measurements in each Field are divided into Classes. A new Class begins for each time a Measure Icon is included, signifying that a new set of measurement data is to be collected. Field and Object measurement data is collected separately for each Measurement Class.

The combination of measurements from different classes is possible using Custom Measurements. The Custom Measurements Menu provides a button to select a measurement from a particular Class number. This makes it possible to arithmetically combine measurements for comparison, e.g. in percentage ratios, or for subtracting background values.

The Tree View can be minimized to increase the size of the data view by clicking the **Tree View Icon**  in the Data Views toolbar on the left side of the Data Document View Toolbar.

Playback Toolbar

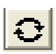


The **Playback Toolbar** is used to control animation between data items in a Data Document. If a Data Document contains multiple fields, the Playback Toolbar can automatically animate between similar items in the Document and render them in the current view selected, creating an animated movie effect.

This is especially useful for reviewing Image Data sequences of time lapse or Z sectioned samples, but can also be useful for playing back data from multiple Fields in the form of graphs or tables.

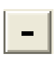
Play  **/ Stop** 

Toggle Stop/Play Mode. Plays continuously until stopped.

Loop  **/ Bounce** 

Toggle Loop/Bounce Mode.

- **Loop** Cycle to the end of the file and begin again.
- **Bounce** Cycle to the end of the file and reverse direction.

Slower 

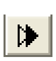
Add a small delay between fields. See the Status bar for feedback.

Faster 

Reduce any delay between fields. See the Status bar for feedback. If running at full speed, speed will increase by skipping fields.

Previous Field 

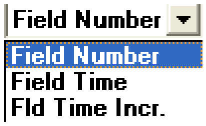
Decrement by one Field. If playing, set playback direction to reverse.

Next Field 

Increment by one Field. If playing, set playback direction to forward.

Playback Range 

Set playback Start, End and Increment.

Playback Field Details 

List the details present in the current field. Select which Field Details to display in the Playback Toolbar.

Playback Search Button 

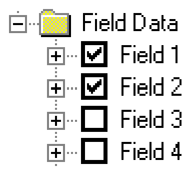
Search for a field to Go To.

Normal 

All fields are included, and the currently selected field's data is displayed.

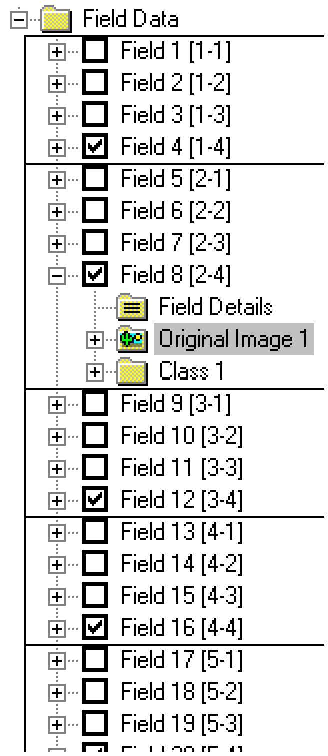
Field Selection 

Only checked fields are included, and the currently selected field is displayed.



Group Selection 

Fields at a given index within their group are selected, and the data from the


Field Data
Field 1 [1-1]
Field 2 [1-2]
Field 3 [1-3]
Field 4 [1-4]
Field 5 [2-1]
Field 6 [2-2]
Field 7 [2-3]
Field 8 [2-4]
Field Details
Original Image 1
Class 1
Field 9 [3-1]
Field 10 [3-2]
Field 11 [3-3]
Field 12 [3-4]
Field 13 [4-1]
Field 14 [4-2]
Field 15 [4-3]
Field 16 [4-4]
Field 17 [5-1]
Field 18 [5-2]
Field 19 [5-3]

Data Document Views

The left most Icon will minimize the Data Document Tree, expanding the View portion to fill the Data Document window.

The data types in a Data Document can be viewed using one or more Views. The views are enabled or disabled depending on whether the view type is compatible with the type of data contained in the currently selected node in the Data Document Tree.

For example, when an **Original Image** node is selected in the data document tree, the available view types include the same eight image views documented under Image Data View Summary.

In addition to the same views available from an image document, a data document's fields can also be viewed as an "image montage", in which several images are combined into a single larger image using:

- **Image Montaging**

Data documents contain not only images, but several other types of data nodes, some of which have their own special view types.

The default views for two of the other data node types are capable of highlighting correlations between graph data and image objects, or between table line items and identified image objects, respectively.

- Field Details Graph:
- Object Measurement Graph : displays an upper graph / lower image split-view once the mouse passes over a histogram bar or clicks a line graph node.
- Object Data node: displays a vertically-tiled table / image split-view once an image data row is selected.
- Dynamic Intensity Analysis results
- Motion Tacking Analysis results

To animate the Data Document Views between Fields in a Data Document, use the Playback Toolbar.

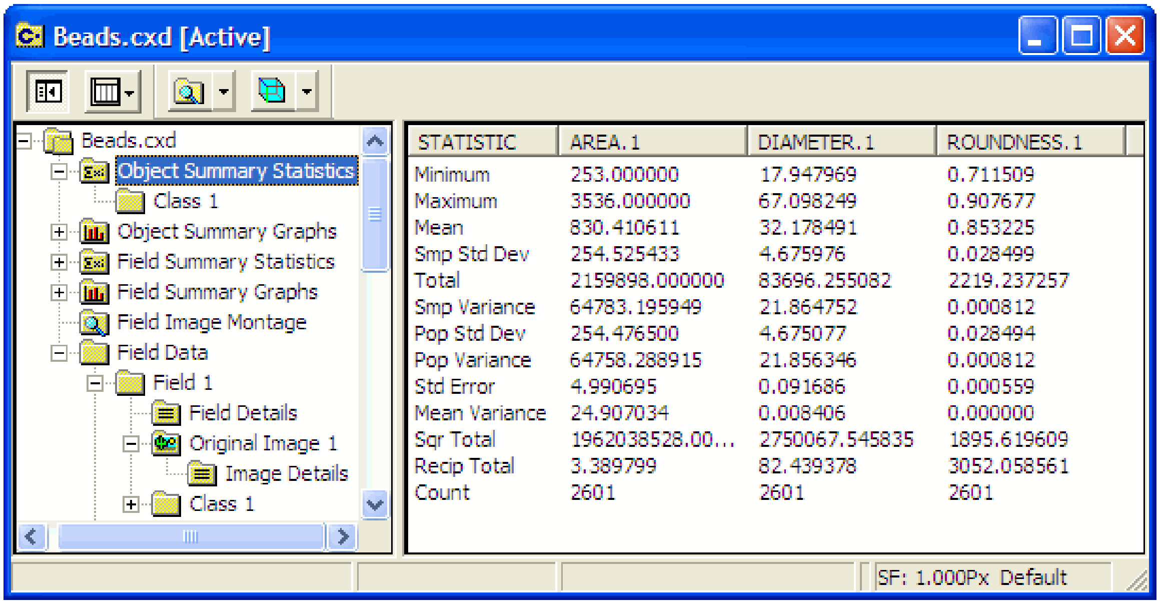
Data Document Data Types

The Data Document file can contain a variety of different **Data Types**. Each Data Type can be displayed in one or more **Views**, and exported to compatible file formats or to the Windows Clipboard for pasting into other applications for further numerical analysis, report generation or for desktop publishing.

The Data Types include:

- **Text Comments or** Capture Settings
- **Object Summary Statistics**
- **Object Summary Graphs**
- **Field Summary Statistics**
- **Field Summary Graphs**
- **Image Data**
- **Field Details**
- **Object Measurement Data**
- **Field Measurement Data**

Object Summary Statistics



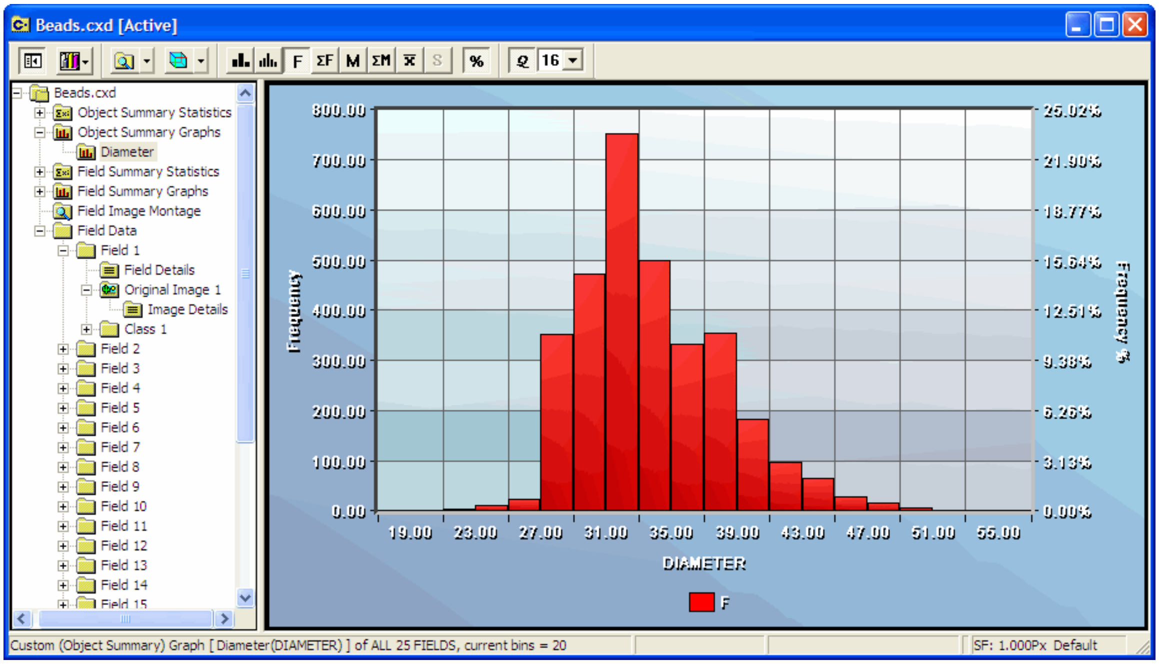
Object Summary Statistics are collected for each of the Object Measurements made for each Measurement Class. As Object Measurements in a Workfile may be selected and de-selected during data collection the Count value may vary between Field Measurements. Each Statistic is computed according to the actual Count of Objects processed for each measurement selected.

This node can be optionally selected for saving in the Data Document. If detailed Object Measurement Data is not required, this can dramatically reduce the size of the Data Document file.

See Statistical Measurements for details on the built-in statistical functions shown above.

Further Statistical parameters can be created by defining a Custom Measurement. The Object and Field Measurement partial and computed Statistics are available in the Custom Measurements menu, and can be combined together as required.

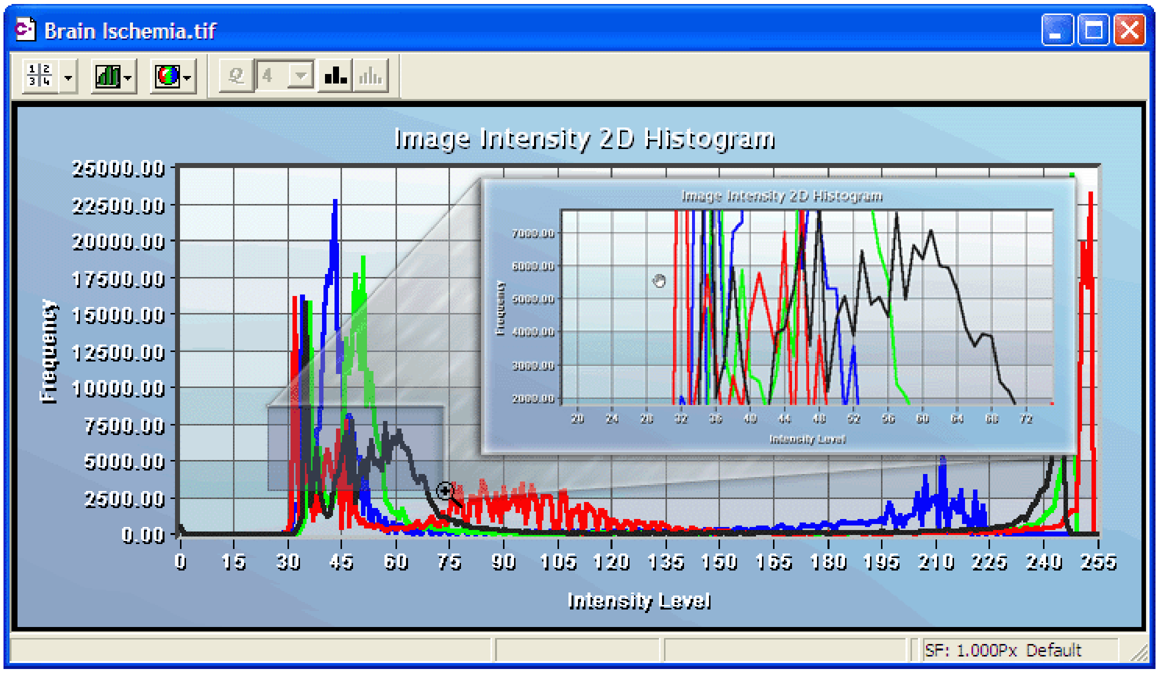
Object Summary Graphs



The **Object Summary Graphs** of the Object Measurement Data of All Fields are available if stored in the Data Document being viewed.

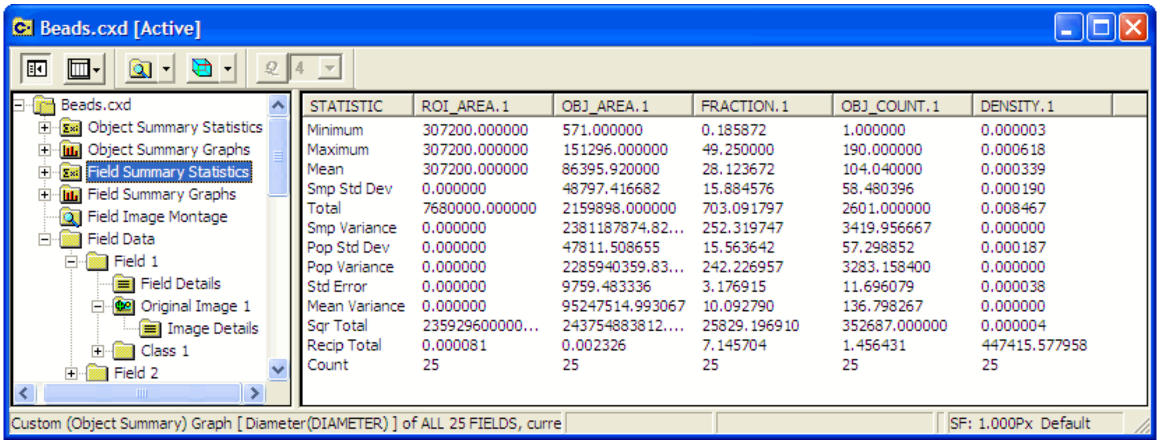
The Graph Toolbar can be used to select the type of measurement data to display. The graph above shows the Object Data plotted as a frequency distribution.

To zoom in on a particular area click and hold the left mouse button as you drag a box around the area of interest. Release the button and the graph zooms to the size of the box. Click the left mouse button to return to the normal view. While in a zoomed position the user can pan by dragging the mouse in the direction they wish to view. In the example below, a portion of a 2D Histogram has been enlarged.



See the section on 2D and 3DGraph Views for further information on how to control the Graph View.

Field Summary Statistics



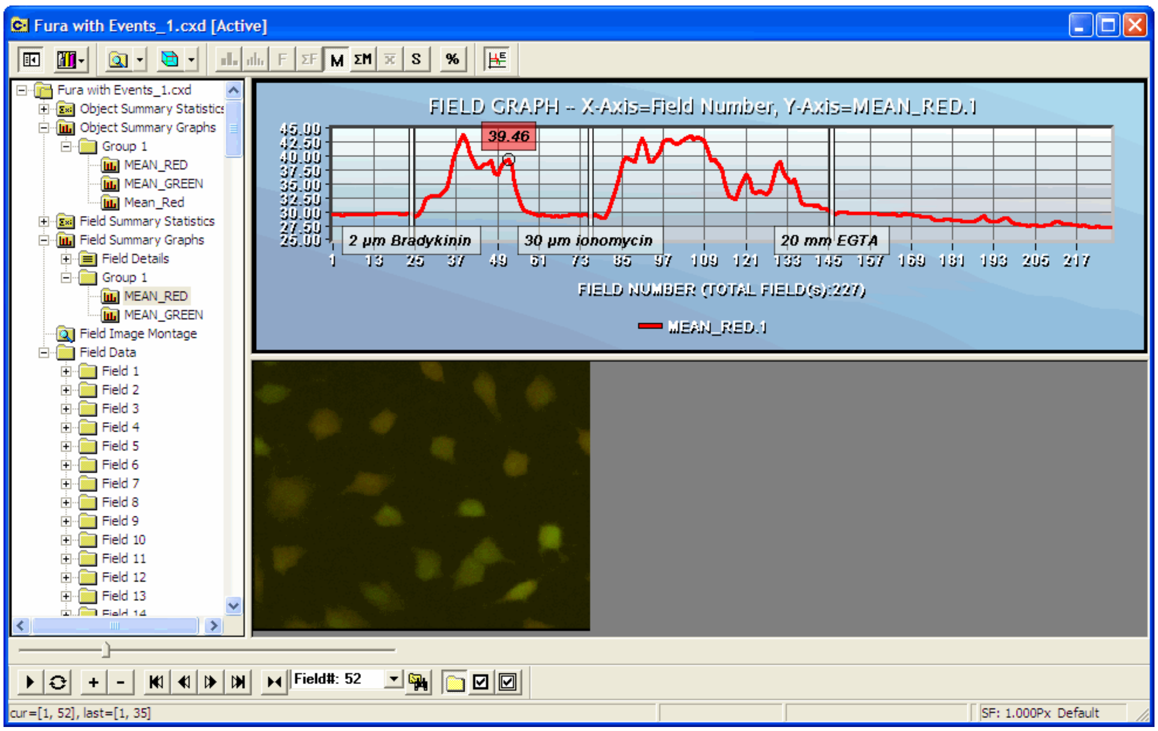
Field Summary Statistics are collected for each of the Field Measurements made for each Measurement Class. As Field Measurements in a Workfile may be selected and deselected during data collection the Count value may vary between Field Measurements. Each Statistic is computed according to the actual Count of Fields processed for each measurement selected.

Opening the **Field Summary Statistics** node will display a node for each Measurement Class present. Selecting the Field Summary Statistics node will display all Field Measurement Classes. Under the Field Summary Statistics node is a node for each Measurement Class. Selecting the Class node will display the Field Measurement Data for the individual Class.

See Statistical Measurements for details on the built-in statistical functions shown above.

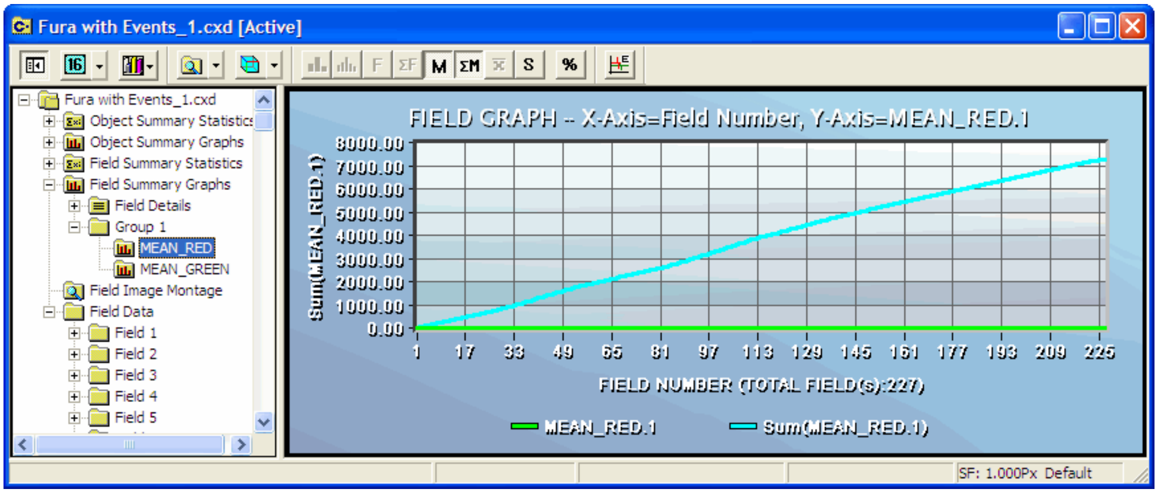
Field Summary Graphs

Field Measurements can be plotted for each Field in the Data Document. The Field Summary Graphs show Field Measurement parameters for each Measurement Class with the Field number as the X axis variable and the Field Measurement as the Y axis variable.

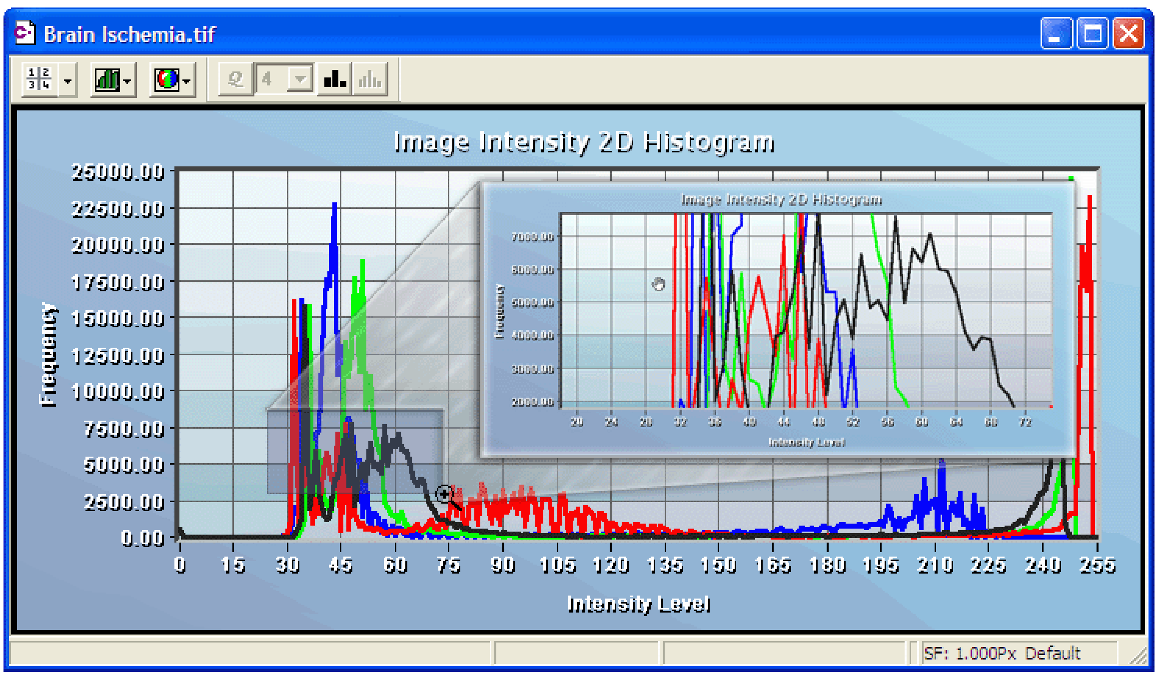


The Field Summary graphs are an easy to use interactive way of displaying and viewing data. The Field Summary graph above may look busy but the information that is displayed is very useful. First the Mean Red Intensity is measured over Time. Second there are three **Event Markers** that indicate specific points during the experiment that an event happened. In this case, we know the name and the amount of the chemicals that were added and the time they were added. The Event Markers may be toggled on and off by clicking the Show Event Markers Icon . The intensity at any given point is displayed by hovering the cursor over a section of the graph. The corresponding image may also be viewed by clicking on a point along the graph. Zoom in on a specific area of the graph by clicking and dragging the mouse over the area of interest. Use the playback functions located on the Playback Toolbar to watch the intensity changes of the sample In order to watch the how the image sequence Using the features in the **Playback Toolbar** we can play the image sequence and visually see the changes in the intensity and how they are plotted on the corresponding graph.

The Graph Toolbar can be used to select the type of measurement data to display. The graph below shows the Field Measurement data plotted as a **Cumulative Distribution**.



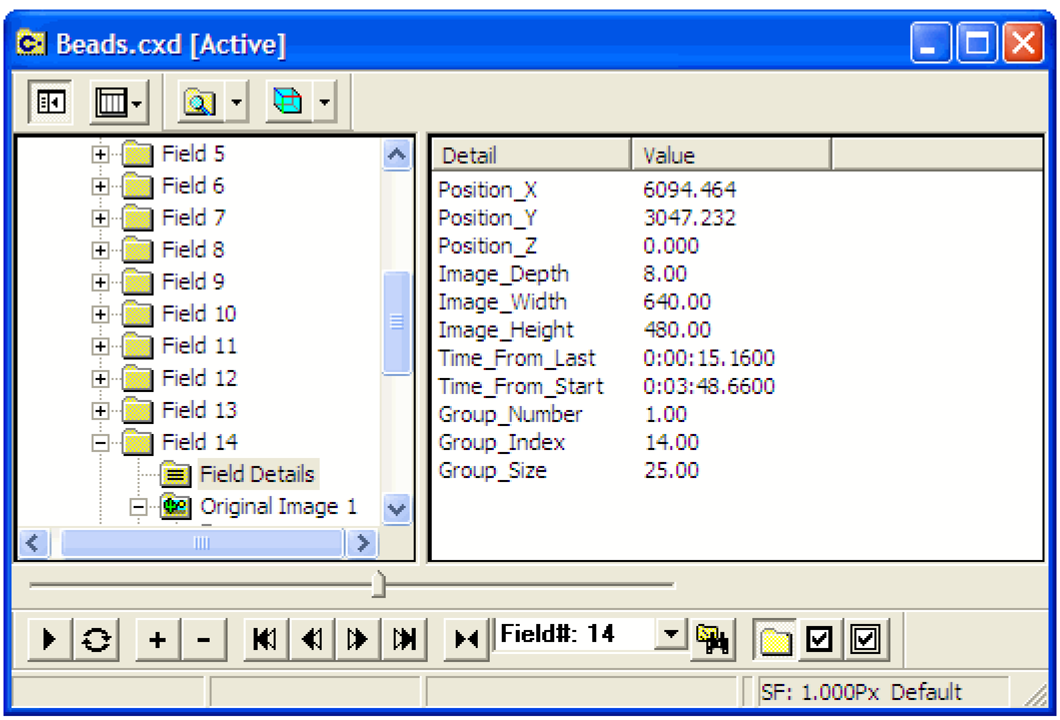
To zoom in on a particular area click and hold the left mouse button as you drag a box around the area of interest. Release the button and the graph zooms to the size of the box. Click the left mouse button to return to the normal view. While in a zoomed position the user can pan by dragging the mouse in the direction they wish to view. In the example below, a portion of a 2D Histogram has been enlarged.



Field Details

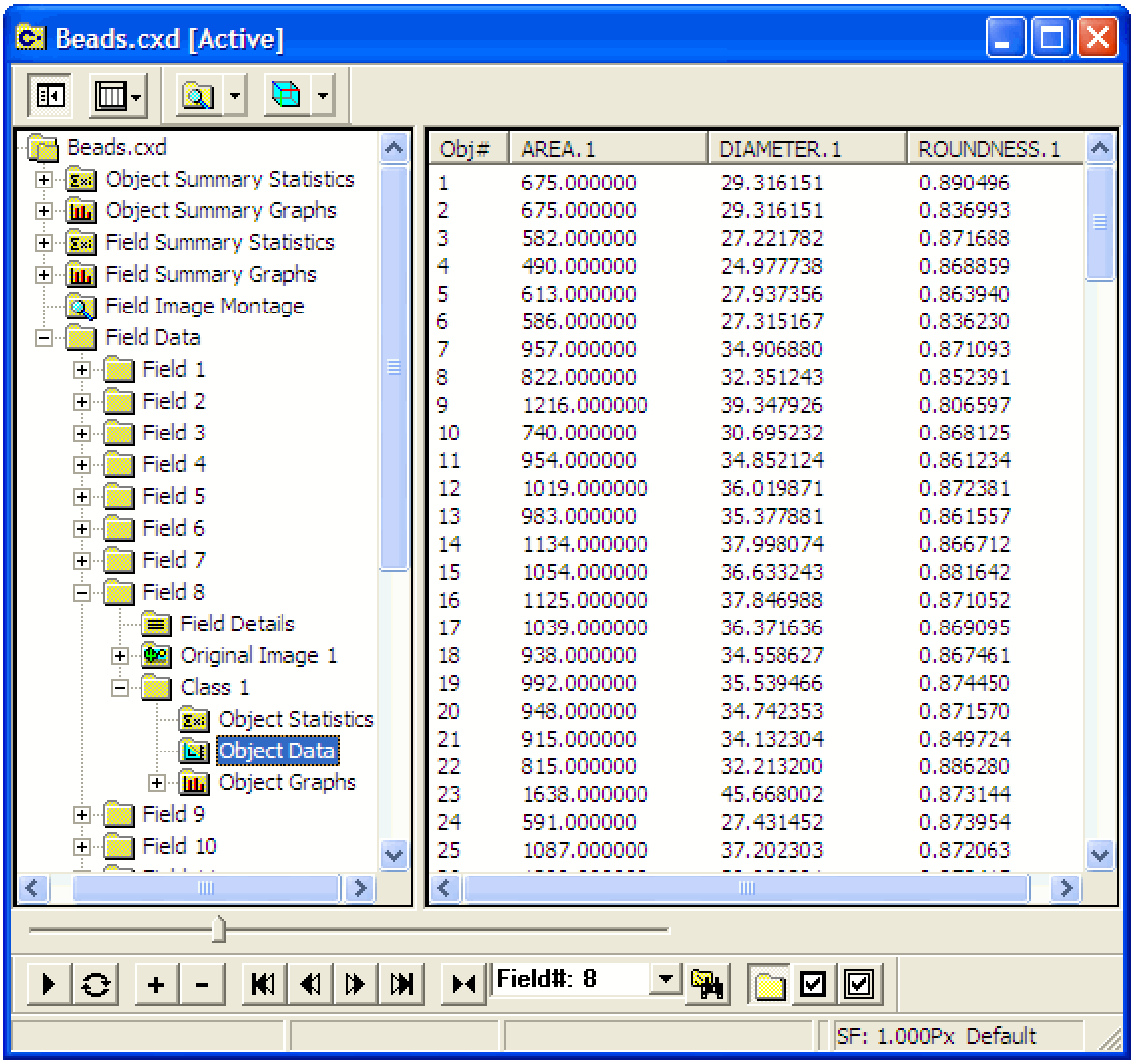
The **Field Details** node contains information for each field about when and where the image was captured. These details can include:

- Computer controlled X,Y and Z Position of a motorized stage in Microns, if a suitable device is configured in the Current System Profile.
- Image Width in pixels
- Image Height in pixels
- Image Depth in bits per pixel
- Time from the start of capturing an Image Sequence in Hours:Minutes:Seconds.Hundredths
- Time since the previous image in a Sequence in Hours:Minutes:Seconds.Hundredths
- Computer-controlled Wavelength used (in nanometers), if a suitable device is configured in the Current System Profile .
- Group Number
- Group Index
- Group Size



The Field Details can be viewed in a Table View or Spreadsheet View and copied to the Windows Clipboard, or in Graphical form.

Object Measurement Data

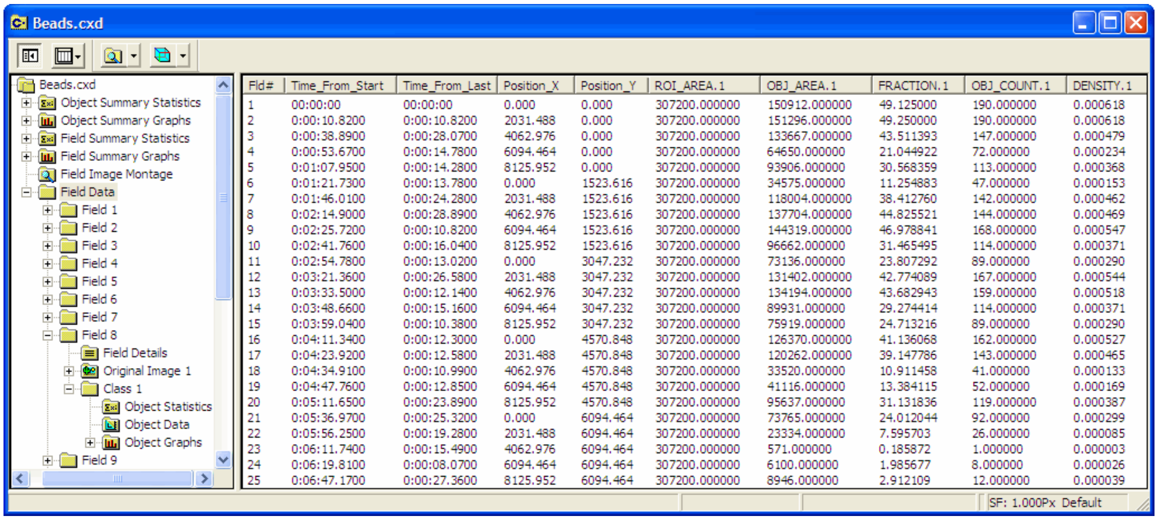


Object Measurement data are collected for each of the Object Measurements selected for each Measurement Class. These data are only saved to the Data Document if they are selected in the Record list accessed in the Collect Toolbar . They can be viewed from the Object Data node of each Field.

Object Statistics and Object Graphs are also available for each field of data measured.

Field Measurement Data

Field Measurement Data are collected for each of the Field Measurements made for each Measurement Class. These data are only saved to the Data Document if they are selected in the Record list accessed in the Collect Toolbar.



Fld#	Time_From_Start	Time_From_Last	Position_X	Position_Y	ROI_AREA.1	OBJ_AREA.1	FRACTION.1	OBJ_COUNT.1	DENSITY.1
1	00:00:00	00:00:00	0.000	0.000	307200.000000	150912.000000	49.125000	190.000000	0.000618
2	0:00:10.8200	0:00:10.8200	2031.488	0.000	307200.000000	151296.000000	49.250000	190.000000	0.000618
3	0:00:38.8900	0:00:28.0700	4062.976	0.000	307200.000000	133667.000000	43.511393	147.000000	0.000479
4	0:00:53.6700	0:00:14.7800	6094.464	0.000	307200.000000	64650.000000	21.044922	72.000000	0.000234
5	0:01:07.9500	0:00:14.2800	8125.952	0.000	307200.000000	93906.000000	30.568359	113.000000	0.000368
6	0:01:21.7300	0:00:13.7800	0.000	1523.616	307200.000000	34575.000000	11.254883	47.000000	0.000153
7	0:01:46.0100	0:00:24.2800	2031.488	1523.616	307200.000000	118004.000000	38.412760	142.000000	0.000462
8	0:02:14.9000	0:00:28.8900	4062.976	1523.616	307200.000000	137704.000000	44.825521	144.000000	0.000469
9	0:02:25.7200	0:00:10.8200	6094.464	1523.616	307200.000000	144319.000000	46.978841	168.000000	0.000547
10	0:02:41.7600	0:00:16.0400	8125.952	1523.616	307200.000000	96662.000000	31.465495	114.000000	0.000371
11	0:02:54.7800	0:00:13.0200	0.000	3047.232	307200.000000	73136.000000	23.807292	89.000000	0.000290
12	0:03:21.3600	0:00:26.5800	2031.488	3047.232	307200.000000	131402.000000	42.774089	167.000000	0.000544
13	0:03:33.5000	0:00:12.1400	4062.976	3047.232	307200.000000	134194.000000	43.682943	159.000000	0.000518
14	0:03:48.6600	0:00:15.1600	6094.464	3047.232	307200.000000	89931.000000	29.274414	114.000000	0.000371
15	0:03:59.0400	0:00:10.3800	8125.952	3047.232	307200.000000	75919.000000	24.713216	89.000000	0.000290
16	0:04:11.3400	0:00:12.3000	0.000	4570.848	307200.000000	126370.000000	41.136068	162.000000	0.000527
17	0:04:23.9200	0:00:12.5800	2031.488	4570.848	307200.000000	120262.000000	39.147786	143.000000	0.000465
18	0:04:34.9100	0:00:10.9900	4062.976	4570.848	307200.000000	33520.000000	10.911458	41.000000	0.000133
19	0:04:47.7600	0:00:12.8500	6094.464	4570.848	307200.000000	41116.000000	13.384115	52.000000	0.000169
20	0:05:11.6500	0:00:23.8900	8125.952	4570.848	307200.000000	95637.000000	31.131836	119.000000	0.000387
21	0:05:36.9700	0:00:25.3200	0.000	6094.464	307200.000000	73765.000000	24.012044	92.000000	0.000299
22	0:05:56.2500	0:00:19.2800	2031.488	6094.464	307200.000000	23334.000000	7.595703	26.000000	0.000085
23	0:06:11.7400	0:00:15.4900	4062.976	6094.464	307200.000000	571.000000	0.185872	1.000000	0.000003
24	0:06:19.8100	0:00:08.0700	6094.464	6094.464	307200.000000	6100.000000	1.985677	8.000000	0.000026
25	0:06:47.1700	0:00:27.3600	8125.952	6094.464	307200.000000	8946.000000	2.912109	12.000000	0.000039

Graphs of the Field Measurement Data of each Field are computed dynamically when viewing a Data Document, and are automatically available as individual measurement distributions in the Field Summary Graphs node if Field Measurement Data is present in the Data Document.

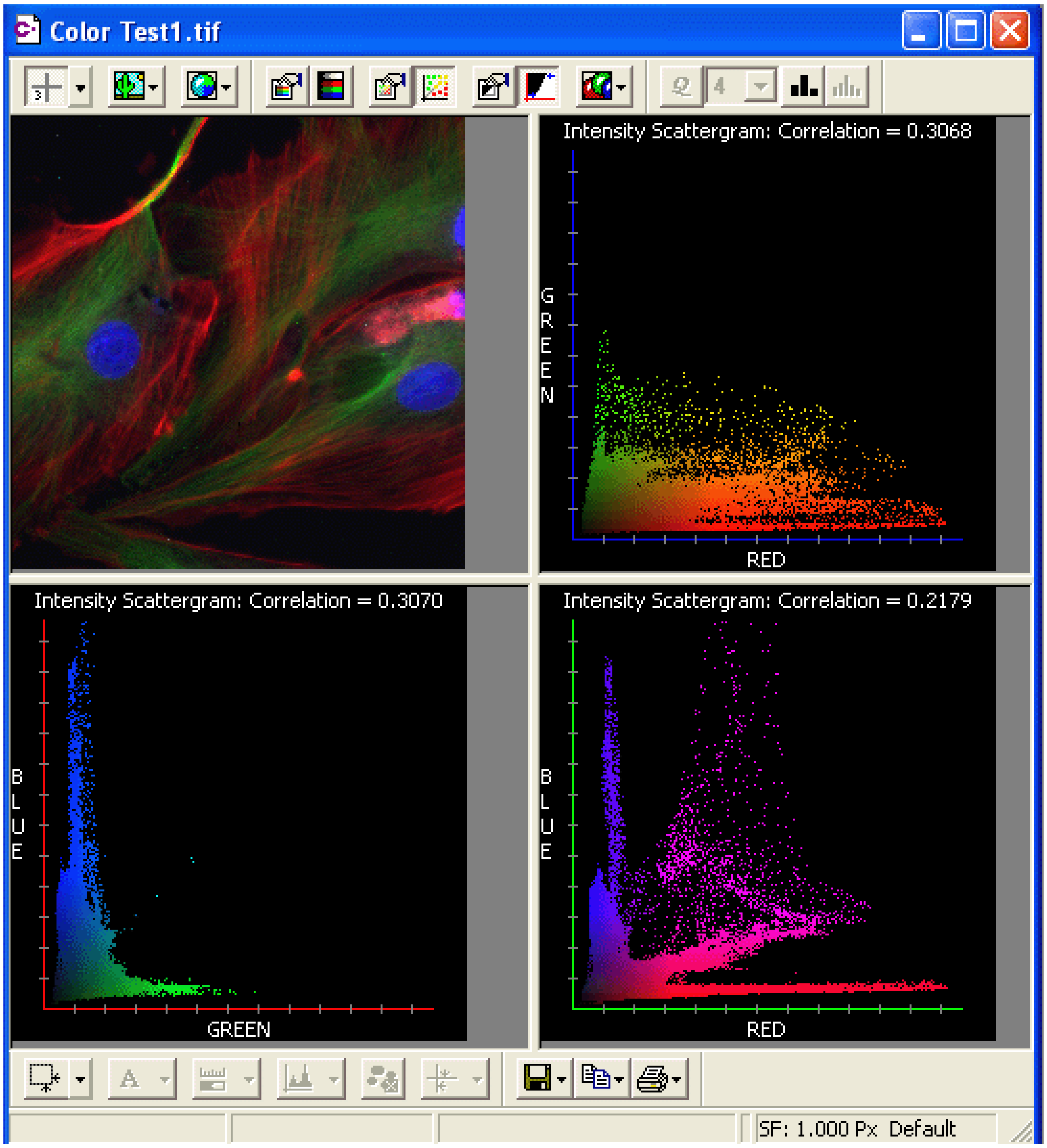
A Summary of the Field Measurement Data for All Fields is available in the Field Summary Statistics node of the Data Document Tree. This node can be optionally selected for saving in the Data Document.

Opening the Field Data node, all Field Data will be displayed in one table. Open the subordinate node to view a single set of Field Measurement Data.

> **Note:** Selecting the Field Summary Statistics node in the Data Document Tree will display All Classes of Field Measurements together. Each measurement title is in the form **Measurement.ClassNumber** making it possible to Copy or Export all Field Measurement in one pass.

Scattergram Overview

Image Intensity data can be displayed as a frequency distribution for one of more color components in an image providing information on the contrast and dynamic range properties of the image. The Image Scattergram allows Image Intensity information to be correlated for two color components at once.



The above split-view shows an contrast-enhanced image in the top-left split-window pane and Scattergram displays in each of the other window panes. Each scattergram shows the correlation of the intensity values in one-color channel vs. the intensities in another color channel.

Click **Decrease Bins** to merge bins together creating an overview

Click **Increase Bins** to separate bins for more detailed analysis

Scattergram Properties and Colocalization

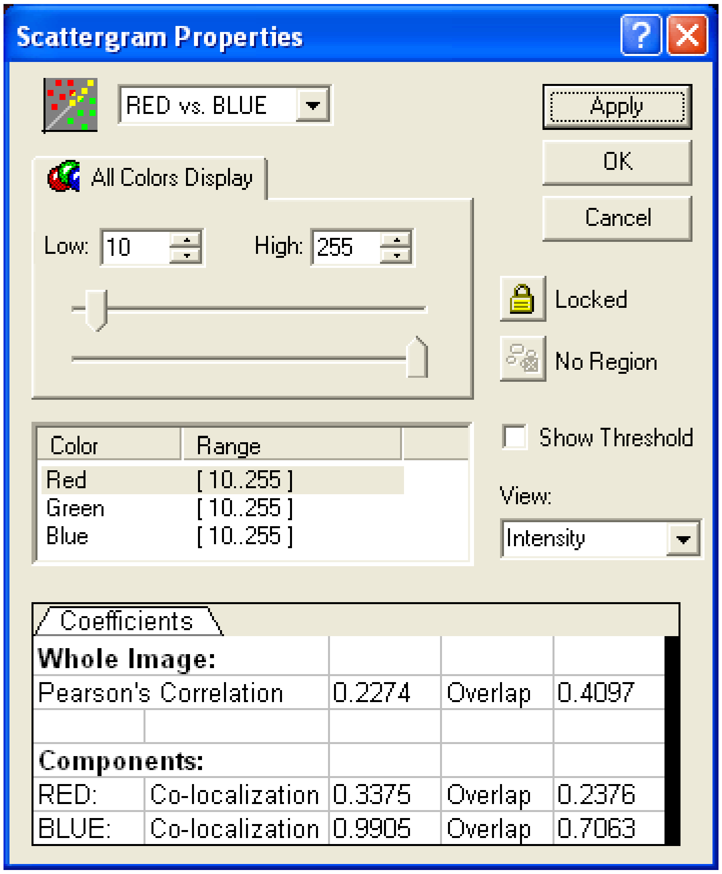


Image Capture

The **Capture Dialog** provides a flexible and comprehensive method to access the many options for controlling how images are acquired and displayed.

Change your Active Capture Device by selecting from a list of available cameras.

Choose the number of channels to capture up to five.

The Capture Dialog layout is based on a number of Tabs or Pages in a Property Sheet allowing many Dialogs to be available with just one click even during image capture. To change between the different pages, click on the tab at the top of the Property Sheet.

At the top of the Capture Dialog is the **Capture Toolbar** providing constant access to a variety of imaging controls during acquisition. On the right side of the Dialog is a column of buttons for controlling the Image Capture and selecting other options and Dialogs. At the bottom are the control buttons for leaving the Capture Dialog.

The Dialog may include a number of **Control Tabs** to control installed Devices:

File Control- Active Capture Device set to Disk.

- The **Disk Capture** Tab controls retrieval of images from Disk.

Camera Control - Active Capture Device set to Camera.

- The **Sensor** Tab functions include sensor exposure, gain and black level adjustment settings. May include digitizer controls: digitizer gain and predigitization reference offsets. Controls vary depending on the selected camera's capabilities. Includes setup of the Filter/Shutter/Wavelength combinations to customize the illumination of the sensor.

Device Setup - Change device settings of Image Capture Device

- The **Device Setup** Tab functions include acquisition mode, triggering, binning, sub-region acquire, bit depth, and other special camera functions. Images can be overlaid with a registration binary image for precise image alignment while capturing.

Processing Control - Active Capture Device set to Camera

- The **Processing** Tab controls how images are enhanced during focusing and acquisition by incorporating image processing operations during or immediately after capture. The operations include Noise Reduction, Image Correction and Split Image Registration.

Stage Control- Current profile includes stage device

- **XY Stage** Tab - Current profile includes XY stage but no Z-stage.
  Provides 2D control of the Stage's XY position for accurate positioning of the sample relative to the objective while in **Live Mode**, before final image Capture.
- **Z Focus**Tab - Current profile includes Z stage but no XY-stage.
  Controls the Z Focus position from the Capture Dialog for accurate positioning of the sample while in **Live Mode**, before final image Capture.

- **XY(Z) Stage** Tab - Current profile includes both XY and Z stages.
  Provides both XY and Z control.

Microscope Control- Current profile includes microscope device.

- The **Microscope** Tab controls the many automated microscope features, including lighting, filters and Stage positioning.

The Dialog includes a number of **Capture buttons**:

- Capture

  The **Capture** button will initiate Image Capture using the Active Camera and the settings for each of the Capture Control Tabs including the **Processing** Tab and **Display** operations. If Sequential image capture is selected acquisition will cycle through the images. Image processing will be applied. During Capture, other Tabs can be selected and their options changed, for instance moving the XY stage to target a sample.

  When **Capture** is pressed the **Stop** and **Abort** buttons appear. Click **Stop** to stop at the end of the current Capture cycle. The **Abort** button is used to terminate image capture when the nominated capture device is not responding or to interrupt an inconveniently long exposure.

- Capture1

  The **Capture1** button will initiate a single capture cycle including image processing and data display operations.

  When **Capture1** is pressed the **Abort** button appears. Click **Abort** button to terminate image capture when the nominated capture device is not responding, or to interrupt an inconveniently long exposure.

- Copy to Document

  If selected, **Capture1** will copy to each captured image to an image document.

- Live

  As its name suggests, the **Live** button is used to quickly align and focus a sample prior to Capture.

  When **Live** is pressed the **Stop** and **Abort** buttons appear. Click **Stop** to stop at the end of the current Capture cycle. The **Abort** button is used to terminate image capture when the nominated capture device is not responding, or to interrupt an inconveniently long exposure

- New Scan

  The **New Scan** button allows image sequences to be acquired without running a Workfile. The **Sequence** can be based on Time Lapse, X,Y or Z movement, or wavelength scanning depending on the equipment installed in the **Current System Profile**. The method for setting up the Scan parameters for acquiring the image sequence is detailed in the **Scan Wizard**.

  When the **Sequence** is running, the images for each Field will be automatically acquired to the **Image Display** and saved as Image Data to the target Data Document . After **Sequence** acquisition is complete, exit the **Capture** Dialog to see the Data Document where the images can be examined using random access using the Data Document Tree, or animated using the **Playback** Toolbar .

- **Default Scan**

  The **Default Scan** button allows a pre-selected scan to be displayed on the Default Scan button. The scan is selected from a drop-menu on the side of the button. The saved sequences can be used without re-answering all the options of the Scan Wizard. Saved Scans can be based on Time Lapse, X,Y or Z movement, or wavelength scanning depending on the equipment installed in the **Current System Profile**. The method for setting up the Scan parameters for acquiring the image sequence is detailed in the **Scan Wizard**.

  When the **Default Scan** is running, the images for each Field will be automatically acquired to the **Image Display** and saved as Image Data to the target Data Document . After **Sequence** acquisition is complete, exit the **Capture** Dialog to see the Data Document where the images can be examined using random access using the Data Document Tree, or animated using the **Playback** Toolbar .

- Calibration

  Images can be calibrated with Spatial Calibration for accurate measurement of distances and areas using real-world units, and with an Intensity Calibration for correcting non-linear image intensities with a standard scale for accurate densimetric or intensity related measurements.

  Note, the intensity calibration tab will only be visible if your Workfile's capture operation

- **ROI**

  Create a Region of Interest. ROI can restrict the Capture or Measurement of the image when applying the Workfile for data collection. This feature is also available from the Image Display Toolbar.

- Runtime Menu

  The Runtime Menu checkbox determines if the Capture Dialog is to be displayed every time an image is captured when running a Workfile in Collect Data mode.

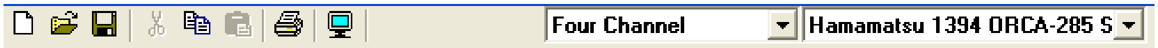
  If the box is checked the Capture Dialog will pop-up for each new field to be acquired. Note the image is NOT automatically captured, it is necessary to use the Capture control buttons (Capture , Capture1 , Focus) to store a new image.

  If the box is not checked a new image will be captured automatically with any image processing selections at each point that the Workfile is due to capture a new image.

A **right-click menu** is available for more detailed access to the Image Display for Copy, Paste, Print and Save of specific image components.

Note: To save your settings to the current Workfile, please be sure to Exit the dialog using the **OK** button. X-ing out of the dialog will cause your changes to be discarded.

Capture Toolbar



The **Capture Toolbar** provides a variety of facilities to control and modify the contents and display properties of the Image Display, plus a convenient way to switch between different configured Capture Devices. Also the right-click context menu and the Save/Copy/Print Toolbar in the Image Display can be used.

The following options by default access the Intensity or Color image in the Image Display. More detailed access is available to each color component in an image and optionally to merge the overlay graphics into the image using the **Right Click Context Menu** in the **Capture Menu** or in any of the icons in the **Task Toolbar** .

The controls in this Toolbar include:

Clear - The pixel intensity values in the Image display will be cleared to zero, normally displayed as black.

Load - Open a dialog for loading an image into the image display. Note the image will be converted according to its original depth and loaded into the current image depth as defined in the Capture Device Setup.

For example, an 8-bit monochrome image will be expanded by loading it into all three components to fill a 24-bit color display, and a 24-bit color image will be converted to Greyscale to fit an 8-bit monochrome display.

Save - The current image on display will be saved into a user specified file. Note the image will be loaded according to its color depth on the display, and converted if necessary into the target image depth as defined by the selected **Image File Format**.

Copy - To copy the entire image on display, use the **Copy Image** button. The image will be copied to the Windows Clipboard for pasting into this or other Clipboard aware applications as an image. The image depth may be converted to match the data types available for images on the Windows Clipboard.

Paste - The Paste Image button will paste any image data from the Clipboard to the Image Display. Note the image will be converted according to its original depth and loaded into the current image depth as defined in the Capture Device Setup. If the image is smaller than the destination A cursor and rectangular outline will appear on the Image Display. Click and Drag the rectangle to place the image in the Image Display before pasting.

Print - The current image on display will be sent to the current selected **Windows Printer Driver** for printing. This can be selected in the **File>>Print Setup Menu**. Only the Greyscale or Color image data will be sent using this button.

Less/More - The Less button will collapse the Capture dialog to a single column, and the More Button will return the capture dialog to the full size, and allow adjustment of all settings.

Toggle Image Display - The **Image Display** can be removed and restored using the Toggle Image Display button in the Main Toolbar, or from the Image Display right click menu.

Image Capture Depth - Select the number of channels you would like to capture**.**

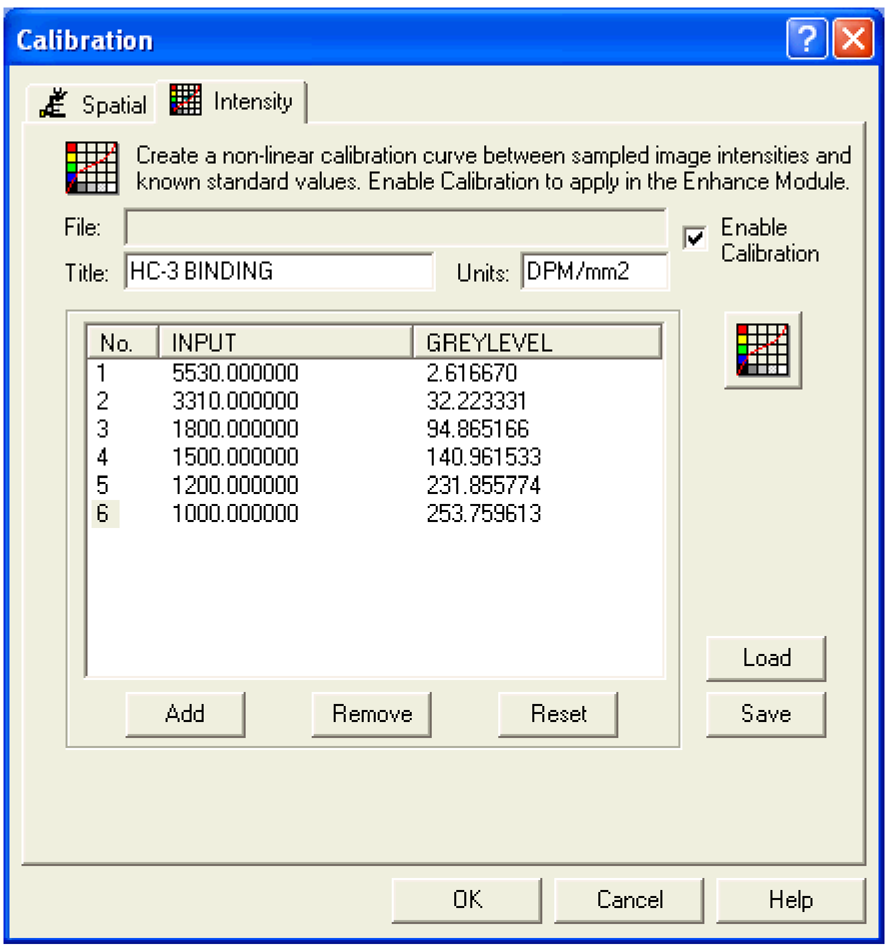
Active Capture Device- The drop-down list allows rapid switching between configured Capture Devices. Multiple Capture devices are installed in the Device Setup Tab of the Current System Profile . Further customization of installed Capture devices is done in the Capture Device Setup Menu.

Calibrate

The **Calibrate** button is used to determine a new Calibration Factor for the current selected calibration. First, an image must be acquired which has an object of known distance, usually a calibration graticule or scale ruler. Clicking the button will bring the Image Display to the front if hidden. Move the cursor to the start of the known distance and click. Dragging the mouse will draw a rubber-band line, which should be joined to the other end of the known distance and the mouse button released. Then answer the question "How long is the line" by entering the number of calibration units indicated by the line. The Calibration Factor in the Spatial Calibration Menu will be updated.

Intensity Calibration

The aim of this process is to create a transform function that relates the image intensity that we sample with the camera to the known standard values that may have a nonlinear relationship.



The **Intensity Calibration Dialog** allows the nonlinear calibration of grey level intensity values to calibrated units, to quantify parameters such as Optical Density or X-ray Film exposure. To create the transform function we first have to identify a list of points representing the known calibrated values and the sampled image intensity. Then choose a curve fitting function in order to compute the intermediate calibrated values for each intensity value, and transform the resulting image ready for MEAN DOSE and TOTAL DOSE object measurements.

The transform function is computed for each of the possible grey level values in the image, and is used to map the image captured from the camera (or from disk), into a new image that linearly relates the calibrated values to grey level intensity values. This enables the measurement of mean and total intensity to be converted into MEAN DOSE and TOTAL DOSE values.

Known standard values are numerically entered into a list in the menu. The corresponding intensity values are sampled from the image currently on display, which may typically consist of several intensity calibration areas corresponding to the known standard values. The average intensity of each area is sampled under the control of the operator, and the data is displayed in the second half of the list. Matching pairs of known standard values and measured intensity values are entered and sampled to create the list of points in the transform to which a curve function is fitted, computing the calibrated values for each possible intensity. The resulting transform function is then used to process the image on display and create an image where the calibrated values are linearly proportional to intensity, for accurate calibrated measurements.

File - Name of the file in which the Input List and Grey Level List data and Titles are stored. If this field is blank, the data is not stored. Use the Load and Save buttons to create and retrieve disk files for the calibration standards.

Title - Name of the calibration standard being used.

Units - Calibration units of the calibration standard being used.

Load - Load calibration parameters from a previously saved file. The compatible filenames have the suffix .STD. All information displayed in this form is loaded from the file.

Save - Save the calibration parameters to a file. The filename has the suffix .STD. All information displayed in this form is saved in the file. Use this option to save a file with the calibration standard values normally used, to avoid re-entering the values for each similar experiment.

Intensity Calibration List

- **Input -** List of known calibrated values. Clicking the LEFT or RIGHT mouse button on any cell in the form will pop-up a prompt to enter the calibrated value. Clicking the scroll buttons on the right side of the list will move up or down the list of possible entries.

  In some cases the **Input List** values and title are constant for many experiments where the same calibrated standards are used, and need only be entered once - see the **Save** button below.

- **Gray Level -** The observed intensity of each of the known calibration values in the Input List. In general these values are not known, and have to be measured from the image.

  - **Interactive Grey-level Entry**

    When the Left mouse button is clicked on an entry in the Grey Level List, a Region Of Interest (ROI) form will pop-up to enable a ROI to be created. The ROI should carefully outline the area of the image containing the intensities representing the standard calibrated value being measured. Selecting Okay from the ROI menu will measure the average grey level intensity of the image in that region and put the value in the Grey Level List. Note that as each Grey Level List entry is made, the prior ROI shape is remembered, and the MOVE option can be used to simply drag the ROI to the next standard location. Each value in the Input List should have a corresponding Grey Level List entry.

  - **Numerical Grey-Level Entry**

If the Grey Level List values are known, **right-click** on a cell to enter the value numerically. Clicking the scroll buttons on the right side of the list will move up or down the list of possible entries.

Note: Right-click on the list titles (INPUT and GREYLEVEL) will allow the operator to change these names to more relevant names for display. A **left-click** on the titles will sort the data.

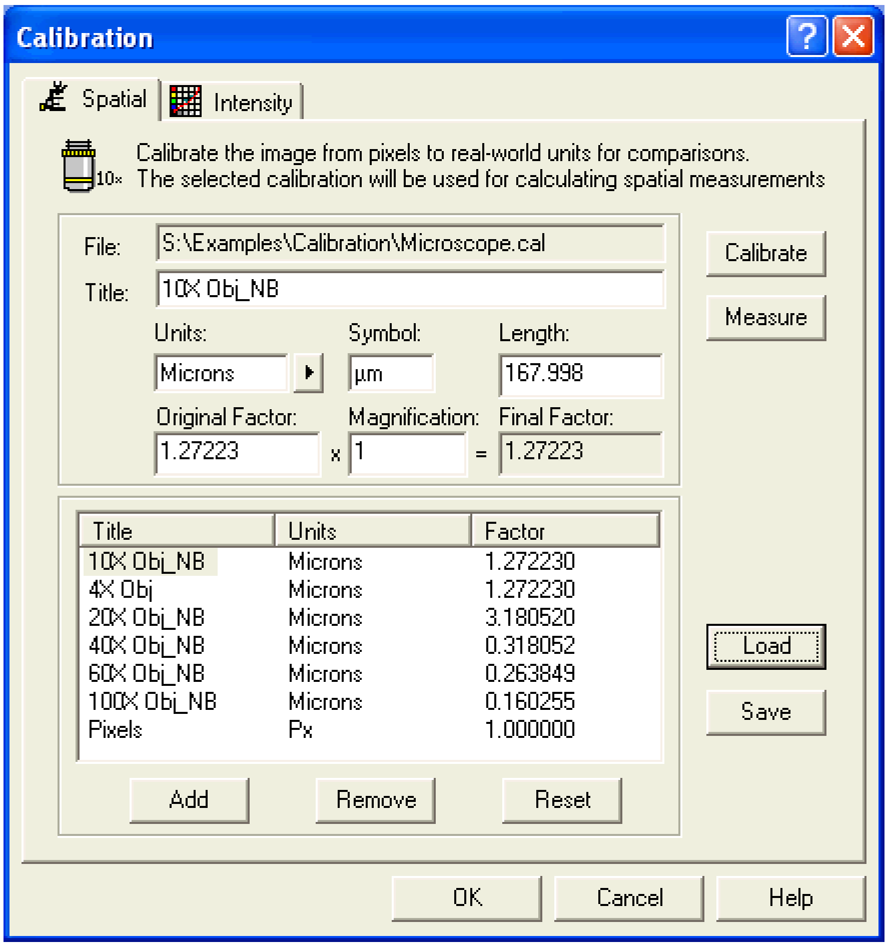
- **Add -** Add a new point to the list. The known standard values are entered in numerically, while the image intensity values are measured using a Region of Interest over an area of the image which contains the intensity from the corresponding standard.
- **Remove -** Remove specific values from the list**.**
- **Reset -** Clear all of the parameters in this List.

Enable Calibration Determines if the Calibration Transform is to be computed and made available for image Calibration in the Enhance Menu. If the checkbox is checked, the currently displayed parameters are remembered for the duration of the application. If the checkbox is not checked, no transform will be available.

Transform Preview

Spatial Calibration Menu

Spatial measurements of Object and Field parameters are made in pixels by default. It is useful to **calibrate** these measurements into more convenient units for real-world comparisons. This option allows the operator to calibrate the image using a microscope reticule or macroscopic scale ruler.



Title- Title of the current calibration. Typically this would be set to indicate the objective magnification, and perhaps an intermediate turret mounted magnifier - "10x obj. + 1.5x Mag". Visible in various data displays to show the current calibration selected as a reminder.

Units- Spatial units of the current calibration. Typically this would be set to some normal spatial units e.g. "Microns", but can be set to any arbitrary scale. Visible in various data displays to show the current calibration units selected as a reminder.

Factor - Scale Factor of the current calibration: the number of units per pixel in both the horizontal and vertical screen directions. Used when measuring spatial parameters, e.g. Length or Area. Visible in various data displays to show the current calibration selected as a reminder. This can be entered directly or computed using the the Calibrate By Pointing button.

> **Note:** The Scale Factor value should be set within the range 0.01 to 100. Other values can be used, but may create too many decimal places for accurate viewing when viewing Area

or Volume measurement. If the value is outside this range, use different units, e.g. Millimeters instead of Microns.

Length - The default size (calibrated in Units) of the Calibration Scalebar created in the Righ-Click Context Menu. A Scalebar of pre-determined size can be saved and recalled easily according to the magnification selected. The value used should create a Scalebar that has enough pixels to be recognizable, and not extend greater than the maximum size of the current selected Capture Device.

Note: This is only the default Scalebar value, and does not restrict the Scalebar sizes that can be created.

Symbol - A two character symbol used in various data displays to show the current calibration selected as a reminder. Typically this would be set to match the Units selected above, e.g. "um" for microns.

Spatial
The Calibration list displays the title and details of the current calibration. Clicking in the lower list will select a different calibration to be displayed for modification.

Any of the entries can be edited by clicking on the slot and typing the new values, notice the blinking cursor, it indicates which slot you are currently editing. The calibration units and calibration symbol are displayed in the status bar and when measurements are displayed. The Calibration Factor can be entered numerically if required, or measured from a known distance in the image using the Calibrate button.It may also be necessary to check the accuracy of the calibration, and an option is given to make linear measurements in calibrated units to verify the calibration factor, the Measure button.

Calibrate

Measure

Calibration File Management
Multiple calibrations can be computed and stored at one time. These calibrations are saved in a file on disk of type ".CAL"; the default name for the file is "CIMAGING.CAL". Any of the different calibrations in the current file can be selected at any time from this form.

Load - The Load button allows a calibration file to be loaded from disk. A list of the available calibration files is displayed. Select one of the files and click OK to load this file into the Spatial Calibration Menu on display. Select Cancel if you change your mind. All Spatial Calibration files have the suffix ".CAL".

A typical use of multiple calibration files is to store calibrations for each objective of a microscope nosepiece, for each zoom factor if there is an optical zoom attachment, and for each capture device used.

The name of the currently selected calibration file is stored in the current profile file at the end of the program, and will be reloaded when the program is executed the next time.

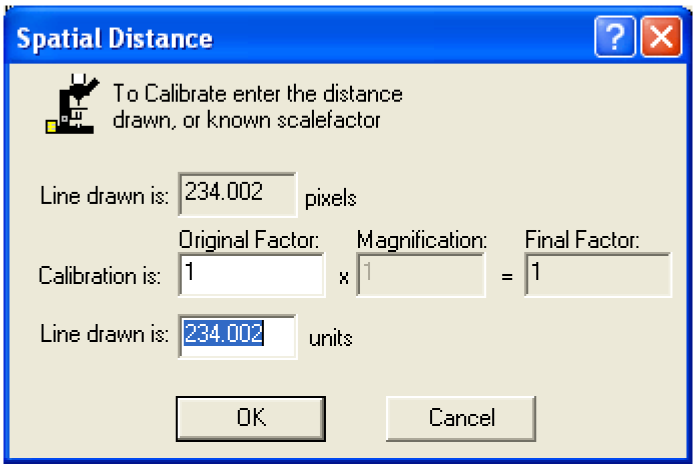
Save - The Save button allows a calibration file to be stored to disk. The File Type should always be ".CAL", which is an ASCII text file and can be viewed with the NOTEPAD utility. These files may be edited, but it is recommended to only change the files using the Spatial Calibration Menu form.

Measure Spatial Distance

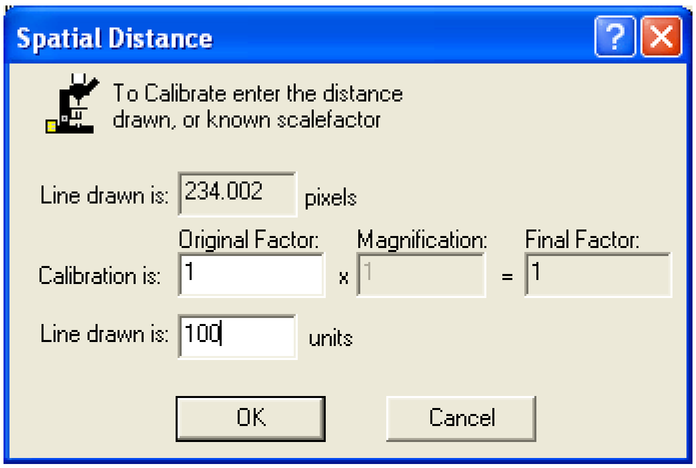
To check the accuracy of the calibration, or to make linear calibrated measurements, use the **Measure** button. Clicking the button will bring the Image Display to the front if hidden. Move the cursor to the first point of the line to be measured, and click, then drag the rubber-band line to the other end of the line and release. The measured distance will be displayed in a dialog.

Spatial Distance Dialog

After drawing a line using the Measure or Calibrate buttons, the Spatial Distance dialog is displayed.

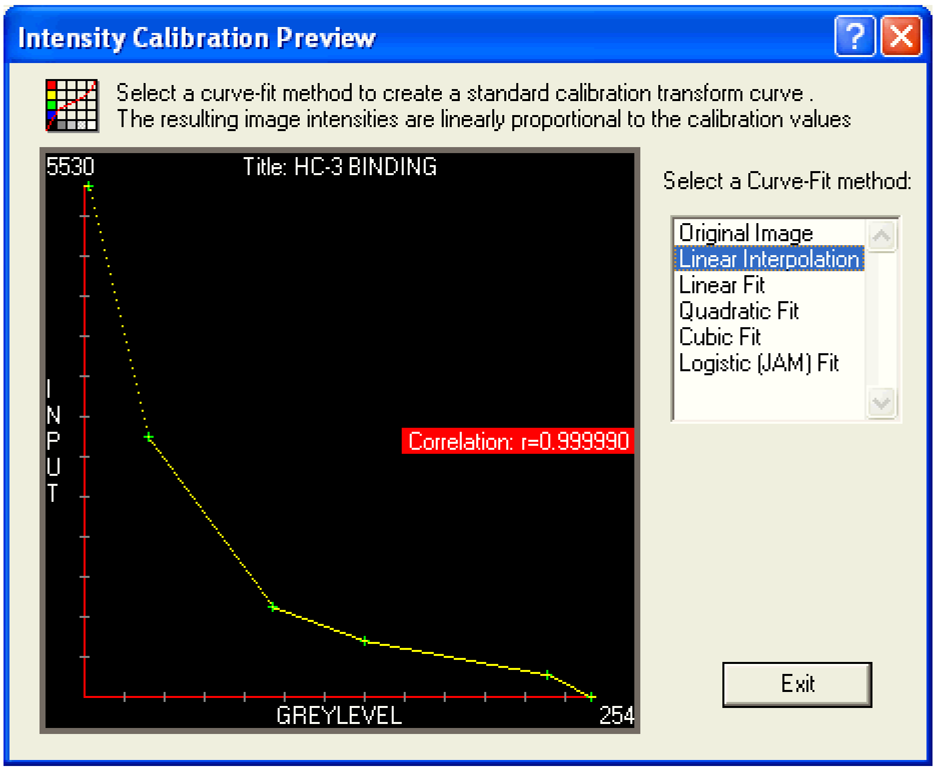


When calibrating, enter the known distance in the "Line drawn is" box. The Calibration Factor will then be calculated and automatically updated on clicking Okay. Click Cancel to ignore this value if the line is inaccurate and needs to be re-drawn.



Intensity Calibration Transform Preview

The **Transform Preview** can be used to select from the available curve-fit methods to be applied to the standard/intensity pairs of values entered in the Intensity Calibration List. Activating the Transform Preview will display a graph showing the fit between the Calibration Standard values on the Y-axis and the measured image intensity values on the X-axis.

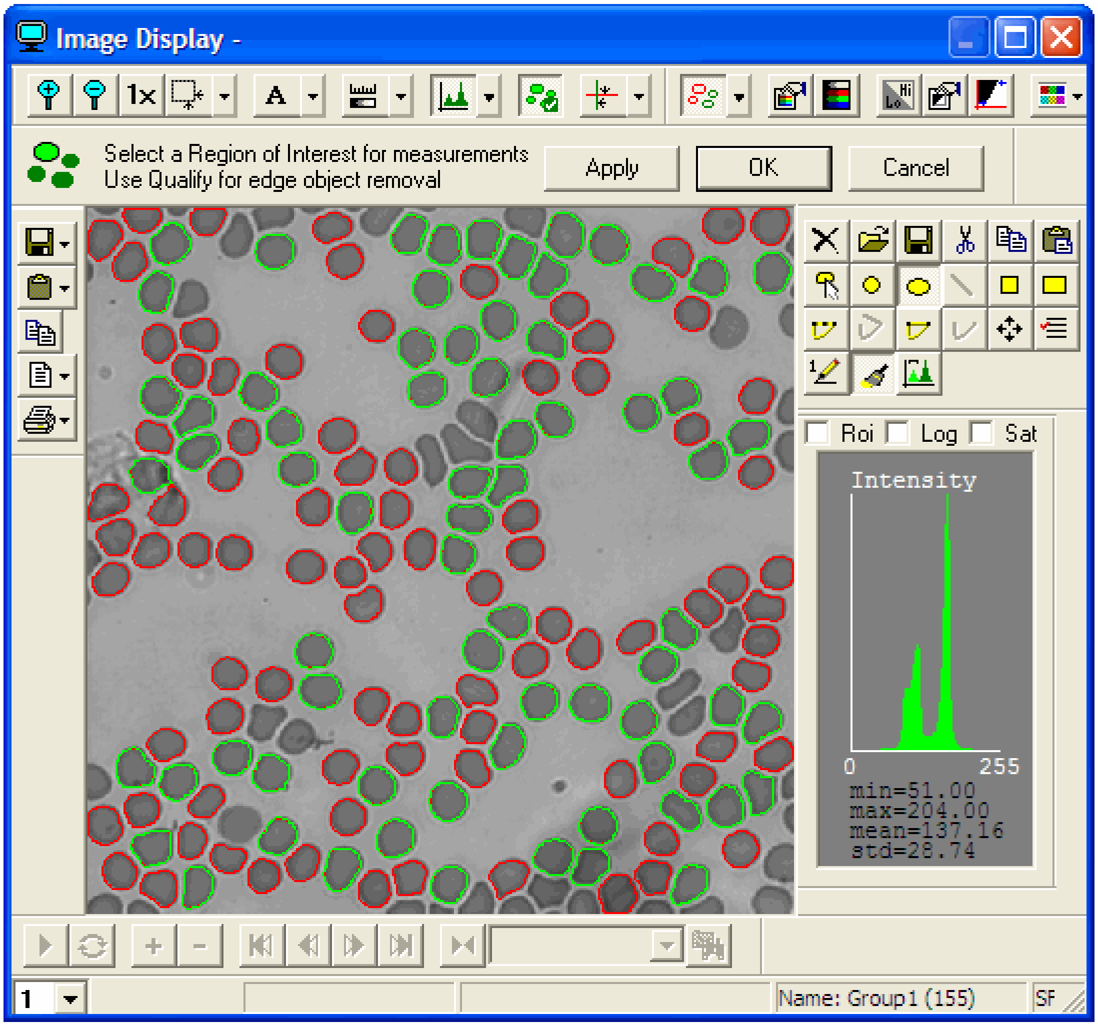


The preview includes a choice of curve-fit methods. Selecting a method will re-draw the graph showing the curve fit and the correlation factor value, and, if imaging is enabled, the image display will temporarily show a preview of the transformed image.

> **Note:** Hovering the mouse cursor over each point will display a tool tip showing its coordinates.

Select **Exit** to return to the Intensity Calibration dialog. The selected curve-fit method is saved in the Density file.

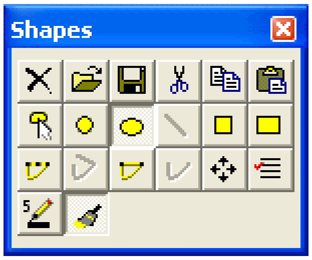
Region Of Interest



In imaging applications it is often necessary to exclude portions of an image from processing and analysis. This can speed execution time and reduce the likelihood of biased results from unwanted objects. This is achieved by using the Region Of Interest, or ROI.

When in the **Capture Dialog**, the ROI is shown in the Image Display overlaid on the current image, as a graphical outline. The ROI can be toggled on or off, and filled using the buttons in the Image Display Toolbar. This ROI Menu contains a variety of shape icons for changing the ROI. The same ROI can be set once and used repeatedly from the Workfile, or the ROI can be adjusted for each field to be processed and analyzed if Capture is used interactively with the Run-Time Menu.

See the Image Editing Toolbar for details on creating, moving, copying, pasting and deleting shapes that make up the ROI.



The Image Editing Toolbar for defining Regions of Interest.

When the ROI button is selected the Shapes Toolbar is visible on the Image Display. Use these controls to create the ROI. If shapes have been created then they can all be deleted by selecting Reset All Shapes from the menu option.

Selecting a ROI other than FULL FRAME, means that when object measurements are taken, only pixels in the ROI will be considered, rather like a "cookie cutter". Objects may be truncated by the edge of the ROI, or by the edge of the image if the ROI is FULL FRAME. The EDGE REMOVAL option can be selected in **Qualify** to remove all objects touching the outline of the ROI after the ROI has been applied, but before object measurements are taken.

The ROI menu is used at several times during the program

- When creating a Measurement Region Of Interest for limiting the image area over which measurements are made in **Qualify** .
- When creating a **Training ROI** to sample the grey or color intensity information of target objects in **Identify** and **Intensity Calibration**.
- The ROI is used for measuring the sample area and other intensity related parameters – see **Field Measurements**

Apply

The current size and coverage of the ROI will be accepted, measured and displayed.

Okay

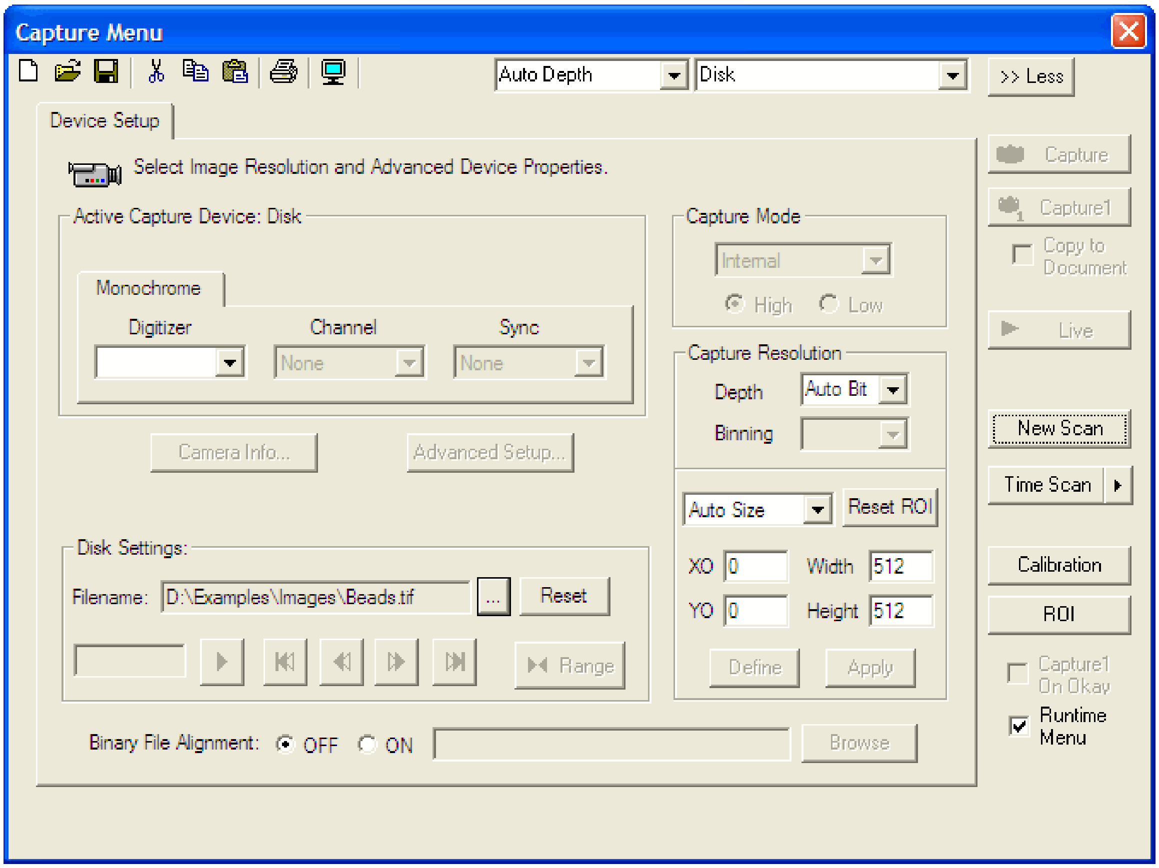
The Okay button will save the current ROI details in the current Workfile, and exit the Dialog.

Cancel

The Cancel button will recall the previous ROI selection from before this form was opened, canceling any changes, and return to the CAPTURE form.

Disk Capture

Images can be loaded from Disk resources on an individual basis using the File Open icon on the Capture Toolbar, or as a sequence of images for Playback on the Image Display.



To analyze a Disk Sequence, use Collect Data and create a Scan List of all images required. See the section on **Scan Disk Sequence** .

To load an image sequence for playback, use the **Browse** button to identify the file name of the first image to load.

The **Playback Toolbar** is enabled when the File opened is a data document or an image document which ends with a number (and therefore a potential disk sequence). When a file sequence has been selected the total number of images in the sequence is automatically detected and displayed, and the first image is loaded. The other buttons in the Disk Capture allow a Range of images from the image sequence to be selectively loaded and displayed on the image display. Use the First or Last button to jump the First or Last image in the current defined range. Use Prev(ious) and Next to increment through the image sequence using the current defined increment. Can also use Playback toolbar in Image Display which includes playback speed controls.

Use the **Reset** button to clear the Disk Image Sequence being used. This may be necessary to avoid opening the same Data Document from two places at once, or if the sequence is reported as being

How to Create a Binary Image Registration Overlay

This option allows a Binary image to be created or loaded from disk as a registration overlay when capturing images. There are three main ways to create the registration image:

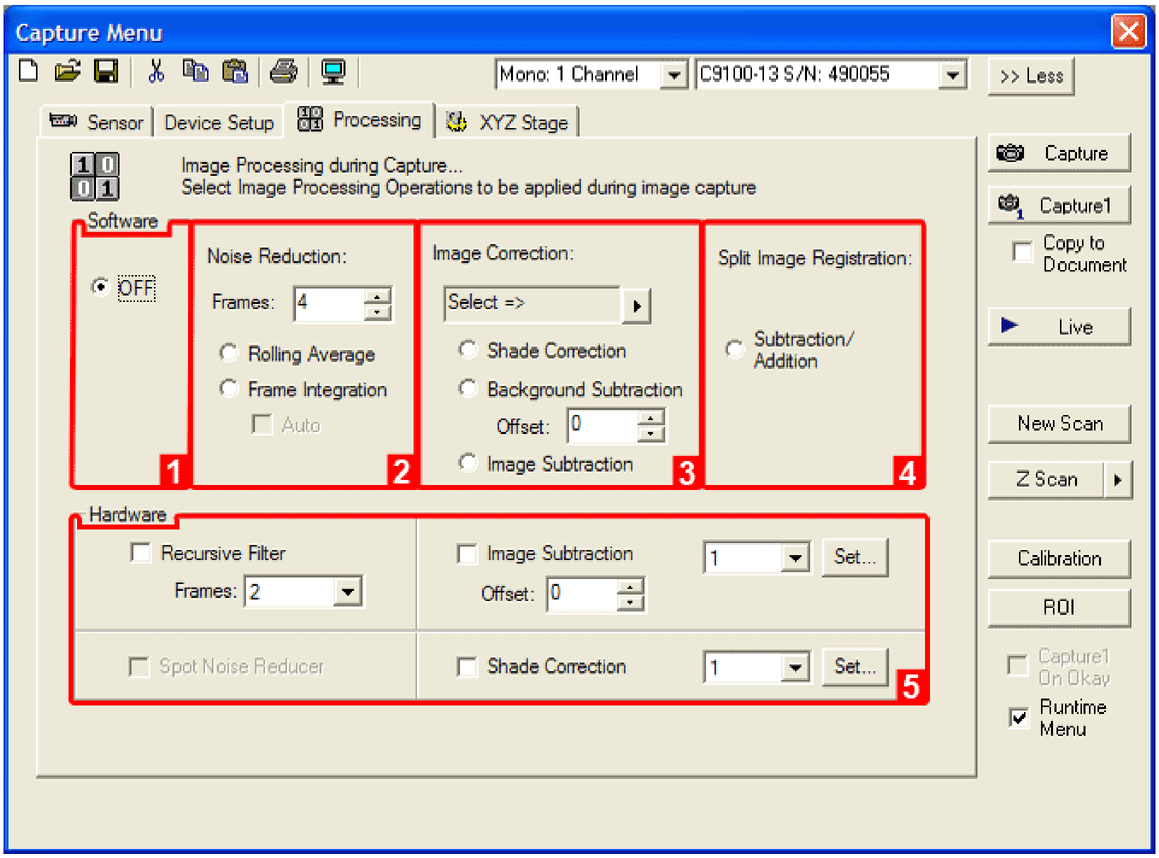
1. Use the **Editor** button to hand draw key outlines and save to a Binary image file on disk. The filename will be displayed and this image will automatically be loaded each time Capture Menu is opened. This file name is stored in the Workfile.
2. Create an Alignment target image in the Modify Menu using the Grid options, where a combination of the Concentric Circles and Cross-hair Grid styles can be used to make a target suitable for centering objects in the field of view.
3. If the registration alignment template is of a complex boundary of one or more objects, an image can be automatically created using a combination of Enhance, Identify and Modify.

1. First, Capture an image of the target objects.
2. Then use an edge enhancement filter in the Enhance Menu, e.g. Sobel or Kirsch to highlight the boundaries of the objects.
3. Threshold the edges into a Binary image in the Identify Menu .
4. In the Modify Menu reduce the edges to thin lines using Skeletonize or Erode operators.
5. Save the Binary image to a file on disk from the Modify Menu with a fixed name, e.g. "Registration.TIF".

The resulting image file is now suitable for selection with Binary Image Alignment Browse button in the Device Setup tab of the Capture Dialog.

Processing Tab

The **Processing** tab provides selections to enhance images *during focus and acquisition* by incorporating image-processing operations during or immediately after image Capture. The operations may be at frame rate for some Capture Devices, or may be applied as a sequential step for others. Some operations require multiple images to be acquired and combined before the result is displayed.



To select an Image Processing operation, first select the **Operation Type** in the left most window, then the second window will display a list of choices for that type. Clicking the Capture or Capture1 buttons will then initiate image capture with the selected image processing operations applied.

The **Operation Type** list allows selection from operations falling into four categories of image processing:

1. **No Processing:** Disable image processing within the capture step.

2. **Noise Reduction:** Remove unwanted random noise from the incoming image**.**
   - Rolling Average

     Rolling (or Temporal) **Averaging** works by first capturing a single live image, and then adding sequential live images to the original image weighted by a fraction of their intensity. The effect of this is to create a rolling average of the image coming from the

Capture Device over Time. Images viewed using Rolling averaging are optimized over a period of time related to the weighting factor. If a large weighting factor (e.g. ¼) is used, each new image has a big impact on the image being accumulated. A small weighting factor (e.g. 1/64) allows images resulting in a very low noise component to be built up over a longer period of time. Typically for low noise images a small weighting factor can be used (e.g. 4), but for extremely noisy images a large weighting factor can be used to good effect (e.g. 30 - 60). If the subject moves during accumulation of the image, it will appear to fade away, and then re-appear at its new location. This makes Rolling Averaging unsuitable for moving objects because the display seems sluggish and blurred.

As a guide to the time required to completely optimize a live image using Rolling Averaging, the weighting factor approximates the number of frames necessary to average, e.g. a weighting factor of 1/64 will require approximately 64 frames to produce a fully averaged image. This may be an under-estimate when moving from a bright object to dark objects, as the image intensity change from weighting a dark object is less than a light object. The best criterion is the visual one, if the noise speckle in the image has been removed sufficiently to make the image look good, then click the Stop button in the Capture Menu. The numerical weighting value can be entered in the adjacent slot in the form.

This continuously updating temporal noise reduction method is also referred to as Kalman filtering.

- Frame Integrate

  Frame Integration a selected number of images are acquired and the accumulated image intensities are displayed. The number of images used may be changed in the box above labeled Frames. This has two effects.

  - The image intensity can be significantly increased, which is useful for very dim images, which cannot be increased by other means.
  - The image noise can be somewhat smoothed as we are combining a number of images each with random noise, that will cancel itself out and produce more of a typical result than a single image would. The integration process only updates the display every N frames, giving a stop-action feel to the display.

  Care should be taken with the number of frames to integrate. Large numbers of frames can take a long time to acquire, especially for cameras that have a slow exposure or data transfer rate. If too many images are integrated, the image itself may saturate at the bright end of the scale, reducing the image data that can be discriminated. In this case, use Auto Integrate first, which will sense the approximate number of frames to use to generate a good image.

  Also note that repeated acquisition may result in image fading for delicate specimens.

  In some cases bright areas of the image may be sacrificed to give good image quality in the darker areas of the image, if this is useful consider capturing an image of more than 8-bits of grey scale resolution. To do this, select a Capture Depth of 16-bits per channel in the Capture Device Setup. The resulting image can have a high dynamic range of greater than 256 intensities for display and measurement. Select the portion of the display to be enhance using the Contrast Dialog.

- Auto Integrate

  Auto Integration is similar to **Frame Integration** in that a number of images are accumulated, but Auto Integrate will automatically stop when a value greater than 255 is detected. The number of frames is reported in the numerical value slot to indicate how many frames were used. If Auto Integrate is selected in Capture, each new image captured will be integrated on the basis of its image content. This may affect the accuracy of thresholding as set in the Identify Menu.

. Rolling Average and Frame Integrate require a Frame Count. You may enter the Frame Count in the edit box under Noise Reduction.

3. **Image Arithmetic:** Remove artifacts from the incoming image**.**

- Shade Correction

  If the illumination source for an image presents a bright non-uniform background, as is typical in bright field microscopy, applying a correction for this shading may be helpful for producing an image better suited for measurements. Using **Shade Correction**, an image of the illumination deformity is stored in memory, and subsequent live images from the Capture Device are corrected by it before the result is stored in the display. If at all possible, correct or improve the illumination first by centering the microscope lamp, or using diffusion filters.

  The **Shade Correction** algorithm calculates a correction image in which each pixel's value is a positive integer which, when added to the corresponding pixel in the shaded image, increases intensity value of darker pixels (presumably more shaded) by a greater amount than the intensities of lighter pixels (which are presumed to have been more strongly illuminated).

  corrected image = (**original image)/(arithmetic image**) * (mean value of all pixels in the **arithmetic image**)

  original image = image needing shade correction

  arithmetic image = the background image (such as that which results from the procedure outlined below) selected in shade correction step.

  > **Note**: For color images, the shade correction is performed separately on each color channel (RGB).

- Subtract Background

  If the illumination source for an image presents a dark non-uniform background, as is typical in fluorescence microscopy, it may be necessary to apply a image background subtraction to produce an image suitable for measurement.

  Using **Background Subtraction**, an image of the illumination deformity is stored in memory. Subsequent live images from the Capture Device are corrected by using simple subtraction, and the result is stored in the display. If at all possible, correct or improve the illumination first by centering the microscope lamp, or using diffusion filters.

  Note: This is different than Image Subtraction which scales the subtracted intensities between +127 to -127 into the range of 0 to 255, indicating loss or gain of intensity.

- Subtract Image

  If it is necessary to measure the difference between two images, from two different time intervals, or an experiment versus a control, we may need to generate an image showing intensity loss or gain. Using **Image Subtraction**, an image of the first instant is stored in memory, and subsequent live images from the Capture Device are subtracted from it before the result is stored in the display. This technique can be used for full grey scale image alignment between images.

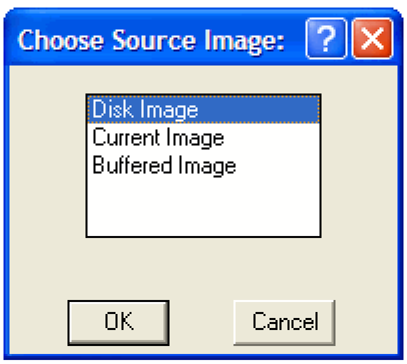
  When subtracting two images with grey levels from 0 to 255, we can arrive at grey levels of -255 to +255. In **Image Subtraction** These grey levels are scaled into the resulting image by scaling grey level -255 equal to 0 (Black) and grey level +255 equal to +255 (white), thus grey level 127, is equal to mid grey, and reflects no change between the two images.

Note this is different than Background Subtraction which does a simple subtraction of the background intensities from the captured image then truncates negative values to zero.

In order to process the incoming live image with Image Subtraction, it may be necessary to miss some frames, and so the number of frames per second (fps) of the live image may be reduced. Use this option only if live Image Subtraction is necessary. If it is possible to subtract the images later, capture one image using live, and SAVE it, then capture the second image using the Capture button, and exit the CAPTURE Menu. The Saved image and the current image on display are available in the Enhance Menu, where they may be subtracted also.

For Image Arithmetic, you must first choose a source image. You can choose between selecting a Disk Image or using the Current Image in the display. If the Current Image is chosen, it becomes the Buffered Image.

To change the source image, click on the arrow  under the Image Arithmetic List.



How to create an arithmetic source image and perform standard Shade Correction

1. Select **No Processing** in the Image Processing Tab
2. Click **Capture** to get a Live image
3. Click **Stop** to store the image of the background of the sample normally from a position on a blank part of a microscope slide, or from a flat white sheet of paper, or a light box in macroscopic cases.
4. Click **Save** to store the image in memory
5. Select **Image Arithmetic** in the Image Processing Operations
6. Select **Shade Correction** in the Image Arithmetic list
7. Select **Saved Image** as the image source.
8. Click **Capture** to get a live image with Shade Correction applied.

How to collect an arithmetic source image and perform Background Subtraction:

1. Select **No Processing** in the Image Processing Tab
2. Click **Capture** to get a Live image

3. Click **Stop** to store the image of the background of the sample normally from a position on a blank part of a microscope slide.
4. Click **Save** to store the image in memory
5. Select **Image Arithmetic** in the Image Processing Operations
6. Select **Background Subtraction** in the Image Arithmetic list
7. Select **Saved Image** as the image source.
8. Click **Capture** to get a live image with Background Subtraction applied.

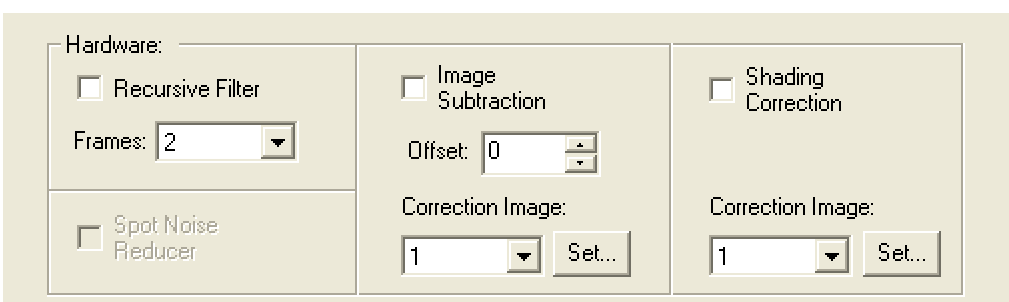
How to collect an arithmetic source image and perform Image Subtraction

1. Select **No Processing** in the Image Processing Tab
2. Click **Capture** to get a Live image
3. Click **Stop** to store the image of the background of the sample normally from a position on a blank part of a microscope slide, or from a flat white sheet of paper, or a light box in macroscopic cases.
4. Click **Save** to store the image in memory
5. Select **Image Arithmetic** in the Image Processing Operations
6. Select **Image Subtraction** in the Image Arithmetic list
7. Select **Saved Image** as the image source.
8. Click **Capture** to get a live image with Image Subtraction applied.

See also: Enhance Menu (for applying Image Processing operations after the image has been captured)

4. **Split Image Registration** is used in conjunction with the Optical Insights Dual View. The processed image in the display is split in two parts A & B. The left side displays (A - B) and the right side displays (A + B).

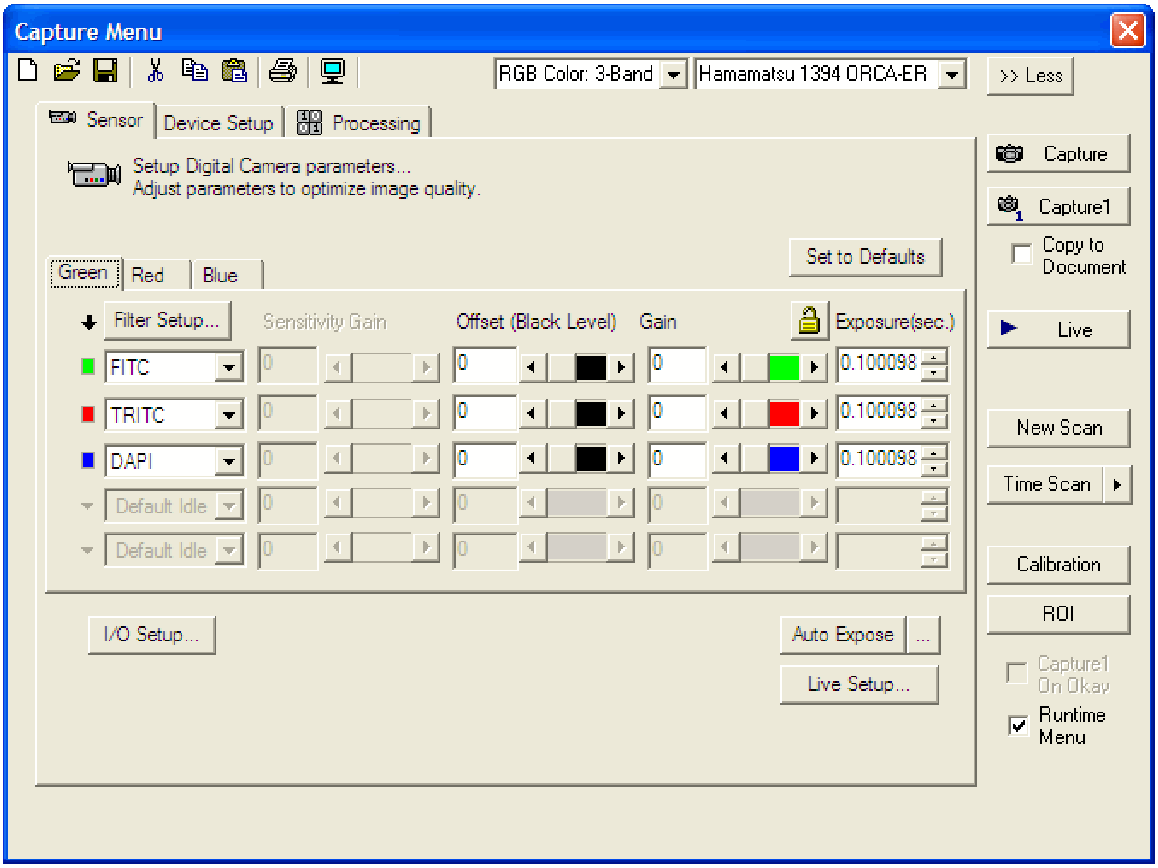
5. 5. **Hardware Processing:** Some cameras also support hardware processing. The Hamamatsu 9100-13 is shown below.



Hardware Processing may be done while **Live**.

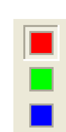
The Camera Sensor Tab

The appearance of this Sensor tab-page may differ slightly depending on the features reported by the selected Capture Device.



- **Set to Defaults -** Sets all camera settings to camera defaults**.**
- **Filter Setup**
- **Filter Selection -** Choose a defined filter/shutter position for the current channel from a drop-down list. *SimplePCI* will move to the designated position prior to channel capture.
- **Destination Band -** When capturing a 2 or 3-Band color image, it is possible to choose the Destination Band. You may choose to capture your image in Green-Blue-Red order rather than the Standard Red-Green-Blue order.

  1. Click the colored button next to the Filter Selection List. 

  2. Choose your Destination Band from the drop-down menu. 

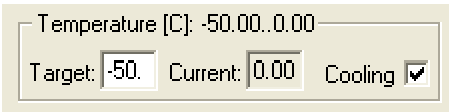
     Note: It is possible to choose a destination band more than once. However, the second image will overwrite the first.

- **I/O Setup**

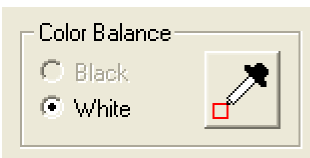
- **Lock Exposures -** Locks current exposure ratio. These ratios will be maintained as any single exposure is changed.
- **Auto Exposure**
- **Live Setup**

In this example, the camera supports Offset, Gain and Exposure Changes.

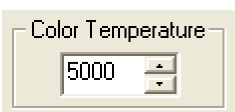
Other Sensor controls not shown in this example:



- **Cooling:** Cooling hardware
- **Target Input:** Temperature controller
- **Current Temp Readout:** Built-in temperature sensor



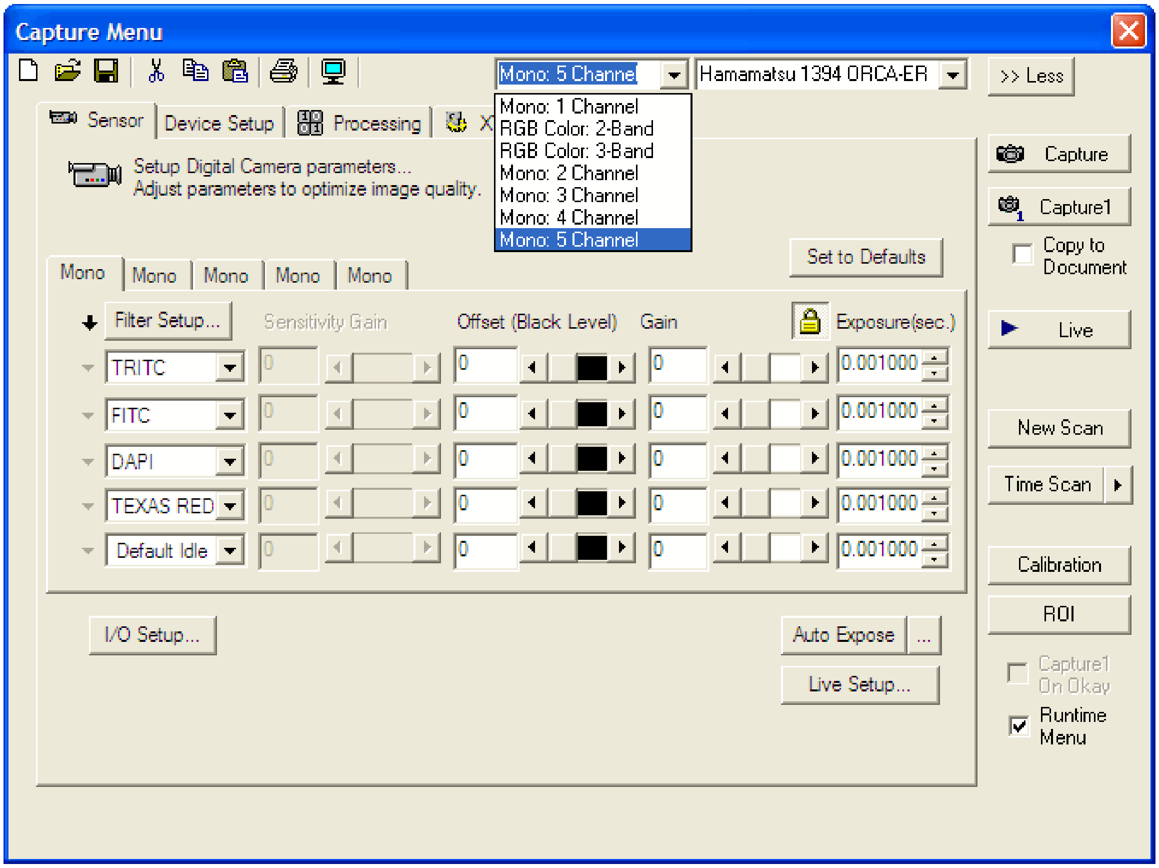
- **Manual region selection-based color correction**: White-balance color correction (Bayer cameras) - Selecting the dropper icon will allow selection of a region in the Image Display which ought to appear white; Black-balance color-correction - As above, except a region which ought to appear black is selected.



- **Color Temp-based color compensation:** Camera equipped with color-temperature compensation, (Hamamatsu 37780, Hamamatsu SX900) Entry of the color temperature of the light incident to the camera will compensate the output to capture objects in their true colors (as they ought to appear given ideal lighting conditions).

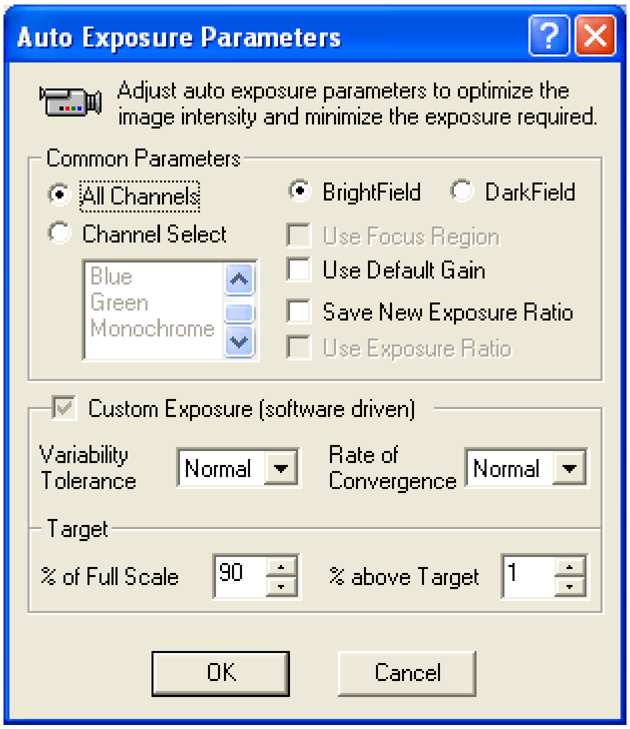
Define Capture Images

User may capture up to 5 monochrome images or a single color image (2 or 3 band).



Auto Exposure

The **Auto Exposure** dialog allows customization of the parameters with which to automatically optimize the image intensities. An iterative process of Adjustment, Capture and Analysis is used to determine the Exposure settings that optimize the dynamic range of intensities in the image. A number of controls in the dialog can be selected to customize this process. Note this process can also be applied using **Auto Digitizer Offsets** for analog cameras.



After the Auto Exposure process has been applied it may still be necessary to use Capture or Capture1 to acquire an image using the computed exposures.

- **Channel Selection:** Auto Exposure can be applied to one or more channels in a color image.
- Bright Field: Well-illuminated transmitted light, typically dark objects on a white background.

  Use this setting for images where the exposures are expected to be short, and where the bright background values outnumber the darker areas in the image.

- Dark Field: Fluorescence or Luminescence illumination, typically bright objects on a dark background.

  Use this setting for images where the exposures are expected to be long, and where the dark background values outnumber the brighter areas in the image. The main purpose of this setting is to reduce the number of iterations, which causes the process to take longer and perhaps increase photo bleaching of the sample.

  Note: For samples using fluorescence illumination, repeated exposure can cause a decrease in the intensity, which causes a longer exposure and so on. Do not use auto exposure on samples that fade quickly. Try setting the exposure on an area of the image which is discardable, then moving to a good area for image capture.

- Use Focus Region

  If this setting is selected the image is only monitored in the Focus Region. Some capture devices will restrict the image acquisition to the Focus Region, decreasing the read-out overhead. This can also be used to restrict the region of interest to avoid an unwanted bright feature in the field of view. See the Focus Setup section for how to select the Focus Region.

- **Use Default Gain** Set camera gain to default
- **Save New Exposure Ratio**
- **Use Exposure Ratio**
- Custom Exposure Parameters

  Some Capture devices have a built-in auto-exposure mode, which is used when the Custom Auto Exposure is not enabled. For devices which do not have a built-in Auto Exposure method, Custom Exposure Parameters is always enabled.

  Image Variability Tolerance

  This setting is used to predict the amount of variation between images with the same exposure. In cases where the image has a wider variability, the Auto Exposure will not try to be as precise. For stable images use the Normal or Close setting.

  Convergence Rate

  This setting controls the number of iterations used to determine the exposure. The slower the convergence value, the more images will be captured and tested.

  Target – Percent of Full Scale

  This value determines the threshold intensity value for good exposure. A higher value will require a longer exposure, and may require more iteration to meet the requirement.

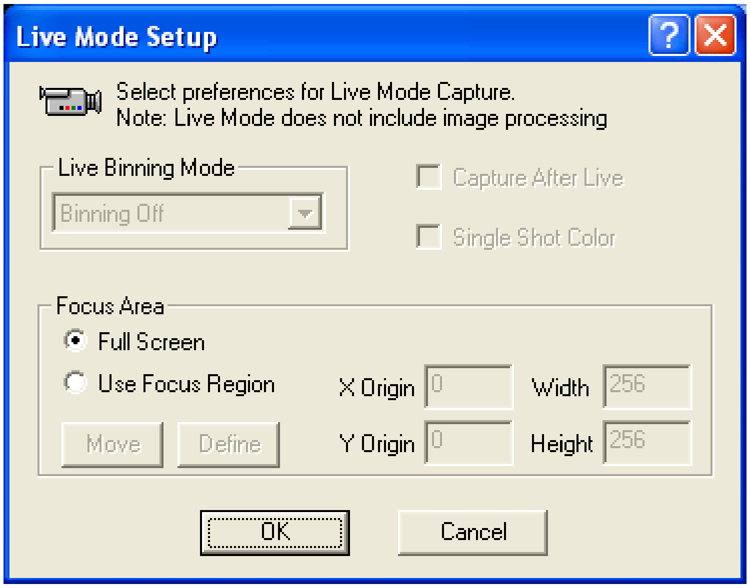
  Percent above Target

  This value determines how much of the image data must be above the Target Percentage. A higher value will require a longer exposure.

Clicking **OK** will activate the exposure process.

Live Setup

The **Live** Mode Setup Dialog allows control of image acquisition when in Live Mode. See the **Live** button in the Capture Dialog.



Live mode is used to align and focus an image without applying processing and other operations which would slow down the rate of capture, making it harder to find the optimum plane of focus. You may adjust the image quality and focus area while in Live Mode.

Adjust Live Mode Image Quality by selecting a **Live Binning Mode:**

- **Binning Off -** No additional binning is applied. The image is acquired at full resolution within the Focus Region. Exposure is taken from the Capture Dialog.
- **Binning Faster -** Increase current binning by a factor of 2 during Live Mode. Images will have one fourth the spatial resolution. Zoom is adjusted so image size remains the same. Exposure is reduced so that brightness will remain approximately the same.

Note: Availability based on current binning and camera capability.

Capture After Live - Capture an image into the Image Display following Live Mode. This option will activate the Capture1 cycle after pressing Stop when in Live Mode, to acquire a full image with processing operations applied.

Single Shot Color - Select for three-color Live Mode instead of monochrome. Applicable to color-capable cameras only.

Choose your Focus Area:

1. Select **Full Screen** to focus using the entire image capture area.
2. Select **Use Focus Region** and do one of the following:

- Enter your region values:

  X Origin Horizontal coordinate of the left edge of the focus region

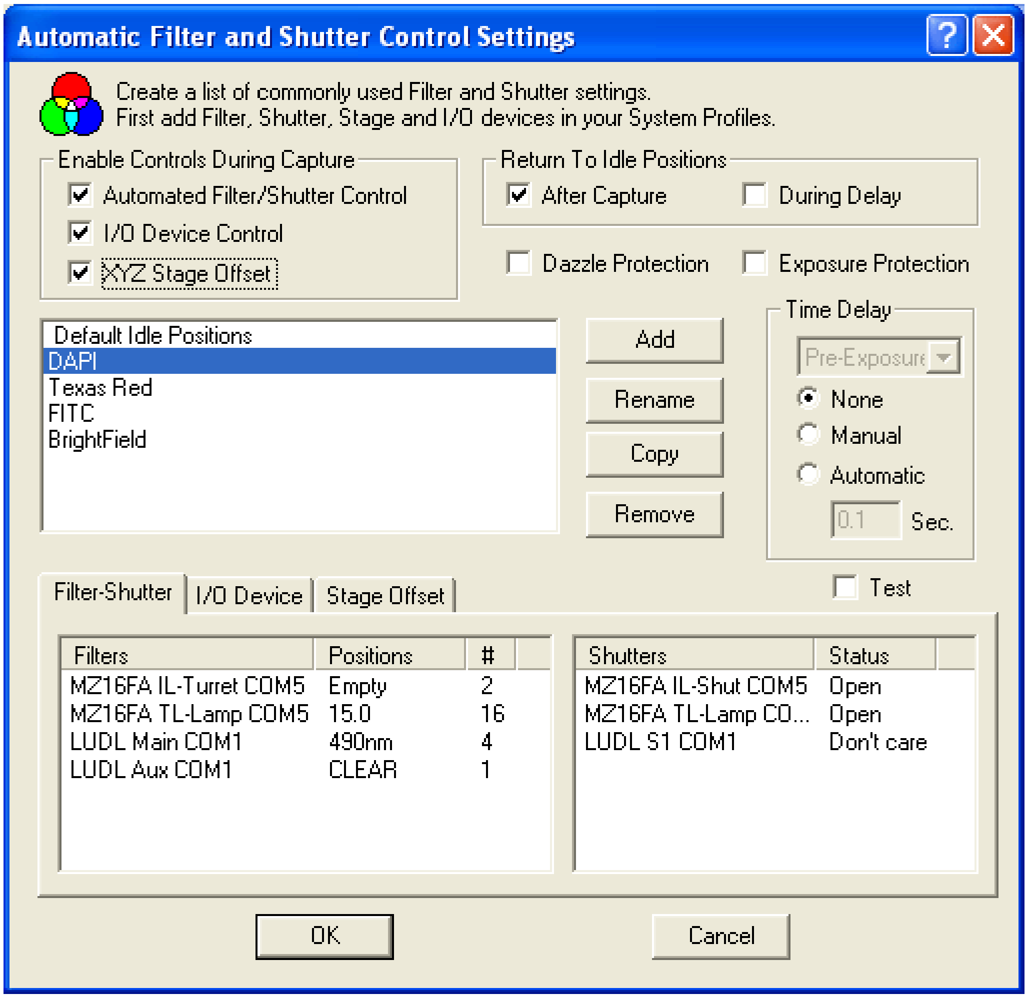
  Y Origin Vertical coordinate of the top of the focus region

Width Horizontal width of the focus region

Height Vertical height of the focus region

- Click **Define**, then Click and Drag the mouse in the Image Display to create a new region.
- Click **Move**, then Click and Drag the mouse in the Image Display to modify an existing region.

Automatic Filter and Shutter Control



The **Automate Filter and Shutter Control Setup** dialog allows customization of a list of positions for each of the computer-controlled Filters and Shutters installed in the Current Profile. Filter and Shutter devices can be added to the current profile using the Device Setup tab of Current Profile's properties (accessed through the main window's **File>>Current Profile...** menu command).

A list of Filter/Shutter combinations is maintained in the Profile along with some parameters relating to each device when positioning for image capture. The customized Filter/Shutter combinations are available in each of the Sensor tabs to select the illumination conditions for the Sensor when capturing each image component.

The **Filter and Shutter Control Setup** dialog includes:

- Enable Controls During Capture
    - **Automated Filter/Shutter Control** Enable/Disable all automatic-during-capture controls
    - **I/O Device Control** Enable/Disable automatic I/O device control during capture
    - **XYZ Stage Offset** Enable/Disable automatic stage device control during capture

Note: I/O Device and Stage Offset tabs will be hidden when de-selected

Note: Even while automatic filter/shutter control is de-selected, it is still be possible to select the various added filter/shutter groups in various capture dialogs. It may be worthwhile to configure filter/shutter group settings information even in a case where the devices used practically cannot be used with automatic filter and shutter control due to the lack of electronic controls.

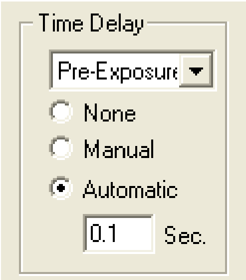
- Return to Idle Positions
  - **After Capture** Filter/shutter controller applies the Default Idle Positions group after each image capture
  - **During Delay** Filter/shutter controller applies the **Default Idle Positions** group during each configured delay

  Return to Idle Positions options can be useful for manual viewing of a specific combination through the microscope eyepieces, or to prevent over-exposing a light-sensitive sample.

- Dazzle Protection
  The shutters will return to the defined default idle state during filter move. This help to protect the sample from an undesired wavelength.

- Exposure Protection
  The shutters will return to the defined default idle state at the end of camera exposure during camera readout. This help to protect the sample from unnecessary light.

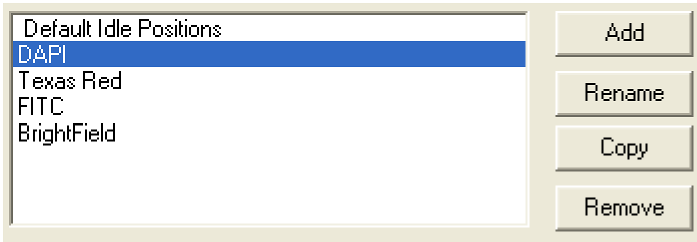
- Filter/Shutter Delay



  For each filter setting, a programmable time delay can be inserted at one of three possible times in the image capture sequence:
  - **Pre-Exposure** Delay is inserted after the shutter opens but before the exposure is made. A common use of this delay is to allow time for the shutter to fully open.
  - **Pre-Shutter** Delay is inserted after the filter command is sent but before the shutter is opened. A common use of this delay to allow time for the filter to reach its intended position.
  - **Pre-Filter** Delay is inserted before the filter device command is sent. To allow a sample to be exposed at a different wavelength

  The delay can be **Manual**, in which case a dialog will pop-up requiring interaction before completing the capture, or **Automatic**, in which case a delay is specified in seconds to wait before completing the image capture.

- Filter/Shutter List



The **Filter/Shutter List** manages groups of Filter/Shutter settings, i.e. sets of configurations listing what state each of the installed Filter and Shutter devices needs to be placed in to obtain the particular lighting conditions necessary for the desired image.

Default Idle Positions is a group of filter/shutter settings defining the default positions for each of the installed Filter and Shutter devices. This group can be utilized during delays, between captures, etc. depending on the state of options such as **Return to Idle Positions**.

- **Add** Prompts for a name to define a new group of filter/shutter settings. All values will be initialized to Don't Care.
- **Rename** Prompts for a new name for the selected group.
- **Copy** Prompts for a name for a new group of settings initialized identically to the currently selected group.
- **Remove** Removes the selected group from the list.

Note: Configure newly added groups in the Filter and Shutter selection boxes.

Selecting an item in the list will display the current position settings for the installed devices in the Filter and Shutter display boxes, where they can be modified.

- Filter Selections

Filters	Positions	#
MZ16FA IL-Turret COM5	Empty	2
MZ16FA TL-Lamp COM5	15.0	16
LUDL Main COM1	490nm	4
LUDL Aux COM1	CLEAR	1

The Selections for each of the installed Filter Devices are displayed in the Filter Selection box of the Filter Setup Menu. Each Installed Filter Device is listed in the left column of the box. Clicking on the Wavelength of any of the Filter Devices will pop-up a list of installed filters. Selecting the required filter will show the Wavelength and Position in the list. New filters are installed in the Current Profile.

Select the **Don't Care** position when no movement is desired, i.e. leave the filter in the previous state.

- Shutter Selections

Shutters	Status
LUDL S1 COM1	Closed
SUTTER Shutter-A C...	Open
SUTTER Shutter-B C...	FIXED: Open

The Selections for each of the installed Shutter Devices are displayed in the Shutter Selection box of the Filter Setup Menu. Each Installed Shutter Device is listed in the left column of the box. Clicking on the **Status** of any of the Shutter Devices will pop-up a list of shutter positions.

The positions available are defined by the way the Shutters were installed in the Current Profile. Each shutter is installed with one of four conditions:

- **None**: Not installed. Not controlled.
- **Active**: Software controlled. User selects position.
- **FIXED: Open**: Software controlled. Shutter is fixed in the Open position
- **FIXED: Closed**: Software controlled. Shutter is fixed in the Closed position

Only those shutters installed as **Active** can have their position selected. Clicking on the Status of an Active shutter will toggle the status between **Open, Closed** and **Don't Care.** Select the **Don't Care** position when no movement is desired, i.e. leave the shutter in the previous state. The shutter will remain open if it was already open and remain closed if it was already closed.

Note: For some devices that do not have a physical shutter, it may be possible to use the **Default Idle Position** in the List as a shutter by blanking the filter position or driving to a inactive wavelength and using the **Return to Idle Positions after Capture** option.

Note: See Timing Diagram for Exposure/Dazzle Protection details.

I/O controlled Filters and Shutters can configured in a similar manner in the I/O Device Tab.

Filters	Positions	#
LPT1 Output	00010100	20

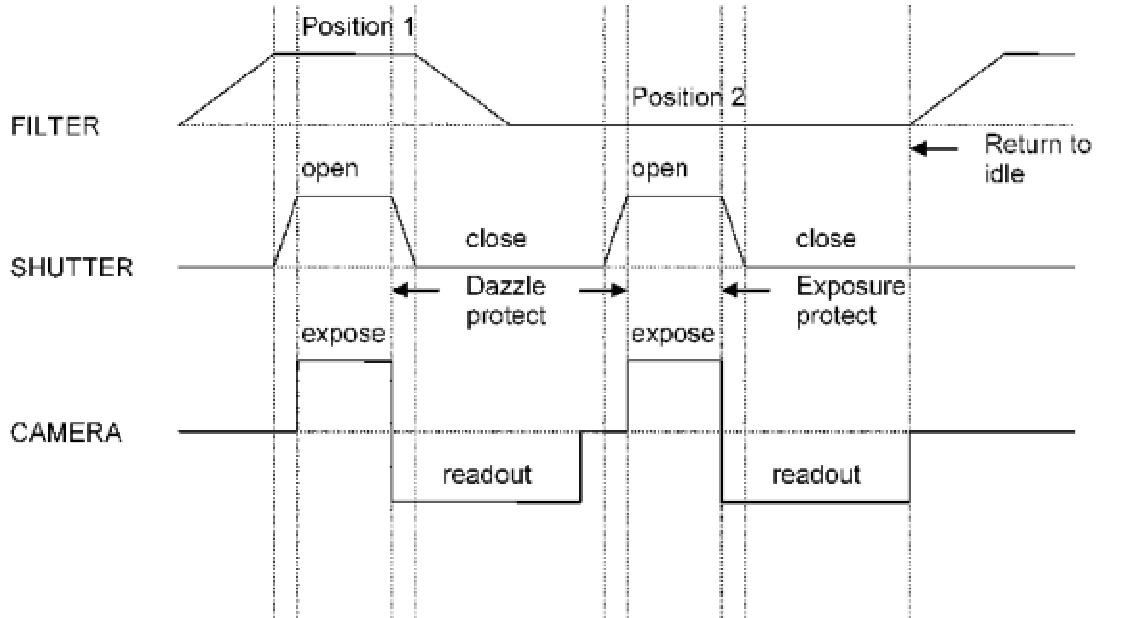
Shutters	Status
LPT1 P2-	Low
LPT1 P3-	High
LPT1 P4-	Don't care
LPT1 P5-	Don't care
LPT1 P6-	Don't care
LPT1 P7-	Don't care
LPT1 P8-	Don't care

Note: Check the **Test** button to provide immediate feedback of filter/shutter positions. Filters and shutters will move accordingly as each position is clicked.

Select the Stage Offset Tab to define an X, Y, Z offset for each filter/shutter position.

Timing Diagrams

Capture 1 with Exposure and Dazzle Protection



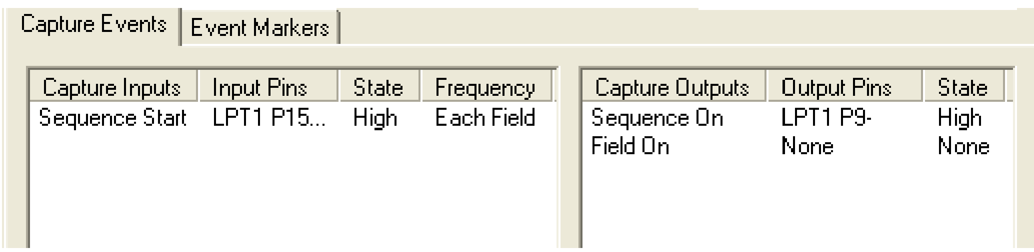
I/O Setup

Under **Enable I/O during Capture**, check **Capture Trigger/Signal** to setup capture triggers and output signals.

You may choose from a list of the installed pins by clicking in the cells under the pin heading. The signal level of the pin can be changed by clicking the cell under the State heading. Each click will toggle the state between High and Low.

For Capture Inputs, click under frequency to choose when to trigger. Each click will cycle through the list of choices: **Each Field**, **First Field**, and **Each Pass**. **Each Field** waits for an input trigger at the beginning of every capture cycle, including Capture and Capture1. **First Field** and **Each Pass** are Capture Sequence specific. First Field waits for an input trigger at the start of the sequence. **Each Pass** waits for an input trigger before the first field of each sequence pass.

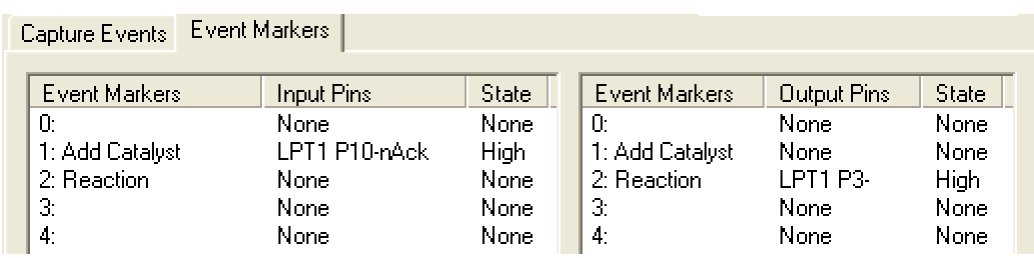
For Capture Outputs, **Sequence On** will set the chosen state of the pin at the beginning of the capture. This pin will remain signalled until the sequence has completed. **Field On** will set the chosen state of the pin at the start of a field's exposure. This pin will signal on and off as each field is captured.



To Trigger each capture with the CTRL-key.

1. Under **Enable I/O during Capture**, check **Capture Trigger/Signal** .
2. Click under **Input Pins** to key-press. The **State** will automatically be set to CTRL.
3. Click under **Frequency** until **Each Field** appears.

Under **Enable I/O during Capture**, check **Event Marker** to setup user-defined event inputs and outputs.

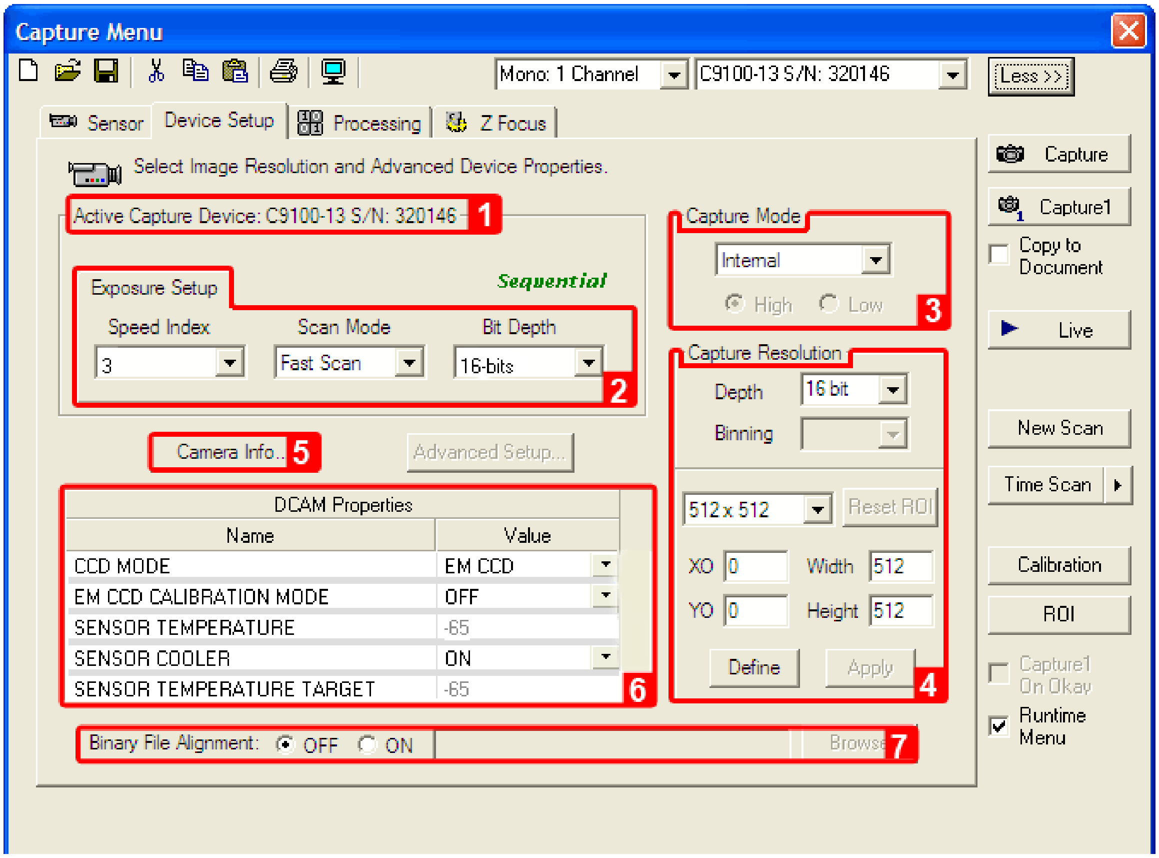


You can customize event names by clicking the Event number and pressing the F2 key.

You may choose from a list of the installed pins by clicking in the cells under the pin heading. The signal level of the pin can be changed by clicking the cell under the State heading. Each click will toggle the state between High and Low.

As you capture a sequence, event markers will be added to your data document when input pins are signalled. If you manually add an Event marker, all appropriate output pins will be signalled.

Camera Device Setup Tab



1. Active Capture Device: Displays current Capture Device. To change the Active Capture Device select from a list of available cameras in the drop-down list.

2. Exposure Setup:Speed Index - Select readout speed for cameras with selectable readout speeds, e.g. Hamamatsu 9100-13 has Speed 1 = 0.69 MHz, Speed 2 = 2.75 MHz, Speed 3 = 11 MHz.

Scan Mode - Select digitizer to use for cameras with multiple digitizers, e.g. Hamamatsu Orca II has a High Speed Digitizer or a High Precision Digitizer.

Bit Depth - Change the format of data from the camera, 8 bit or 16 bit. Use 16 bit data format for 10, 12, 14, and 16 bit digital CCD cameras.

3. Capture Mode: External Triggering can be set to Edge or Level. The trigger polarity can be set to High or Low.

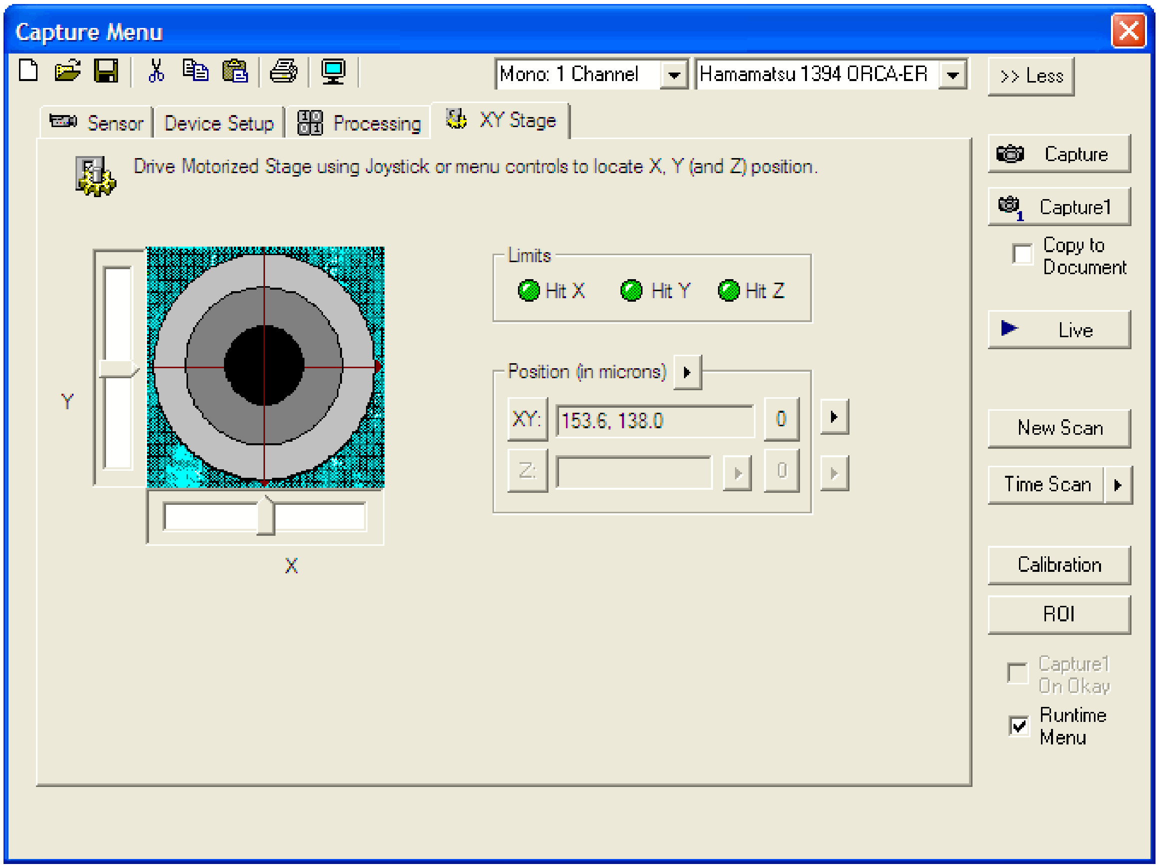
4. **Capture Resolution:** Allows the user to adjust Capture Depth (8 bit or 16 bit) as well as Binning and Resolution (as determined by the camera).

5. Camera Info: Provides camera details including model and driver version.

6. DCAM Properties: Lists all of the properties associated with the DCAM* supported Active Capture Device. Some of the properties have drop-down menus which allow the user to control specific aspects of the functionality of the camera. *This functionality is only available with DCAM supported Hamamatsu cameras. Not all Hamamatsu cameras will display properties in this window.

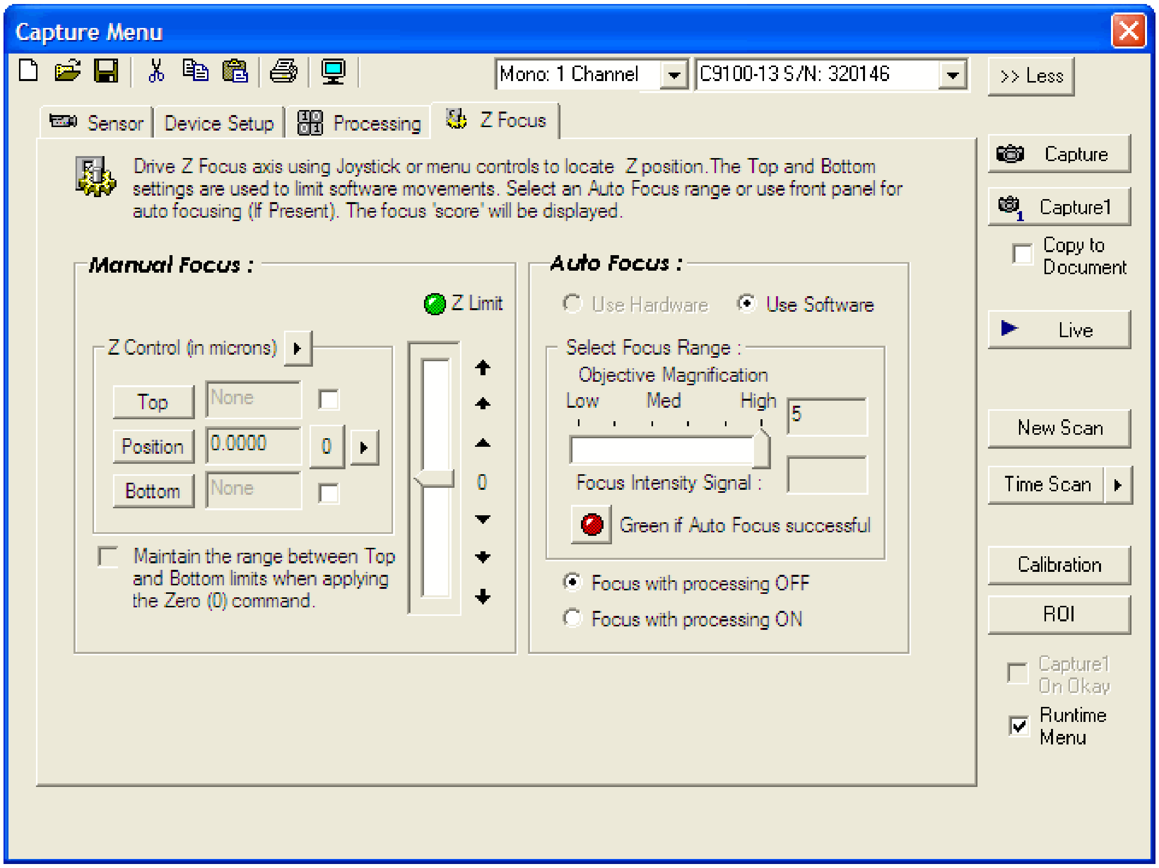
7. **Binary File Alignment:** A Binary image is loaded from disk as a registration overlay when capturing images.

XY Stage Tab

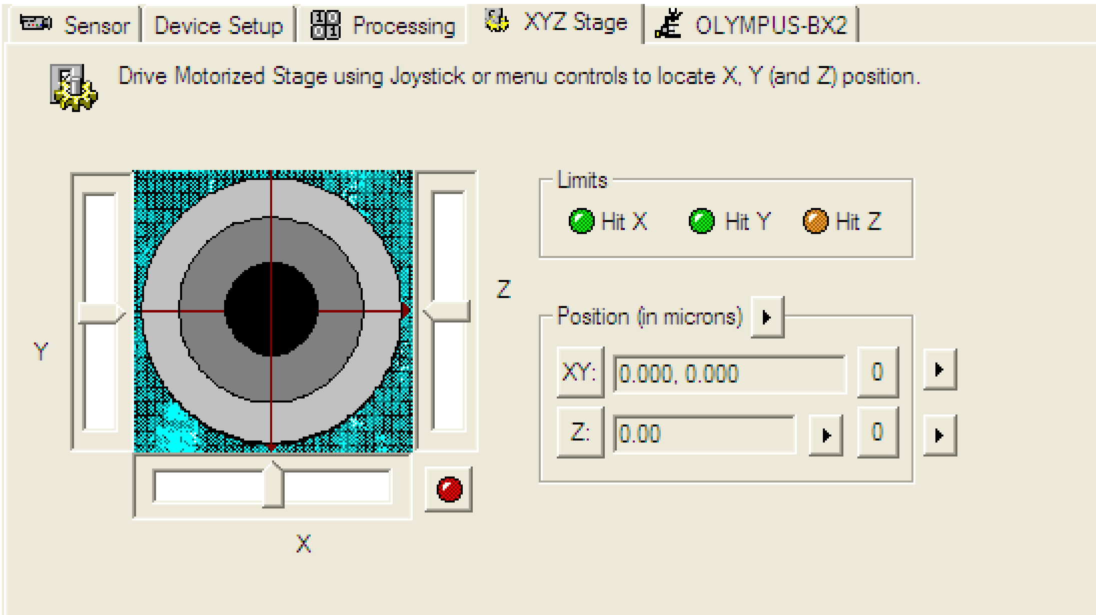


Capture Menu
Mono: 1 Channel
Hamamatsu 1394 ORCA-ER
>> Less
Sensor
Device Setup
Processing
XY Stage
Drive Motorized Stage using Joystick or menu controls to locate X, Y (and Z) position.
Limits
Hit X
Hit Y
Hit Z
Position (in microns)
XY:
153.6, 138.0
0
Z:
0
Y
X
Capture
Capture1
Copy to Document
Live
New Scan
Time Scan
Calibration
ROI
Capture1 On Okay
Runtime Menu

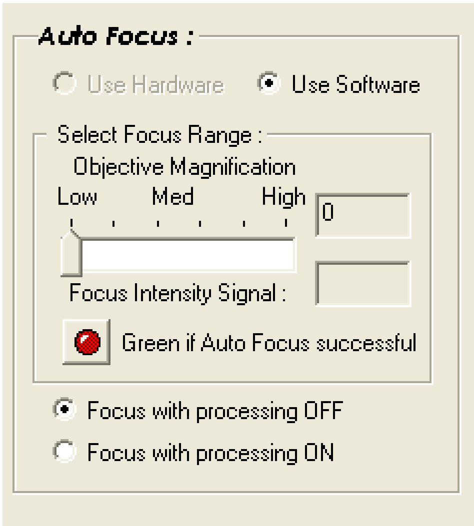
Z Focus Tab


Capture Menu
Mono: 1 Channel
C9100-13 S/N: 320146
>> Less
Sensor
Device Setup
Processing
Z Focus
Drive Z Focus axis using Joystick or menu controls to locate Z position. The Top and Bottom settings are used to limit software movements. Select an Auto Focus range or use front panel for auto focusing (If Present). The focus 'score' will be displayed.
Manual Focus :
Z Limit
Z Control (in microns)
Top
None
Position
0.0000
0
Bottom
None
Maintain the range between Top and Bottom limits when applying the Zero (0) command.
Auto Focus :
Use Hardware
Use Software
Select Focus Range :
Objective Magnification
Low
Med
High
5
Focus Intensity Signal :
Green if Auto Focus successful
Focus with processing OFF
Focus with processing ON
Capture
Capture1
Copy to Document
Live
New Scan
Time Scan
Calibration
ROI
Capture1 On Okay
Runtime Menu

XYZ Stage Tab



Auto Focus Controls



The Auto Focus Controls activate the hardware or software focus functions supported by the installed Z Axis device controllers. If the Auto Focus hardware is detected as installed in the controller the Auto Focus controls are activated. If Auto Focus hardware is not installed, this option is grey. Software Auto Focus is available in both cases. Check the controller documentation to ensure that the Auto Focus hardware is correctly configured.

For Hardware Auto Focus the video signal from a standard video camera viewing the microscope field of view is fed through the Auto Focus controller where the image contrast is measured, and an algorithm is applied moving the Z Axis through a pattern of steps to compute the best focus point.

In the Software Auto Focus case images are captured from any device, including analog or digital cameras, and processed through the focus range to determine the optimum position using a mixture of contrast and edge based information across the whole field of view.

Focus devices are installed in the Device Setup Menu of the Current Profile .

Focus Range

The Focus Range setting controls the size of the steps that the controller makes between focus samples. Typically the Focus range is smaller for higher magnifications. Check the controller documentation for combinations of Focus Range and magnification, or try the different ranges on actual samples.

The Focus range is adjusted using the slider control. Or the hardware controller front panel controls can be used as a manual override if the Use Front Panel Checkbox is checked.

Auto Focus Button

The Auto Focus Button is highlighted if Auto Focus is available. Clicking the button will activate the Auto Focus, during which time the current focus position will be updated.

Focus Intensity Signal

The final score of an Auto Focus operation will be reported in the Focus Intensity Signal box. These values have relative information that is highly sample dependant. A higher value generally means better focus, but in some cases where the depth of field is large, varying values may not provide a visible difference in the focus quality.

Window on Screen (Hardware only)

The Focus Intensity Signal is determined in a rectangular region of the video window. See the controller manual for details on the adjustment of the window position and size. The Focus Window can be combined in the display output of the controller as an indication of which portion of the field of view is actually being considered for the Auto Focus operation.

Note if the video signal is fed from the Camera to the Auto Focus Controller and looped through as the video input to the digitizer, the Window will be visible on the image display. Check this option off to avoid measuring the window as an object!

An additional video monitor can be useful if the video signal is fed from the Camera to the digitizer, and additionally fed to a second video monitor. This enables the live video image to be seen at all times.

Manual Focus Controls

Manual Focus Control is available using software or by using the manual override controls e.g. Joystick or focus controller knob, to allow positioning of the Z axis. Focus devices are installed in the Device Setup Menu of the Current Profile .

Z Position Slider

The Z Position Slider allows the Z position to be changed by clicking and dragging the slider handle. The movement of the slider is relative, not absolute, so dragging the slider further up or down the scale will produce a faster movement in that direction. Note that it is possible to focus up and down, faster and slower without releasing the slider.

Top Focus Limit

The Top Limit can be set to prevent the Focus position from being incremented past a certain value. This is not the case when using the manual Joystick control that will override the limit and force the limit to a new position. Checking the Top limit will assign the current position to the limit, or the position can be numerically entered.

The Top Focus Limit is also used when computing a Z-Axis Scan.

Current Position

The Current Position is constantly read from the controller and displays the current location of the Z-Axis. This number can also be input numerically to instruct the controller to drive to a specific position.

Zero Position

The Zero Position button provides a choice of two functions:

1) Move to the existing Zero coordinate.

2) Set the current coordinate to be the Zero position.

Maintain the range between top and bottom limits when applying the Zero command.

If this checkbox is checked when the Zero current position selection is made, the range between the top and bottom limits will be maintained.

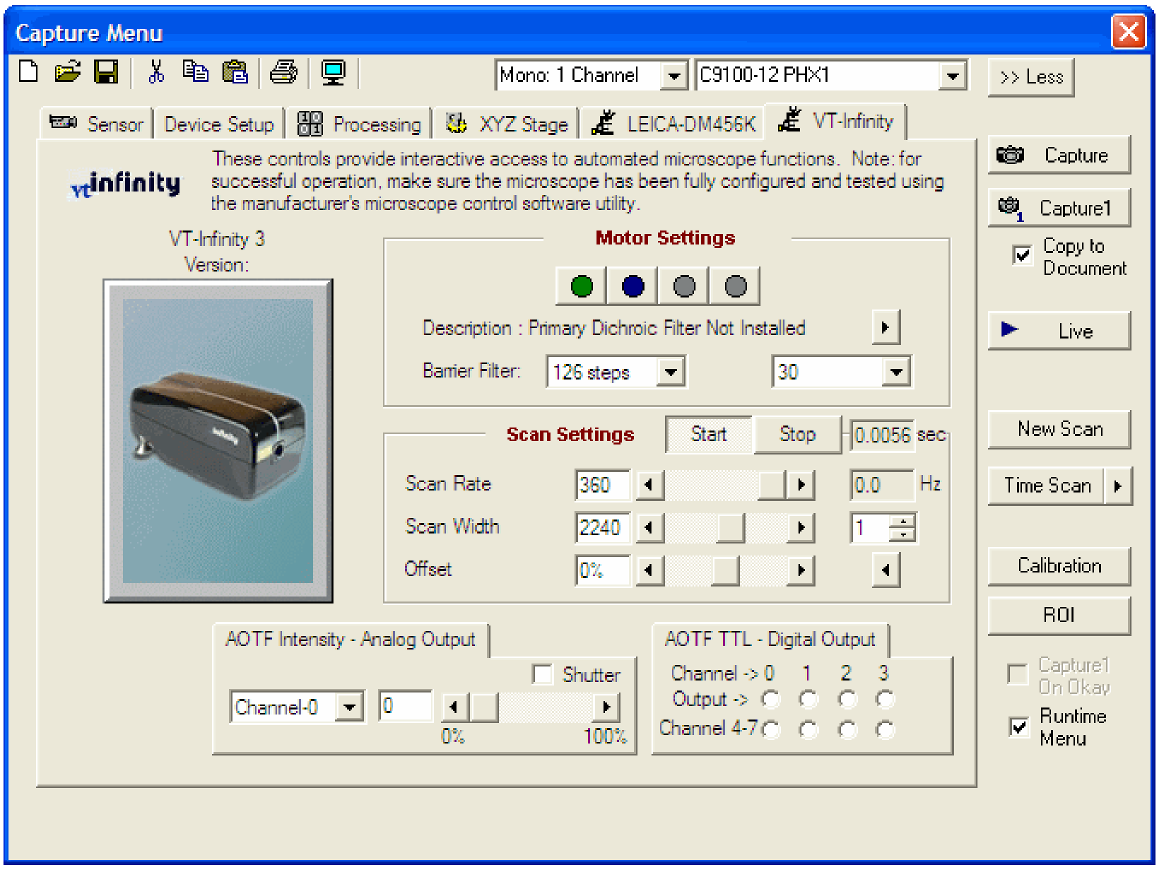
Bottom Focus Limit

The Bottom Limit can be set to prevent the Focus position from being incremented past a certain value. This is not the case when using the manual Joystick control that will override the limit and force the limit to a new position. Checking the Bottom limit will assign the current position to the limit, or the position can be numerically entered.

The Bottom Focus Limit is also used when computing a Z Axis Scan.

Microscope Control Tab

The **Microscope Control Tab** allows control of the automatic and motorized functionality present in the most advanced integrated microscopes. The Microscope Control Tab allows specific control of these functions (for Z position use the Z Focus Tab).



Each device to be controlled can be added and configured in the Current Profile. For convenience, all devices can be added at one time.

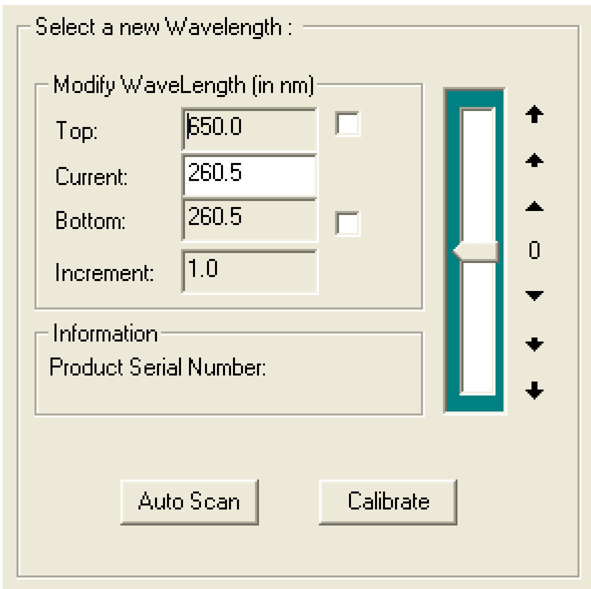
More than one device can be installed and used at once, allowing simultaneous control of excitation and emission wavelengths. Also by controlling Filter and Shutter devices and Capture device settings (Exposure, gain etc.,), it is possible to completely change the image acquisition illumination environment for successive images. Combinations of Filter and Shutter positions are configured and selected in the Filter Setup Menu, accessed from each Sensor Tab as described in the Capture Dialog .

The Microscope pictured here is the Visitech Infinity which provides a variety of automated controls.

Refer to the manufacturer’s Microscope Control Manual for details on the setup and operation of these devices.

Monochromator Control Tab

This page is available in the Capture configuration tab-control when the **T.I.L.L. Photonics** or **ASI Monochromators** are installed in the current profile. It is also available in an enhanced form for the **CAIRN** Monochromator Opto-scan. It provides accurate and convenient control for selecting illumination wavelengths using these devices.



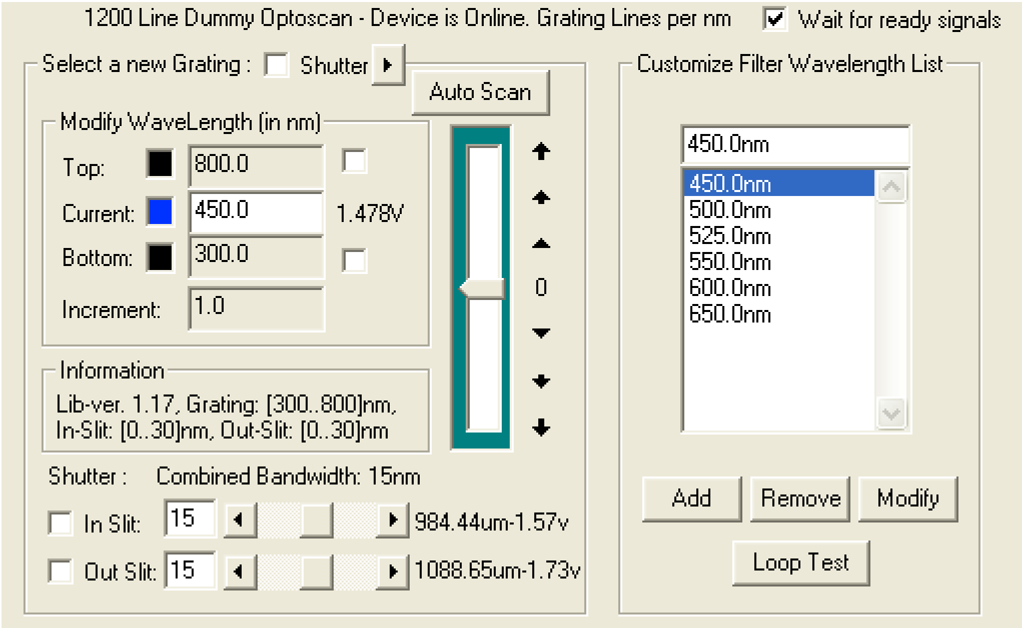
The **Select a new Wavelength** portion of the tab-page has a **Top** and **Bottom** wavelength limit, for which the maximum and minimum limits are factory set and read from the controller hardware. These limits can be overridden to limit the range of wavelengths for a scan. To override top or bottom wavelength limits, check the box to the right of the limit.

The **Current** position is displayed and can be entered as a target. The Current position can be controlled using the slider. Note the slider control is a relative control, not absolute, so clicking and dragging the slider will continue to change the wavelength, and dragging further from the center will increase the step size of the change. The control voltage corresponding to the current position will be displayed to the immediate right of this box.

The **AutoScan** button will continuously scan the wavelength through the spectrum range defined by Top and Bottom. This can be especially useful when trying to determine the best wavelength to use to highlight a particular color stain. The Scan can be done while a live image is on display. Stop the scan when the contrast is optimal to discriminate the target material from the background. Note that clicking on the slider can change the increment of the scan for finer or coarser control.

The **Calibrate** button is used to initiate a wavelength calibration procedure for ensuring the accuracy of the peak light output with reference to physical filters of known wavelength.

The **CAIRN** Monochromator control menu offers a slightly different user-interface to control its setup:



Monochromator Calibration

The Calibration procedure for this device uses two filters of known band pass to identify the computer controlled location of the maximum light output near each end of the illumination range. When these two positions are known, the Calibration command will re-compute the internal controller parameters necessary to match the computer requested wavelength to the actual light output of the Monochromator. If this procedure is not followed, the actual light output may vary from the requested values.

See also the manufacturer's manual for more details on setting up the optical and electrical settings for a Calibration procedure.

Note: The following procedures cannot be undone, if the values are not correct, the Calibration must be accurately repeated in order to get good computer controller values.

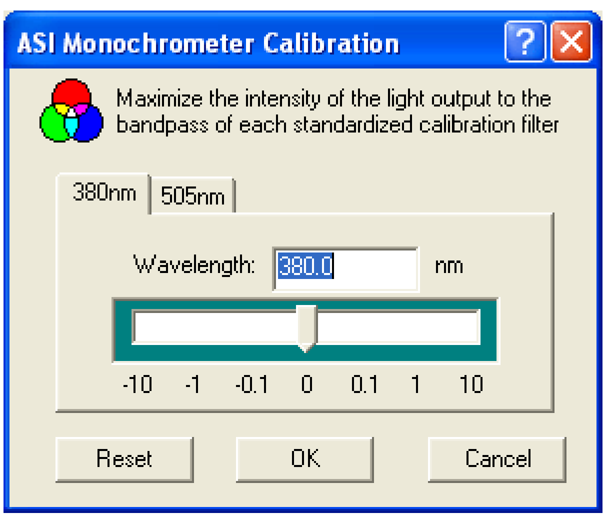
Monochromator Calibration (ASI)

The software steps required to calibrate an ASI monochromator are as follows:

1) Set the 380 nm **position.**

The calibration menu has two Tabs, click on the first Tab, labeled 380 nm, to send the Monochromator to the estimated position for a 380 nm output. With the 380 nm filter installed in the light path (as detailed in the hardware manual), vary the position using the slider on the Tab until the output as measured by the voltmeter is maximized.

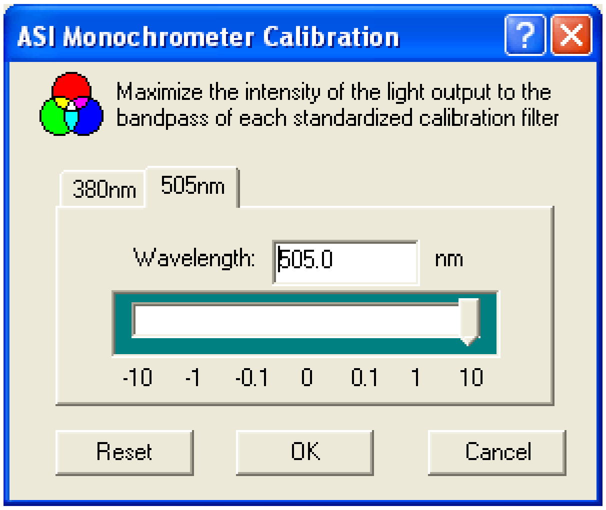
This represents the position of greatest output for 380 nm as defined by the optical filter installed.



2) Set the 505 nm position.

Click On the second Tab, labeled 505 nm, to send the Monochromator to the estimated position for a 505 nm output. With the 505 nm filter installed in the light path (as detailed in the hardware manual), vary the position using the slider on the Tab until the output as measured by the voltmeter is maximized.

This represents the position of greatest output for 505 nm as defined by the optical filter installed.



3) Click on the OK button.

This will accept the two positions and apply the command to internally calibrate the controller. Cancel to abort the calibration procedure and leave the controller calibration unchanged.

The **Reset** button will reset the calibration to the factory default setting and apply the calibration command.

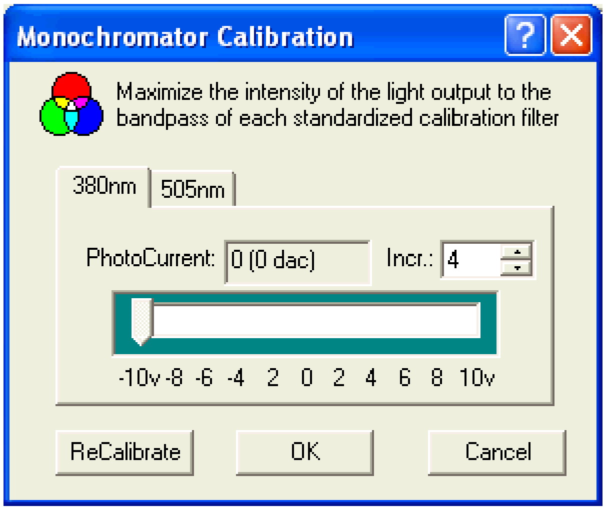
Monochromator Calibration Procedure (T.I.L.L.)

The software steps required to calibrate a **T.I.L.L** monochromator are as follows:

1) Set the 380 nm **position.**

The calibration menu has two Tabs, click on the first Tab, labeled 380 nm, to send the Monochromator to the estimated position for a 380 nm output. With the 380 nm filter installed in the light path (as detailed in the hardware manual), vary the position using the slider on the Tab until the output as measured by the voltmeter is maximized.

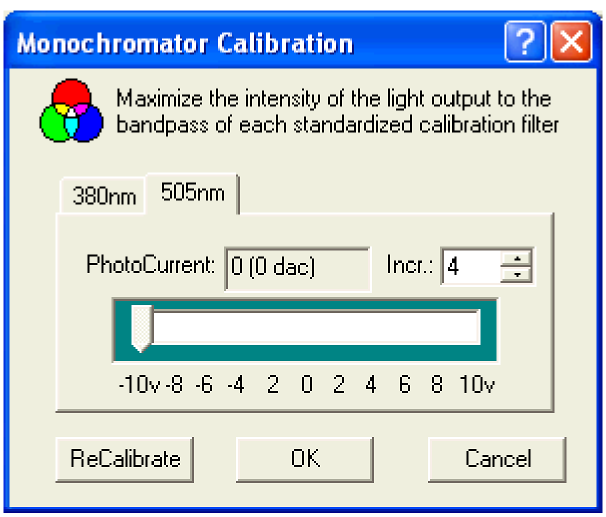
This represents the position of greatest output for 380 nm as defined by the optical filter installed.



2) Set the 505 nm position.

Click On the second Tab, labeled 505 nm, to send the Monochromator to the estimated position for a 505 nm output. With the 505 nm filter installed in the light path (as detailed in the hardware manual), vary the position using the slider on the Tab until the output as measured by the voltmeter is maximized.

This represents the position of greatest output for 505 nm as defined by the optical filter installed.



3) Click on the OK button.

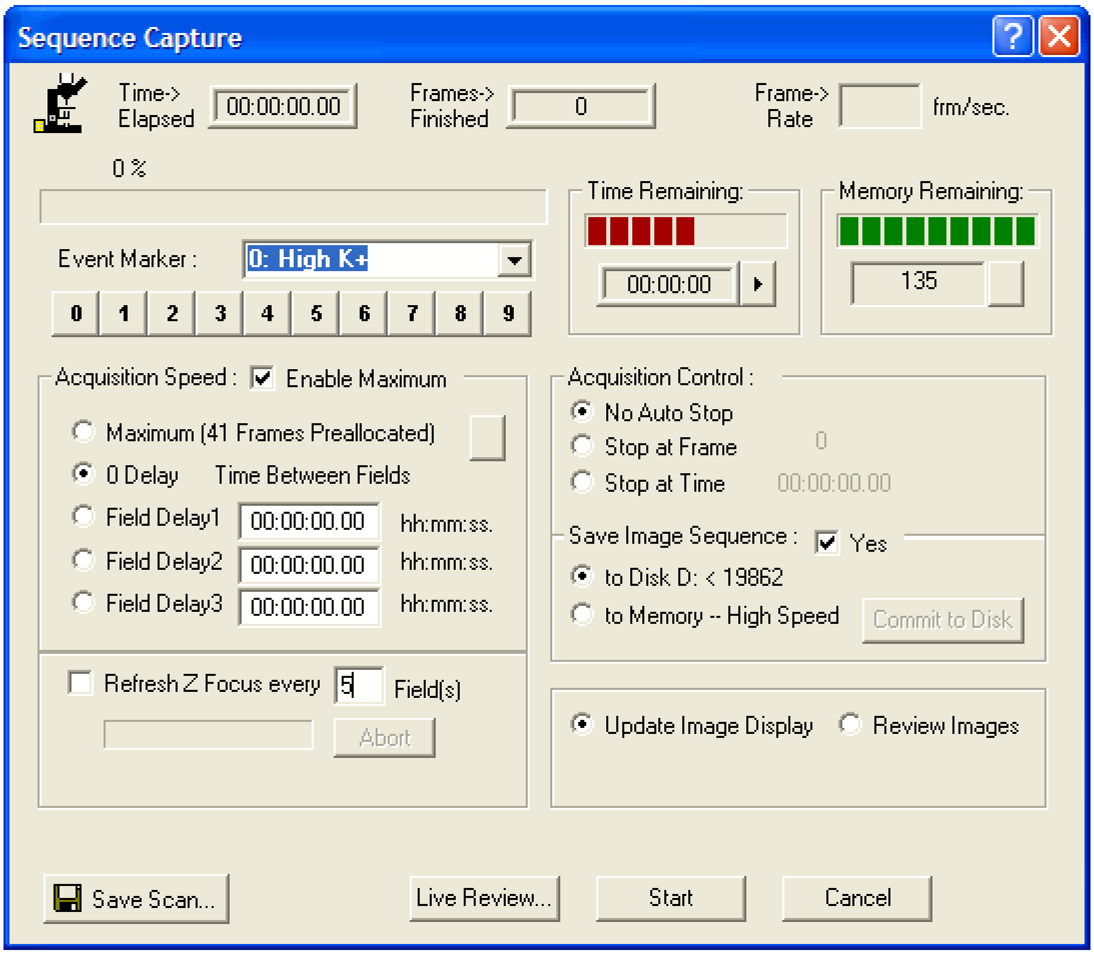
This will accept the two positions and apply the command to internally calibrate the controller. Cancel to abort the calibration procedure and leave the controller calibration unchanged.

The **Reset** button will reset the calibration to the factory default setting and apply the calibration command.

Capture Sequence

A Data Document containing a sequence of images can be created using the **Sequence** button in the **Capture Dialog.** The Sequence button will lead you through the **Scan Wizard** for specifying the type of sequence - X, Y scan, Time series etc., and the parameters of the scan – time delays, file output options etc.

When Scan Wizard for sequence capture is finished, select the data Document file for storing the images. The current Capture Dialog settings will be used for the image capture, including any image processing and Filter changing.



Event Markers can be added to the data document file during acquisition as notable events occur in the experiment. Both the numbered buttons above and keyboard numbers may be used.

Time Remaining is a reference to delay time. The right arrow button may be clicked to exit the delay.

Acquisition Speed: Must check the Enable Maximum box for Maximum Speed to be an option. During maximum speed, items which slow down acquisition will be ignored (Z-focusing, exposure protection..) Several delays can be entered to allow for fast time switching during experiments.

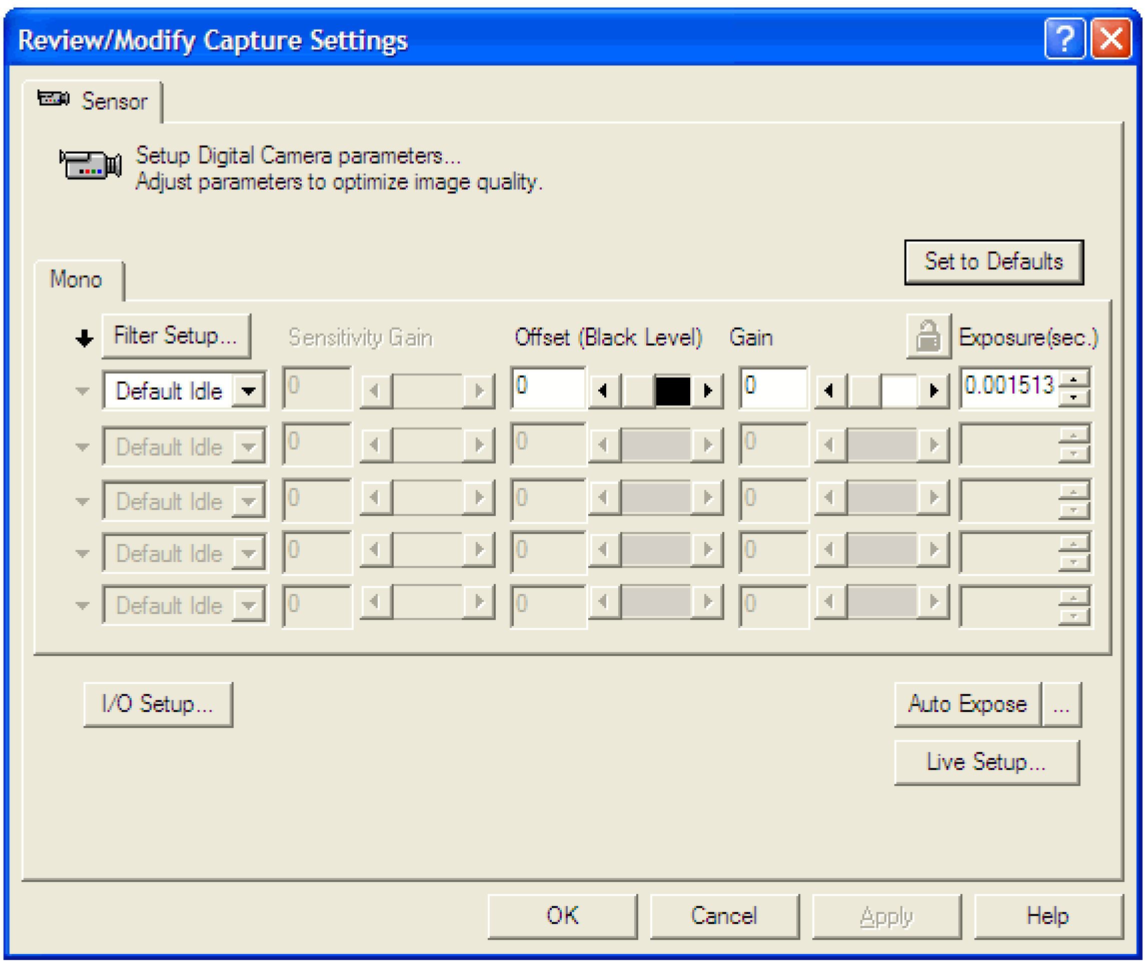
Acquisition Control allows for non-stop acquisition or automatic stop at specified frame or time.

Z Focus can be automatically refreshed every *n* Fields.

Save Image Sequence: Choose between high speed memory acquisition or stream directly to disk. If streaming to memory, you may commit those images to disk while paused.

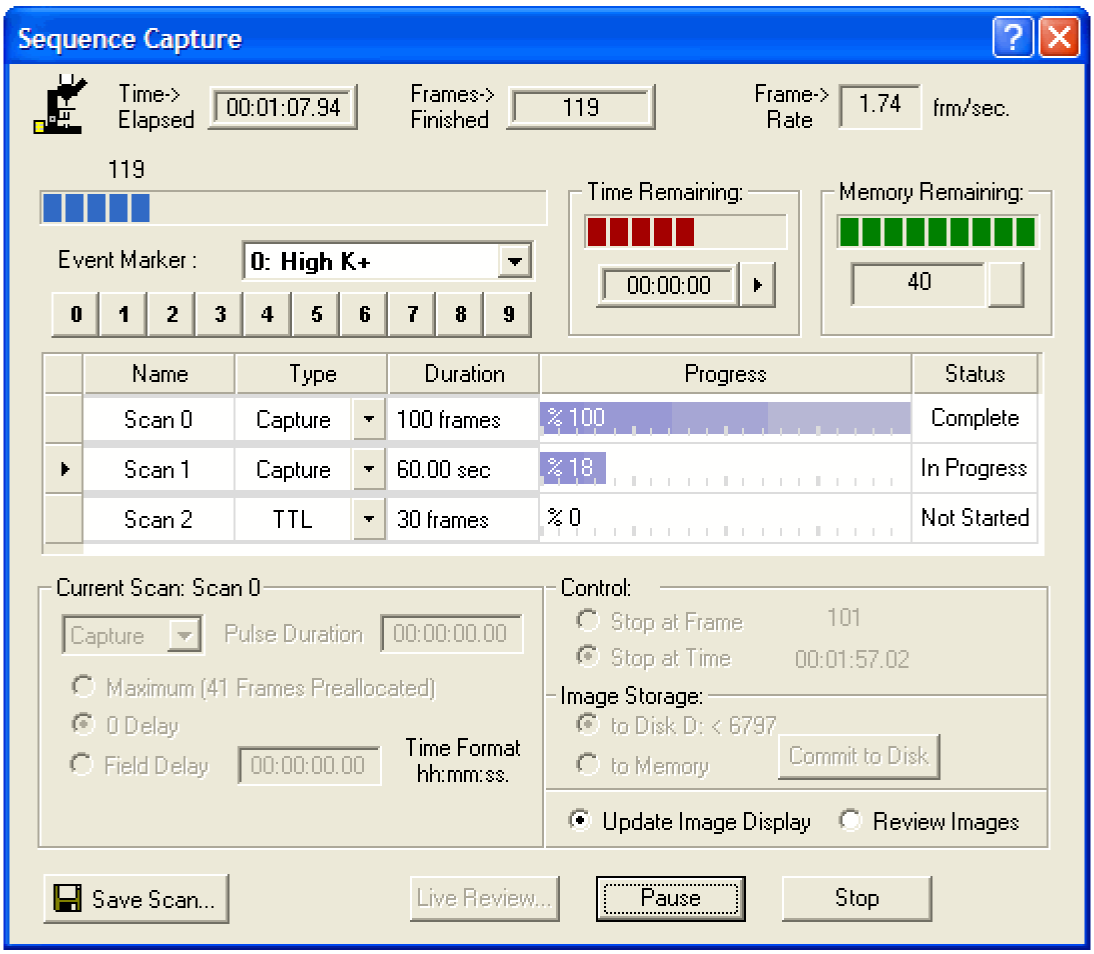
During a sequence, you may choose to review previously collected images by selecting **Review Images.**

Live Review is available when the scan has been paused and opens the **Review/Modify Capture Settings Window**. The Live Review enables the user to review and optimize capture settings such as exposure during an experiment.



Capture Sequence Scheduler

The Capture Sequence Scheduler is an option when choosing a Continuous Time Scan. The Scheduler will setup and run a sequential series of user defined Scans consisting of a combination of TTL pulses and Capture Sequences. Each scan is defined by Type (TTL or Capture) and Duration (Stop at Frame or Stop at Time) which are both defined by the user. The scan may be paused at anytime during the experiment to adjust Capture Settings including exposure and gain. The image below shows a Capture Sequence in Progress that was designed using the Scheduler.



Event Markers can be added to the data document file during acquisition as notable events occur in the experiment. Both the numbered buttons above and keyboard numbers may be used.

Time Remaining is a reference to delay time. The right arrow button may be clicked to exit the delay.

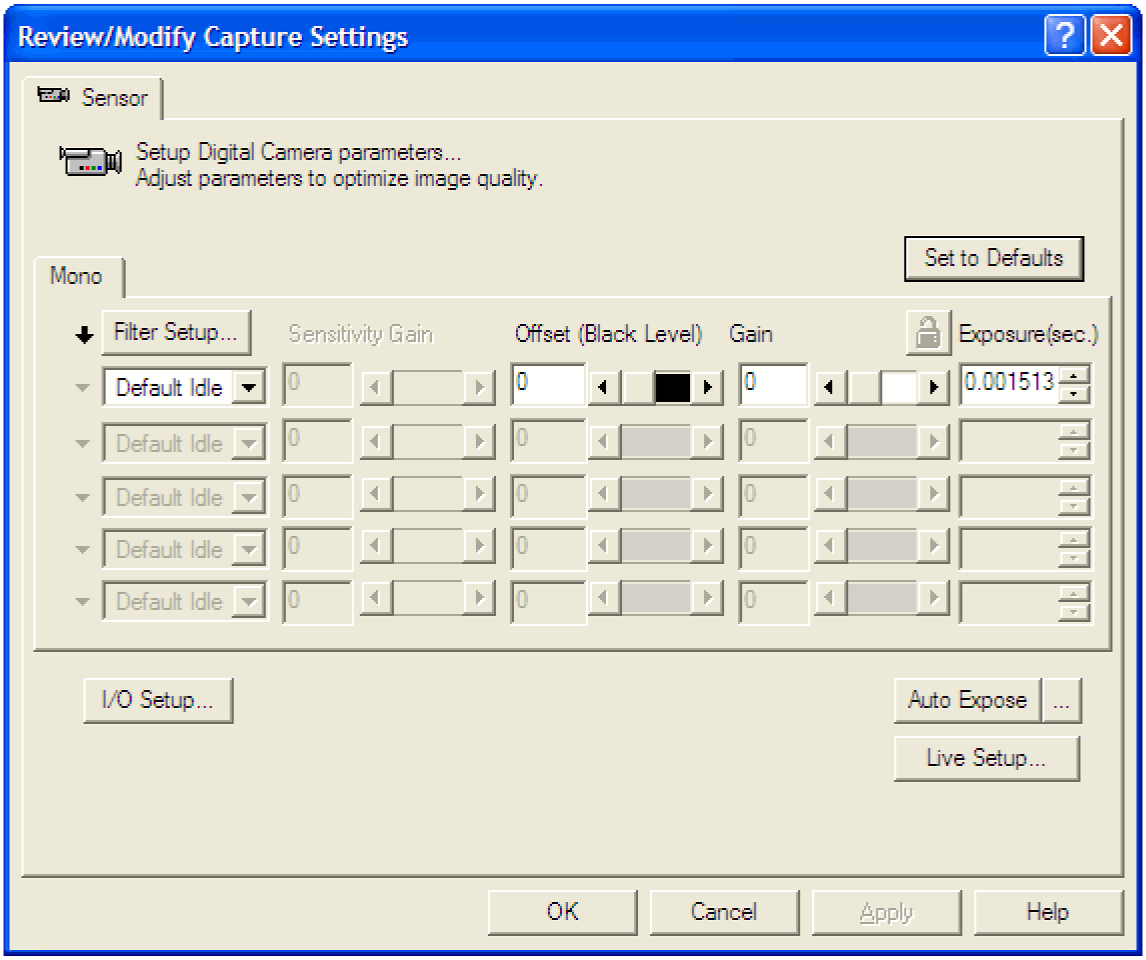
Current Scan: Select the **Type** of Scan from drop-menu. For TTL pulses enter the **Pulse Duration** and a **Pulse Delay** is necessary. For Capture choose **Maximum** for maximum speed, **0 Delay** or **Field Delay**. During maximum speed, items which slow down acquisition will be ignored (exposure protection). Field Delays can be entered to allow for fast time switching during experiments.

Control: Defines a specific Frame or Time point to stop the current scan.

Image Storage: Choose between high speed memory acquisition or stream directly to disk. If streaming to memory, you may commit those images to disk while paused.

You may choose to review previously collected images by selecting **Review Images**.

Live Review is available when the scan has been paused and opens the **Review/Modify Capture Settings Window**. The Live Review enables the user to review and optimize capture settings such as exposure during an experiment.



How to Setup a Scan

First, click on the Scan Type drop-menu and select TTL or Capture. Specify the Duration, for TTL pulses enter the **Pulse Duration** and a **Pulse Delay** is necessary. For Capture choose **Maximum** for maximum speed, **0 Delay** or **Field Delay**. Double-click on the Scan 0 to rename the scan. To add additional scans right-click on Scan 0 and select **Add SubScan**. Once all of the Scans have been setup and Even Markers have been setup click on Start to begin.

Collect Data

When a Workfile has been created, it can be used to control the capture, archiving and analysis of one or more images. The Workfile Icons are applied once for each captured Field.

If only Image Data is to be collected automatically without any Workfile enhancements or measurements, use the Sequence Capture button in the Capture Dialog and open a Target Data Document.

The **Task Toolbar** includes the following controls:

Scan: Initialize the Scan Wizard to guide the operator through the steps in creating an automated or semi-automated Scan**.**

Record: Select the measurement data to be saved to the Active Data Document. Clicking the Record button or opening a New Data Document when starting to Collect Data will display a list of items which can be checked on or off. These include:

- **Object Summary Statistics**
- **Object Summary Graphs**
- **Field Summary Statistics**
- **Field Summary Graphs**
- **Field Data**
- **Original Image**
- **Object Statistics**
- **Object Data**

Start: Enter the Capture Dialog. You are ready to capture the first image in the scan. The Capture Dialog is always displayed to allow a verification of image focus and alignment before committing to a Scan. Once the Scan is underway, use the Stop Button to interrupt the scan.

In the case of acquiring multiple scans at the same time to independent Data Documents, there will be a pause while the Data Documents are created and initialized before the Capture Dialog is displayed. This may take some time for especially large volumes of data to be collected.

Pause/Stop: Choose one of the following

- Continue Collecting: Restart the scan immediately.
- Finish Current Pass then Stop: Finish making measurements etc. and stop before next Field.
- Finish Current Pass then Stop: Finish all fields in the current Pass and stop before the first field of the next Pass.

- Cancel Now and Delete Current Field: If the current field is invalid (e.g. poor focus) abort it and delete any data partially collected.

Delete Last Field: Remove the data collected for the last field from the accumulated statistics and delete the current field of data from the Data Document Tree.

End Collect Data/Stop Collecting: Switch from Collect Data mode to Workfile Editing mode. This can be done at the end of a Scan when data collection is complete, or, in the middle of a scan where some Workfile options need to be tuned before the Scan is re-started.

The Collect Data toolbar is replaced with the Task Toolbar giving access to new modules to be added to the Workfile. The current modules in the Workfile can be accessed immediately by clicking on the icons in the Workfile Icon Toolbar.

> **Note:** Closing the Active Data Document will also **End Collect Data**.

A simple analysis would involve the interactive Capture of images and the identification and Measurement of object measurement parameters. This is the default method for collecting data.

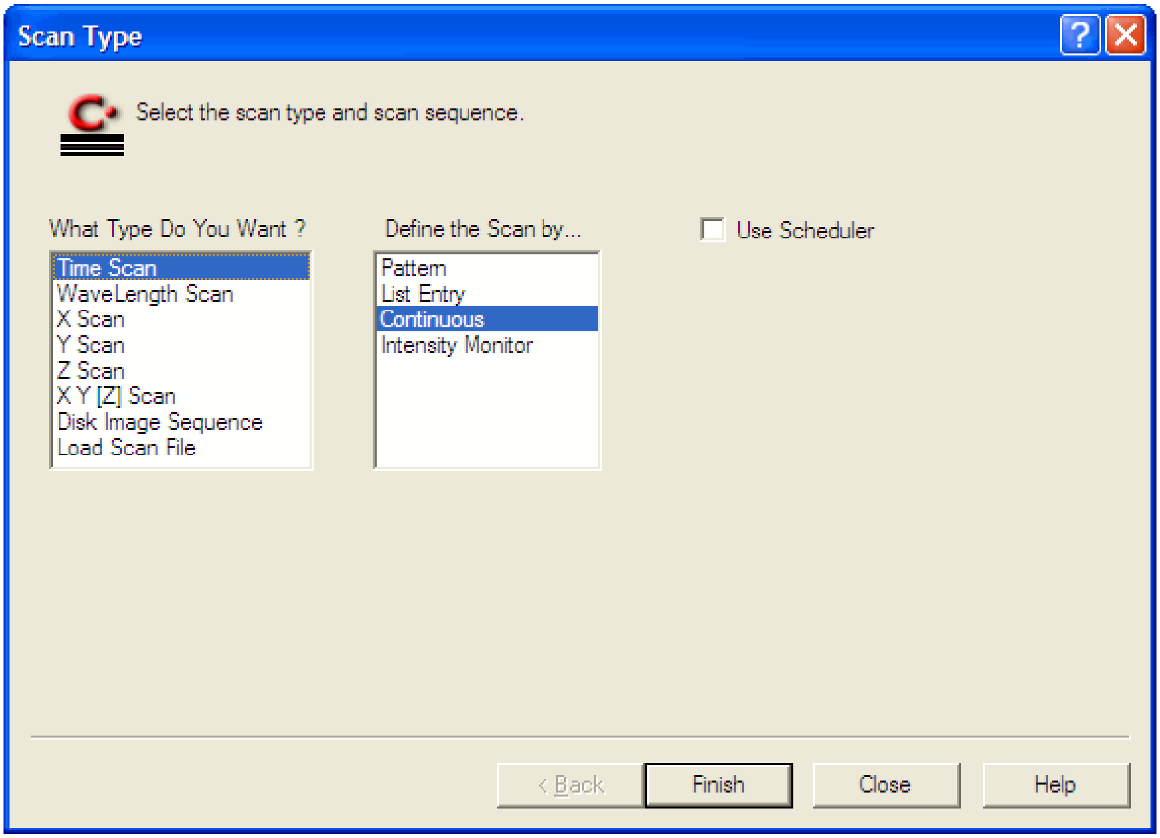
To activate this method use the following steps:

1. Click on the **Collect Data** Icon in the Imaging Toolbar.
2. Select the name and path for the Data Document for storing data.
3. Verify the **Record** settings will include all data required and click Okay to accept.
4. Click on the **Start** Icon to open the Capture Dialog.
5. Click **Capture** to activate a new live image for sample alignment and focus.
6. Click **Okay** to accept the dialog settings and start to run the Workfile.
7. The Workfile will now be applied to the image and measurements made as directed, data measured will be stored in the Active Data Document for review during or after the Collect Data process, and the Capture dialog will be displayed to repeat the process on a new image.
8. When enough images have been captured and analyzed, click **Cancel** from the Capture dialog to stop the loop.
9. Click **End Collect Data** to return to the Imaging Toolbar view.

In the case that images can be captured automatically, using a time lapse sequence or XY stage scan pattern for instance, a **Scan** can be created to automatically control the timing and positioning of image capture, prior to archiving and Workfile processing. Press **Scan** to activate the **Scan Wizard**, which will guide the operator through a sequence of steps to create the list of image locations to capture.

Using the Scan Wizard

The Scan Wizard provides a sequence of steps to configure the capture and processing of a sequence of images.



The Scan Wizard is started from the **Sequence** button in the Capture Dialog. You may also access this wizard from the **Scan** button in the Collect Toolbar.

The Scan can be based on Time Lapse, XY or Z movement, or wavelength scanning depending on the equipment installed in the Current Profile.

Available Scan Types:

- **Time Scan**: Time Lapse Imaging

  The Time Scan Type allows Images to be captured from the Active Capture Device for display, archiving or processing, while incrementing a Time Lapse delay before each image. This can provide the ability to collect temporal information on static objects in the field of view.

- **Wavelength Scan**: Create a spectral scan through a range of wavelengths.

  The Wavelength Scan Type allows Images to be captured from the Active Capture Device for display, archiving or processing, while incrementing the wavelength of the illumination or filter device for each image. This can provide the ability to collect spectral information on static objects in the field of view.

Wavelength changing devices must first be installed in the Device list of the Current Profile.

- **Linear X,Y or Z Scan: Linear spatial scan using a single axis motor drive (X, Y or Z)**

  The X ,Y and Z Scan Types allow Images to be captured from the Active Capture Device for display, archiving or processing, while incrementing a single axis position of the Stage Controller device for each image. This can provide the ability to collect spatial information on static objects outside of a single field of view.

  Stage Control devices must first be installed in the Device list of the Current Profile.

- **XY(Z) Scan**: 2 dimensional XY scan list or pattern, with Z focus control.

  The XY(Z) Scan Type allows Images to be captured from the Active Capture Device for display, archiving or processing, while scanning a two dimensional area with Stage Controller device. This can provide the ability to collect spatial information on static objects outside of a single field of view.

  Stage Control devices must first be installed in the Device list of the Current Profile.

- **Disk Image Sequence**: Load a sequence of images from a Data Document or a series of image files, e.g. image1 to image50.tif

  Allows Images to be loaded from disk based files for display or re-processing. Images contained in a Data Document can be extracted from the Image Data in each Field, or images can be loaded from a sequence of consecutively numbered disk based image files of any supported image format.

Each Scan Type is defined using a **Scan Mode**. This determines how the List of locations is created, automatically or interactively.

- **List Scan Mode** is used where each location is defined interactively. Typically this is used where the locations are irregularly positioned, or not known, such as a series of cells in a culture flask, and must be interactively located.

- **Pattern Scan** Mode is used to automatically create a sequence of locations. Examples are a Time Scan Pattern, where the list of time points use a Start time, and an End time and a time increment, or interval to compute the number of images needed. In a spatial example, the limits of the pattern may be defined by traveling to the Start and End positions of the interesting part of a sample.

- **Continuous Scan Mode** is used only for a **Time Scan**, and allows images to be regularly captured in a Time Lapse sequence without specifying an end point. The sequence will be stopped when resources run out or can be interrupted using the Stop button in the Collect Toolbar.

In both the **Pattern** and the **List** modes locations can be previewed, tested and modified before processing the Scan.

Time Lapse Imaging can be applied for any scan type by processing a scan for **multiple Passes**. This can be selected in the final menu of the Scan Wizard.

> **Note:** The Data Document can be set to **Record** the location of each image in the Field Details option. These details can be used later to verify the actual position and timing of a particular Field of data.

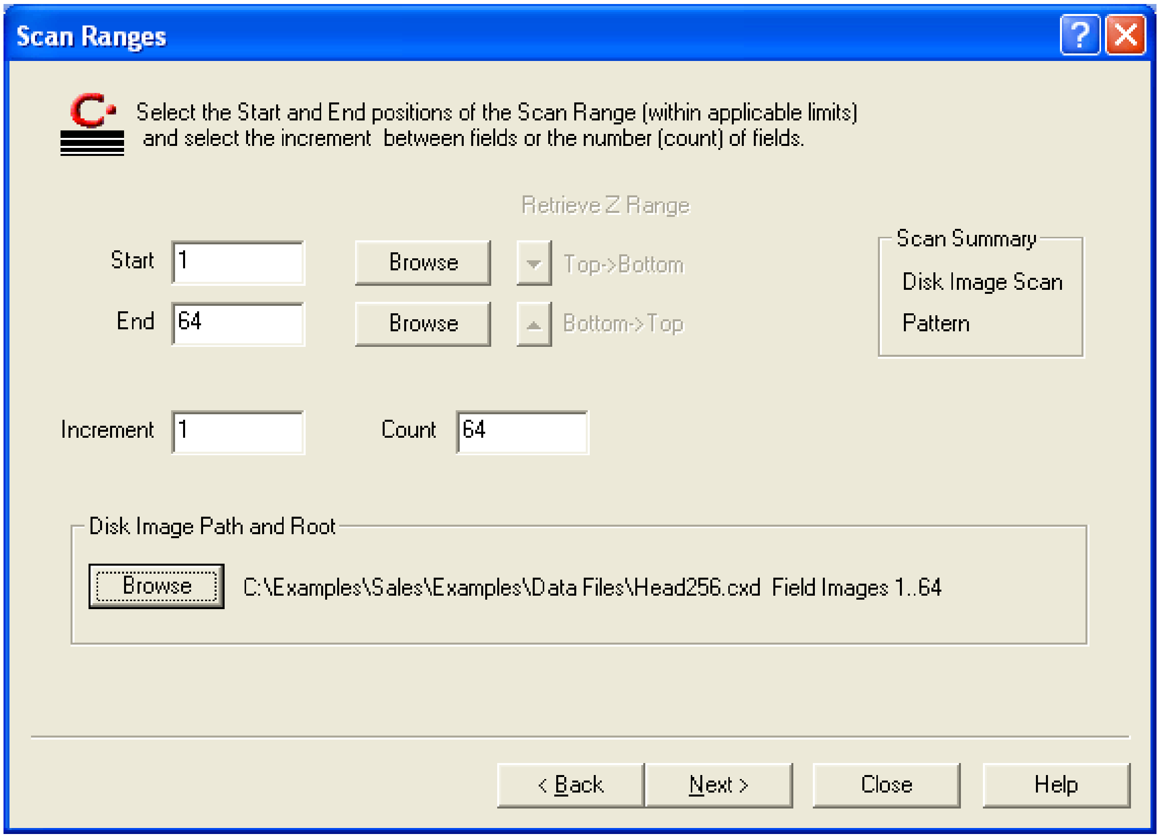
To configure a Scan, follow the steps in the wizard as described for each Scan Type. In each page of the Wizard the **Next** and **Back** buttons are available to travel between the different pages for a scan and modify selections made. The **Finish** button will complete the setup of the current Scan, or to abort the Scan Setup select **Close**. Once the Scan Setup is completed, a Scan List of locations is displayed, activate the scan using the **Start** button.

For more information, see:

- Disk Scan Pattern
- Disk Scan List
- XYZ Scan Pattern
- XYZ Scan List

Disk Scan Pattern

To set up a **Scan Pattern** for a Disk Sequence, use the **Browse** button to locate the file(s) to load.



If the **Browse** button is used to identify a Data Document as the source file, the **Start** number refers to the first field containing Image Data within the Data Document file. The **End** number is automatically computed as the last Field containing image data. The **Increment** defaults to 1, for every field, and the **Count** is the number of Image Fields in the data Document. These parameters can be modified as long as the numbers are within the available range.

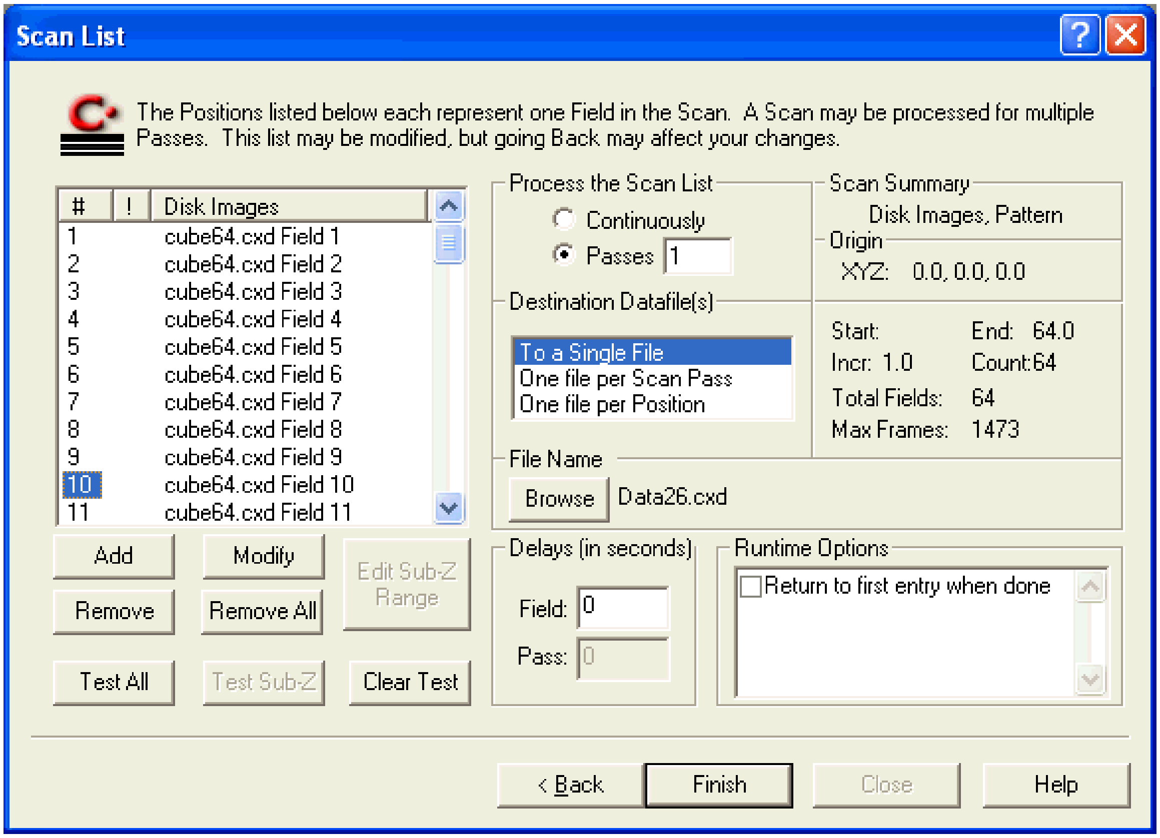
If a sequence of image files is to be read the **Browse** button will allow the **Start** file of the sequence to be selected. Automatically the directory is searched for other files with consecutively numbered filenames, and the **End** number is selected from the last file in the sequence. The **Increment** defaults to 1, for every field, and the **Count** is the number of consecutively numbered Images found in the directory from the Start image. These parameters can be modified to load a portion of the sequence as long as the numbers are within the available range.

Note that when the disk images are loaded, they will be converted, if necessary, to fit the current size and display depth of the **Active Capture Device**.

Click **Next** to see the selected items in Scan List view.

Disk Scan List

The Disk Sequence **Scan List** display allows disk image files to be individually chosen for processing by the Scan. If the Disk Sequence is created using a **Scan Pattern** the resulting list of images to be loaded are displayed in the Scan List, otherwise the List is initially empty.



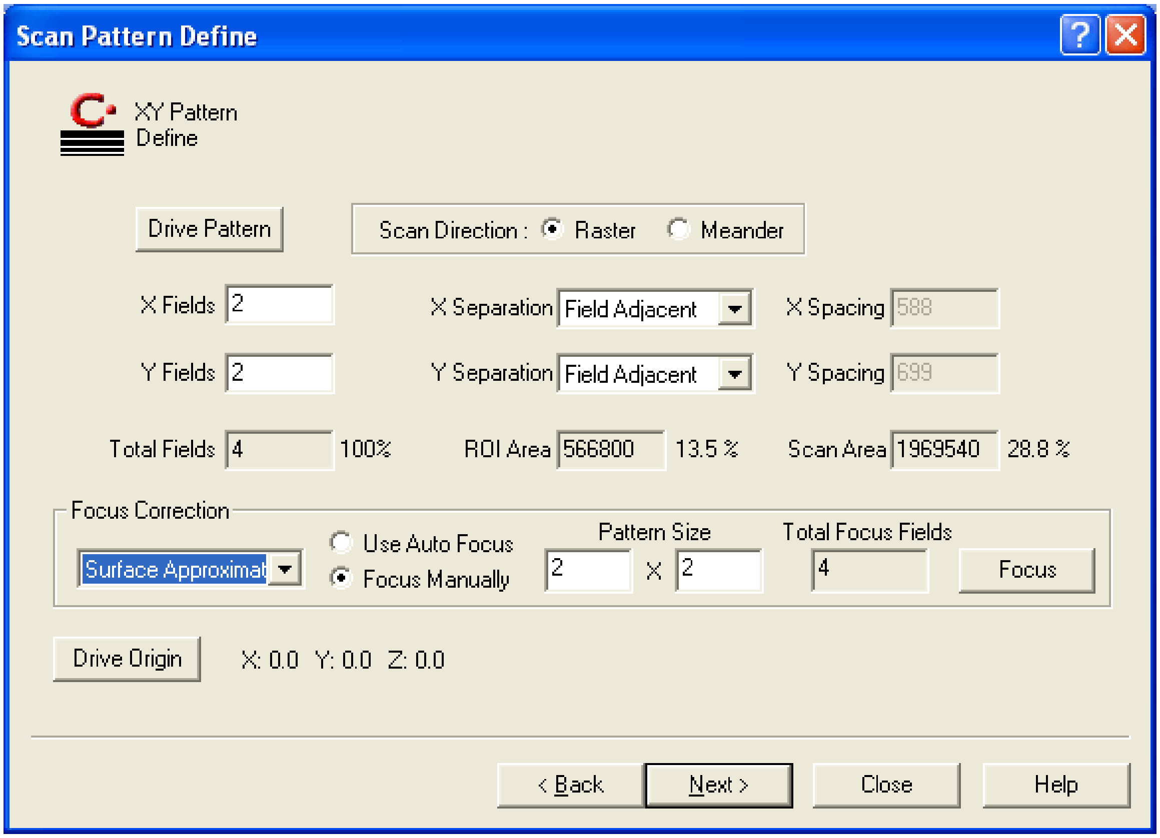
The options in the Scan List page include

Add	Add a new item to the list.
Modify	Modify an item in the list.
Remove	Remove an item from the list.
Remove All	Remove all items from the list.
Test All	Test the location of all items in the list.
Clear Test	As each position is tested, a mark is placed next to each tested position. Clear Test will delete these marks.

To select an item in the list, click on the item number in the left most column.

XYZ Pattern Scan

The **XY(Z) Pattern Scan** can be created by entering the number of fields to capture in each direction, and the increment between the positions, or by interactively driving the stage device to the limits of travel of the sample area, and automatically creating a scan that includes the bounding rectangle of the scan area.

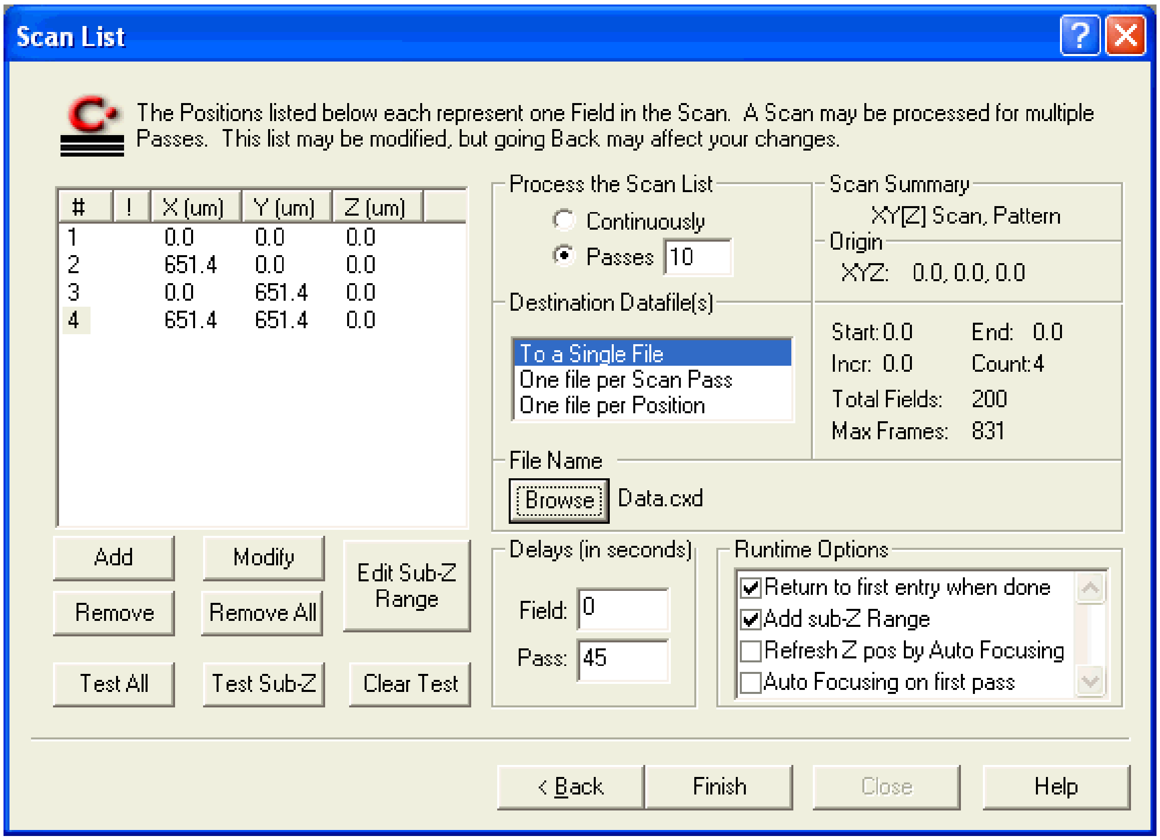


Focus control can be applied to each location in the XY scan using AutoFocus or Manual Focus methods.

Click **Next** to see the selected items in the Scan List.

XYZ Scan List

The **XY(Z) Scan List** display allows specific positions to be individually chosen for processing by the Scan. If the List is created using a **Scan Pattern**, the resulting list of image locations is displayed in the Scan List, otherwise the List is initially empty.



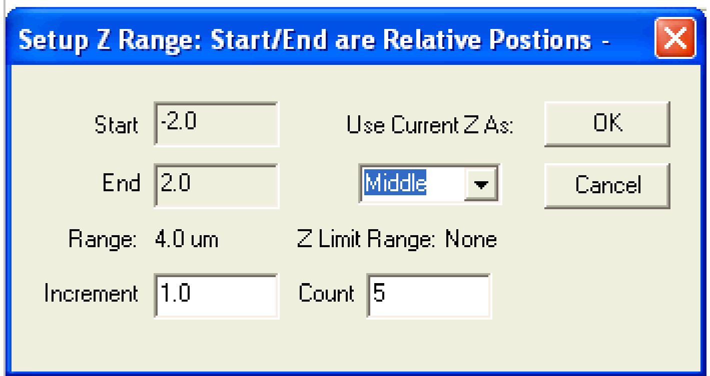
The scan list can be processed once, for a specified number of passes or continuously.

Pass delays may be defined for Multipass scans.

Field delays may be defined for all scans.

List options include:

Add	Add a new item to the list.
Modify	Modify an item in the list.
Remove	Remove an item from the list.
Remove All	Remove all items from the list.

Edit Sub-Z Range	Define a Z-Range to be scanned at each XYZ position.  Define the increment and count of the Sub-Z range. The current Z position can be chosen as the Top, Middle or Bottom of this Sub-Z Range. The Runtime Option **Add sub-Z Range** must be checked to enable this option.
Test All	Test the locations of all items in the list. You may choose to include or exclude the Sub-Z range in this test.
Test Sub-Z	Test the Sub-Z range positions only.
Clear Test	As each position is tested, a mark is placed next to each tested position. Clear Test will delete these marks.

To select an item in the list, click on the item number in the left most column.

Runtime options include:

Return to first entry when done	Stage will return to first X, Y, Z position when scan is complete.
Add sub-Z Range	Select to enable Sub-Z Range scanning.
Refresh Z pos by Auto Focusing	Auto-Focus will update the scan's Z-position.
Auto Focusing on first field/pass	Select to Auto-Focus on first field or scan pass.

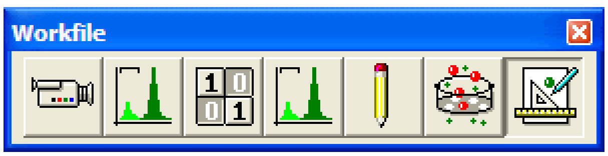
Workfile Document Overview

The Workfile Document is the basic storage mechanism for the options controlling image capture, processing and measurement operations. The Workfile contents can be considered as a macro language, however great pains are taken to shield the operator from having to deal with a textual interface for what is usually a visual imaging problem.

Workfile File Access

The Workfile document is a .enh file that can be saved, loaded, edited and printed. When the application is run a Workfile is loaded or a new default Workfile is created. This is the Active Workfile. The default Workfile to load on start-up of the application is controlled by the File Paths options in the Current Profile.

Workfile Icon Toolbar



There is always a single Workfile open at a time, which is visible as a Workfile Icon Toolbar and a Workfile Document.

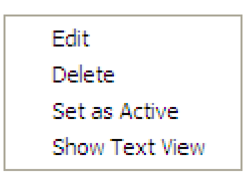
By default, the Workfile Icon Toolbar is docked towards the top and the Workfile Document is minimized at the bottom of the application workspace. You can maximize the Workfile Document for textual details on current workfile settings.

To modify the Workfile, select an Imaging Icon from the Task Toolbar . Each icon in the Task Toolbar will open a dialog providing options for image manipulation. Selected options are saved in the Workfile. New icons are appended as new tasks are added. In this way, a sequence of operations can be created to analyze simple or complex images.

The Active Icon is shown in a pressed state. New Icons added to the Workfile will be appended or inserted after the Active Icon. Click on any Icon to open the Imaging Dialog for that Icon and modify its settings.

Workfile Menu

Right-click on a Workfile Icon to display the Workfile menu.



Edit - Open the selected Icon Dialog for Editing. If Imaging is currently enabled, the Icon will actively perform the imaging operations on the current image.

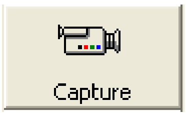
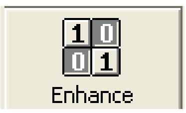
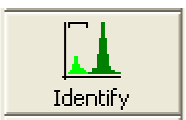
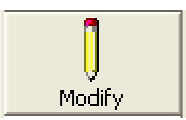
Delete - Remove the selected Icon from the Workfile. All settings will be lost.

Set as Active- Make the current Icon the Active Icon. This allows a particular Icon to be set as the Active Icon without opening its Dialog, as with a Left click. This is useful after some modifications have been made to Icons in the middle of the Workfile, to move the Active position to the end so that new Icons can be added.

Show Text View - Show the settings of the Imaging Icons in detailed numerical and text form. Open the Workfile Document and jump to the selected Icon location.

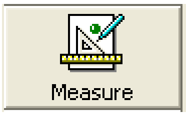
Creating a Workfile

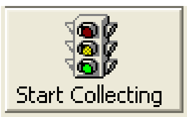
A sequence of steps is necessary to collect object measurements. The order of the Icons in the Task Toolbar follows the typical order of these steps, although not every icon has to be used. These steps are typically recorded in a Workfilefor convenient routine saving and loading but can be performed manually on an Image Document.

	Acquire an image from an installed camera or from Disk. May only be used once per workfile.
	Improve the image or combine it with other images.
	Threshold the pixels into a Binary image, which should represent the objects to be measured.
	Improve the Binary image or combine it with other Binary images.
	Remove objects from the Binary image on the basis of some object characteristic, e.g. Area.

After the above steps have been taken, the options in the Workfile will be set, and a Binary image will have been created ready for object measurements.

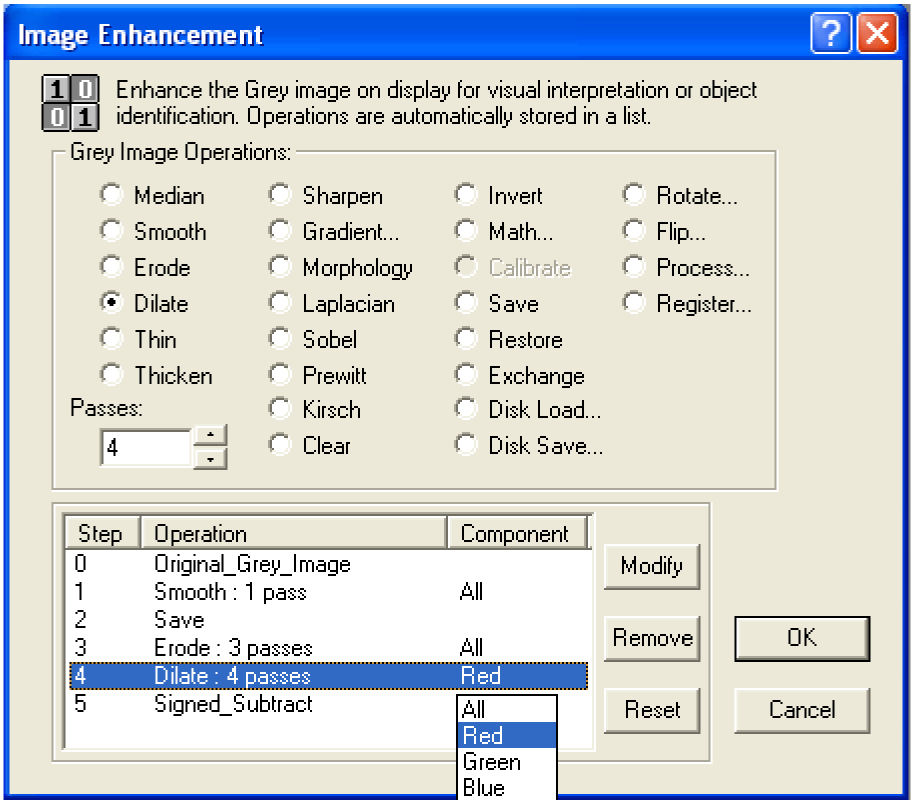
In order to make Object Measurements, the pixels detected in the Binary image must be related together as object. This is done by object detection techniques, one technique is called **Labeling**, where every pixel belonging to the same object is given a value, and every object has a different value. Another technique used is boundary tracing to identify characteristics of an object based on shape. The measurement algorithms can then compute the measurements selected for each object, and store them for statistical analysis.

	Select some object measurements to collect from the original (or enhanced) image pixels which lie within the objects (as defined in the binary image overlay resulting from the identify and modify tasks) which survive the qualification task. Multiple uses will send results into corresponding multiple measurement class nodes in the output data document.

	Apply the Workfile generated or modified by configuring the dialogs resulting from any steps included above to one or more images from capture devices or loaded from disk storage, generating Object and Field measurements and their statistics and writing them into a **Data Document**.

Enhance

The Enhance Dialog allows a combination of image enhancement operations to be applied sequentially to a image.



To select an operation for the current image on display, click on the radio button checkbox of the operation. The operation selected will be applied to the current image on display.

Each time an operation is applied to the current image, the operation is added to the List. Selecting an operation from the list will jump to the appropriate place and allow editing of the operation sequence.

Some Enhance operations can be re-applied repetitively to strengthen their effect. These operations are located in the left hand column in the Enhance Dialog. At the bottom of the column is a counter labeled "passes". This **Pass Counter** shows the number of times that the currently selected operation has been applied. To specify the number of passes click on the operation multiple times, use the spin control arrows or type the desired value. The maximum number of passes allowed per Enhance operation is 255. Notice that the number of passes applied counts as only one Enhance operation.

For color images, may operations can be applied per component.

The second column of options in the Enhance Dialog are image processing operations that are not normally applied repetitively, although this is possible by re-selecting the same option more than once.

The latter two columns of Enhance options are for controlling the storage of a second image in memory, for arithmetic operations between the stored and display images, and for loading and saving disk images.

Enhance Operations:

See Image Enhancements.

List Operations:

To **Modify** an operation in the list, select the item and click Modify. A dialog will pop up listing other possible operations for selection. The new imaging operation will be applied and replace the original operation in the list.

To **Remove** an item from the list, select the item and click Remove. The selected item will disappear from the list and the image will be processed to represent the previous item in the list.

Click **Reset** List to remove all items from the list.

Note: the top most item in the list represents the original image and cannot be modified or removed.

Enhance Operations

Convolutions

- Median

  Useful for detecting single point noise pixels and replacing them with a value more similar to the Grey level values in the surrounding neighborhood. This can reduce noise spikes, dark or light, without affecting the quality of the image sharpness. Repetitive operations of the Median may not yield much improvement over the first pass.

- Smooth

  Replaces each pixel with the average of its neighbors. This process can be useful in reducing noise speckle in the image when using low light conditions. Effectively the techniques applies a "blur" to the image, making conspicuous pixel intensities merge with their neighbors. This operation can be applied for a multiple number of passes, progressively smoothing the image.

- Erode

  Shrinks dark objects by one pixel for each pass applied.

  Can be used to Erode small dark objects until they disappear so that they will not be detected by Identify and counted later.

  Shape of objects may change after many passes.

- Dilate

  Grows dark objects by one pixel for each pass applied.

  This can be used after Erode to Dilate objects back to approximately their original shape and size, so that smaller objects will have been removed and will not be detected by Identify and counted later.

  Shape of objects may change after many passes.

- Thicken

  Grows dark objects by one pixel for each pass applied, but prevents objects from growing together.

  Use on samples which have linear objects. Shape of objects may change after many passes.

- Thin

  Shrinks dark objects by one pixel for each pass applied, but prevents objects from disappearing.

  Use on samples which have linear objects. Shape of objects may change after many passes.

- Sharpen

  Increases the contrast between each pixel and its neighbors, giving the image a "sharper" visual appearance.

  With images containing a degree of noise, the result can be an increase in the sharpness of the noise, making the image useless. This operation may be combined with Smooth or Median to reduce the noise beforehand.

  A particular application of this operation is for **Halo Removal**, where different grey phases in an image have some blur at the edges which creates a halo of a different apparent phase. Sharpening the image can remove this Halo to allow separate detection and measurement of the different phases.

Edge Operators

- Gradient Operators

  The **Gradient** operators can be used to detect an increase or decrease of grey level intensity in one of four directions.

  - 0° horizontal
  - 45° left-down to up-right
  - 90° vertical
  - 135° left-up to right-down

  Note: The result of a Gradient filter is to enhance edges in the image on a pixel by pixel basis. A positive change in grey level intensity in the selected direction will result in a bright pixel value. A negative change in grey level results in a dark pixel value. No change in grey level results in a mid-grey pixel value. Objects frequently will show a bright edge on one side and a dark edge on the other side with mid-grey inside the object (no change) and mid-grey in the background (no change). The results of this digital image processing operation are often compared to the results of the optical Nomarski illumination effect.

- Kirsch Filter

  Shows object boundaries based on their contrast with the background by measuring both positive and negative changes in grey level within the pixel neighborhood in ANY direction result in a pixel intensity proportional to the size of the change. No change in grey level results in a black pixel value.

  Objects frequently will show a bright edge all around with black inside the object (no change) and black in the background (no change). The Kirsch operator has a more intense reaction to edges than the Morphology, Sobel and Prewitt operators.

- Laplacian Filter

  Like the Kirsch filter, the current display image is enhanced by object boundary intensities shown proportional to the amount of contrast to the background in ANY direction, but unlike the Kirsch, information on the direction (sign) of the change is retained. A positive change in gradient is indicated by a bright pixel intensity, a negative change has a dark pixel intensity. No change in grey level results in a mid-grey pixel value.

  Objects frequently will show both a bright edge and a dark edge all around with mid-grey inside the object (no change) and mid-grey in the background (no change). It is the separation of the direction of grey level change (gradient) that distinguishes the Laplacian operator from the other multi-directional operators, Morphology, Sobel, Prewitt and Kirsch.

- Morphology Filter

  The Morphology operator's reaction to edges is similar to but less intense than the Sobel, Prewitt, and Kirsch operators.

- Prewitt Filter

  The Prewitt operator's reaction to edges is similar to but more intense than the Morphology and Sobel operators, and less intense than the Kirsch operator.

- Sobel Filter

  The Sobel operator's reaction to edges is similar to but more intense than the Morphology operator, and less intense than the Prewitt and Kirsch operators.

Image Arithmetic

- Invert

  Reverses the image intensity values relative to the minimum and maximum possible values at the given bit-depth. This creates the effect of a photographic negative, and can be used to Invert x-ray film to positive intensity.

  This option actually changes the grey levels themselves, unlike the Display Contrast functions in the Capture Dialog, which only alter the way in which the grey levels are presented on the display. If an image is saved to Disk at this point and re-loaded later, the inverted image grey levels will be retained. To immediately Invert the image back to normal contrast, click the **Remove** button.

- Math

  Choose from the list of available Math operation choices for combining the Saved and current images.

  Average (A+B)/2

  Arithmetically combine the current image with the Saved image. The addition of two Grey images with grey levels from 0 to 255, creates grey level possibilities from 0 to 510. These values are scaled back into the current display image from 0 to 255, so the resulting image is like a double exposure of the two images.

  Signed Subtract (A-B)+(MaxGray/2 - 1)

  Arithmetically combine the current image on display with the Saved image. The subtraction of two Grey images with grey levels from 0 to 255, creates grey level possibilities from -255 to 255. These values are scaled back into the current display image from 0 to 255, by scaling grey level -255 equal to 0 (Black) and grey level +255 equal to +255 (white), thus grey level 127, is equal to mid grey, and reflects no change between the two images. The dark and bright portions of the resulting image represent loss or gain in intensity, useful in comparing x-rays, or images taken at different times during the growth of a sample.

  Subtract (A-B)

  Arithmetically combine the current image with the Saved image. The subtraction of two Grey images with grey levels from 0 to 255, creates grey level possibilities from -255 to 255. Positive values are scaled back into the current display image from 0 to 255, negative values are truncated to zero intensity.

  Sum (A+B)

  Arithmetically combine the current image on display with the saved image. The addition of two images with grey levels from 0 to 255, creates grey level possibilities from 0 to 510. Values are NOT scaled back into the current display image, and so bright values may saturate at 255. This can be used as a method of image integration.

  Minimum Value Min(A,B)

  This function replaces each pixel in the current image with the Minimum value of the current and saved image. No scaling is applied. This method can be used to create a minimum projection of a series of images, perhaps at different focal depths of a sample.

  Maximum Value Max(A,B)

This function replaces each pixel in the current image with the Maximum value of the current image or saved image. No scaling is applied. This method can be used to create a minimum projection of a series of images, perhaps at different focal depths of a sample.

Add Constant A+N

Each pixel of the current image is added to the value and the result used to replace the pixel in the displayed image.

This function may be used to apply a background offset to remove uniform background intensity, or to equalize two images.

Subtract Constant A-N

Each pixel of the current image has the constant value subtracted and the result used to replace the pixel in the displayed image.

This function may be used to apply a background offset to remove uniform background intensity, or to equalize two images.

Multiply Constant A*N

Each pixel of the current image is multiplied by the constant value and the result used to replace the pixel in the displayed image.

Shift Up Constant A>>N

Each pixel of the current image is shifted up by the constant value and the result used to replace the pixel in the displayed image.

Shift Down Constant A<<N

Each pixel of the current image is shifted down by the constant value and the result used to replace the pixel in the displayed image.

File I/O Allows Disk Save/Load operations, and can be used to store temporary images to disk for use in the Workfile process. These operations are the only means by which to apply imaging arithmetic calculations involving more than two images. Each File I/O operation is available in both Query mode (interactive, asks for filename) and Fixed Mode (filename is stored and re-used).

- Disk Load...

  Loads an Image from a disk file into the image currently on display.

  Note: Image formats will be converted as necessary to fit the target image depth.

- Disk Save...

  Saves the Image currently on display to a disk file.

Memory I/O (for Image Math)

- Save

  Makes a duplicate copy from the grey image currently on display to memory, replacing any image previously saved by this option

- Exchange

Swaps the saved grey image in memory with the image currently on display.

- Restore

  Copies from the saved grey image currently in memory to the display, replacing the current image on display

- Clear

  Clears all pixels to a zero intensity value.

The saved image can be used for arithmetic combination (Grey Level Math) with already enhanced images or with a further image loaded in to the current display using **Disk Load...** The saved image can be returned to the display later by using the Restore option. If the image on display should not be replaced, use the **Exchange** option.

Only one image can be saved to memory at one time, if logical operations involving more images is required, use File I/O.

Rotate

Rotate the image clockwise

- 90°
- 180°
- 270°

Flip

Flip the Image

- Horizontal
- Vertical

Process

- Separate Background

  Enter level variance and minimum spot area.

- Ratio Shade Correction

  Choose Background Image. Original Image is divided by the Background Image and then multiplied by the mean of the Background Image.

- Spatial Average

  Enter Noise Threshold. If the grey-level intensity difference between the center pixel and any of its eight direct neighbors is greater than the Noise Threshold, then the pixel is given the average grey level of its neighbors instead.

- Background Subtraction

  Choose Background Image. The Background Image is subtracted from the current image.

- Cosmic Ray Removal

  Enter Threshold and Dilation values (number of dilation passes: *d*.)

  Mask original image from threshold value to max gray value.

Dilate mask by *d* passes – Looking to encompass non-thresholded rise around ray

Measure the diameter in the mask image. Find the radius of the ray: *r*. Apply Median from original to destination for *r* passes – passes should be half-diameter of ray.

Conditional copy of original outside mask to replace non-masked values with original data

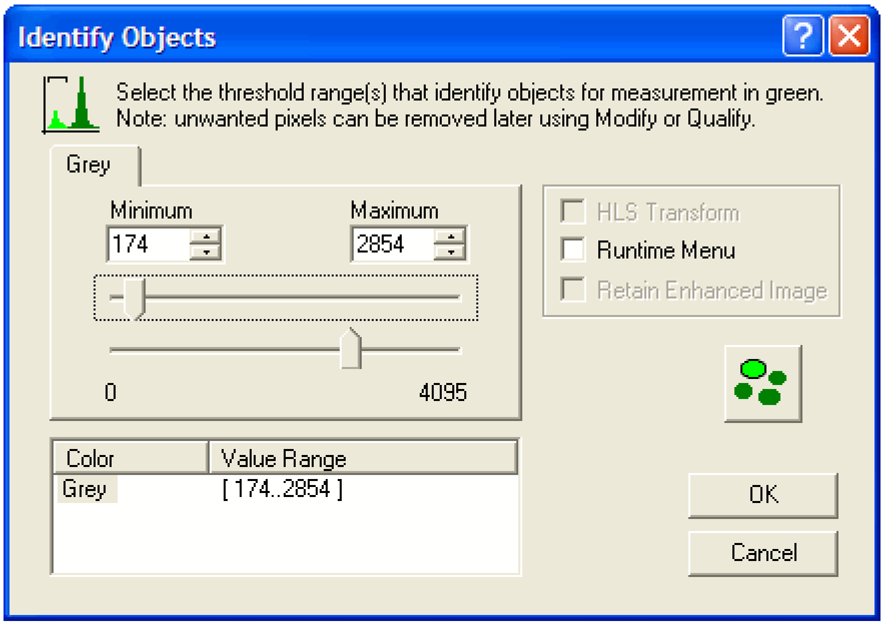
Register

Perform a pixel-shift

- Horizontal: Shift in the X direction (negative value to the left; positive value to the right.)
- Vertical: Shift in the Y direction (negative value up; positive value down.)

Identify Dialog (Summary)

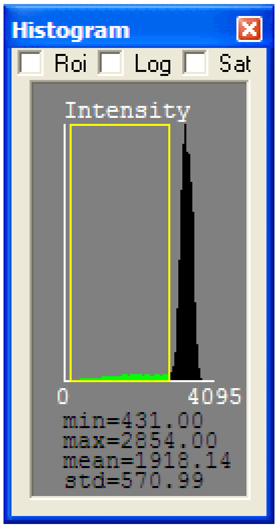
The **IDENTIFY Dialog** options control the way that a BINARY image of objects is created for later editing, modification and measurement. This is done using a technique called THRESHOLDING, or SEGMENTATION. The Binary image is a map of all detected pixels that may later be combined together into objects for measurement.



In a monochromatic **Identify**, a range of GREY level intensities called the THRESHOLD RANGE is selected. Each pixel in the image is tested against the minimum and maximum values in the range. If the pixel intensity is between these two values (inclusive), it is considered part of an object, and its value is set to **one** or **ON** in the current BINARY (1-bit) image on display. Pixels at all intensities outside the THRESHOLD range now represent the background. Object pixels are displayed as GREEN on the **Image Display,** while the original image pixels are displayed everywhere else. The aim is to correctly color GREEN only the pixels that ought to be considered part of the objects targeted for measurement.

Identify can also be performed on color images, in either RGB or HLS color-coordinates.

The **Identify** Dialog has a Training ROI button designed to assist in the identification of the threshold range. The user need merely draw a shape over the area of interest and the threshold range will be automatically calculated.



The **Image Histogram Display** shows the current image histogram(s) with a box outlining the current selected MINIMUM and MAXIMUM values. The current values are used for thresholding, and all identified pixels are displayed in GREEN.

*The behavior of the following dialog elements in the monochromatic **Identify** Dialog are identical to (and documented within) the color **Identify** case:*

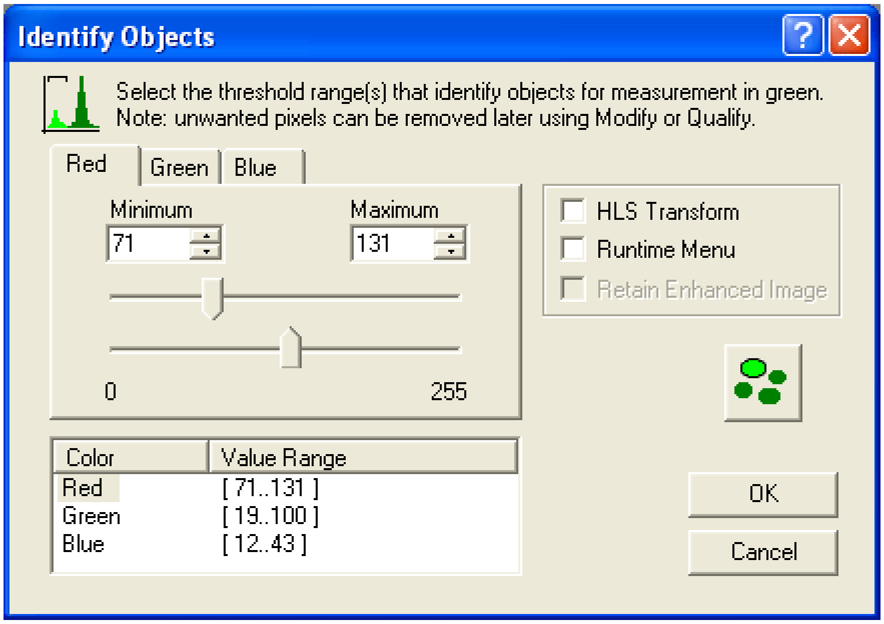
- Minimum
- Maximum
- HLS Transform
- Runtime Menu
- Training ROI

OK

The **OK** button will save the options in the WORKFILE being created or changed and exit the Dialog.

Identify (RGB)

The **IDENTIFY Dialog** options control the way that a BINARY image of objects is created for later editing, modification and measurement. This is done using a technique called THRESHOLDING, or SEGMENTATION. The Binary image is a map of all detected pixels that may later be combined together into objects for measurement.

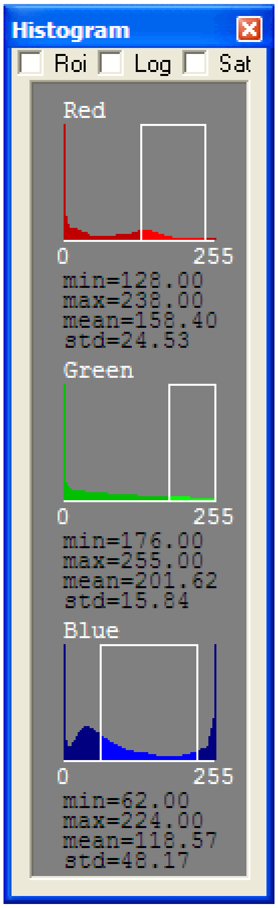


In the case of COLOR images three threshold binary images are defined, one for each color band, and these are combined together via an OR operation to include each pixel which is within all three ranges before identifying those pixels in GREEN. Objects may also be identified in monochrome images.

In **Identify**, a range of GREY level or RGB intensities called the THRESHOLD RANGE are selected to represent the objects in the image. All other intensities represent the background. Each pixel in the image is tested against the minimum and maximum values in the range. If the pixel intensity is between these two values (inclusive), it is considered part of an object, and its value is set to **one** or **ON** in the current BINARY (1-bit) image on display. If the pixel, is outside of this range, its value is set to **zero** or **OFF**. All pixels that pass the THRESHOLD RANGE test are displayed as GREEN on the **Image Display**. All pixels that fail are transparent, and the GREY or RGB image will be displayed. The aim is to correctly assign only the pixels that belong to objects for measurement as GREEN.

The **Identify** Dialog has a Training ROI button designed to assist in the identification of the threshold range. The user need merely draw a shape over the area of interest and the threshold range will be automatically calculated.

Multiple tabs are used in the case of color images where thresholding can be done on each individual color component using RGB or HLS color space.



The **Image Histogram Display** shows the current image histogram(s) with a box outlining the current selected MINIMUM and MAXIMUM values. The current values are used for thresholding, and all identified pixels are indicated with brighter colors.

In the case of RGB COLOR images three threshold ranges are defined, one for each color component, and are combined together to determine if each pixel is within all three ranges before it is identified in GREEN.

*The options in the **Identify** Dialogs are described below:*

Minimum

The MINIMUM intensity is displayed in the left hand slot in the Identify Dialog and the upper slider. Pixel intensities have to be above or equal to this value in order to qualify as "object" pixels and be set to GREEN.

Maximum

The MAXIMUM Intensity is displayed in the right hand slot in the Identify Dialog. Pixel intensities have to be below or equal to this value in order to qualify as "object" pixels and be set to GREEN.

The Minimum and Maximum values can be entered or changed using the spin controls or dragging the sliders. As these values are stored as percentages so that changing the bit-depth after choosing a value will retain the relative positions along the sliders. For instance, choosing a value of 127 when the relevant bit-depth is 8-bits (half of the maximum 8-bit value of 255) will become 32768 (half of the maximum possible 16-bit value) if the relevant bit-depth becomes 16.

The maximum slider value depends on the relevant image bit-depth. For the identify dialog reached from the workfile's task toolbar, the relevant bit-depth is that of the capture device configuration specified in the **Capture** icon's **Device Setup**. On the other hand, for the identify dialog available from the Region of Interest's shapes toolbar, the relevant bit depth is the display bit-depth of the image display that the ROI shape layer is associated with. If the image document or field contains 16-bit (per color channel) data, this bit-depth can be adjusted using the bit-depth display button  from the Image Data View toolbar.

Training ROI (Region of Interest)

In the case of GREY images, selecting the range is probably easiest using the HISTOGRAM adjustment method. An alternative, which is the preferred method for COLOR images, is to outline a TRAINING REGION OF INTEREST (ROI) which includes the one of the objects to IDENTIFY.

The Training ROI is a collection of shapes created using the **Image Editing Toolbar**.

Once a TRAINING ROI has been selected from the ROI Dialog, clicking OK will sample the current image, find the intensity ranges for the object, and apply them to the entire image. If the identification range is not quite correct, fine adjustments can be made by using the slider controls. If the ranges are not good, use the TRAINING icon again. Be careful not to include any of the background intensities, as the automatically sampled ranges will include the background values as well as the object, which will not give an accurate identification.

When using color images, if the identification is not good using the RGB thresholds, try with the HLS Transform selected.

Runtime Menu

When this checkbox option is selected and a Workfile is being executed, an interactive Identify Dialog will pop-up. This allows the operator to check and correct the MINIMUM and MAXIMUM values for the particular image during execution. The pre-set values are applied and changes to these values are saved to the work-file when the OK button is used to exit the dialog.

If variation in object intensity between images is small, or if the object to background contrast is very high, this is not likely to be necessary. If however, the objects and background intensities overlap between images, the BINARY image of objects using the fixed Identify threshold values will cause objects to be under or over identified, and further processing and measurements may be incorrect.

This option can be used to start a Scan with interactive supervision of the Identify values, and then checked OFF when it is clear that the values are stable and do not need to be changed anymore.

If Identify is found to give a poor BINARY image, the Workfile execution can be interrupted, and the **RUN-TIME Menu** turned on to regain control.

Retain Enhanced Image

This option is simply a checkbox that allows control of which image is displayed during data collection. If the **Retain Enhanced Image** option is checked, the resulting image after the Enhance operations have been executed will be retained on display for operation on by subsequent workfile operations. If this option is not selected (the default), the original image of the sample will be recalled when the object measurements and statistics are on display.

Unchecking **Retain Enhanced Image** is useful in the case that the Enhance operations create a visually distracting image simply to improve object detection in Identify, perhaps by using the Sobel operation. If Sharpen is used for example, this image may be an improvement visually, so checking this option will display this image with the object measurements instead of the original.

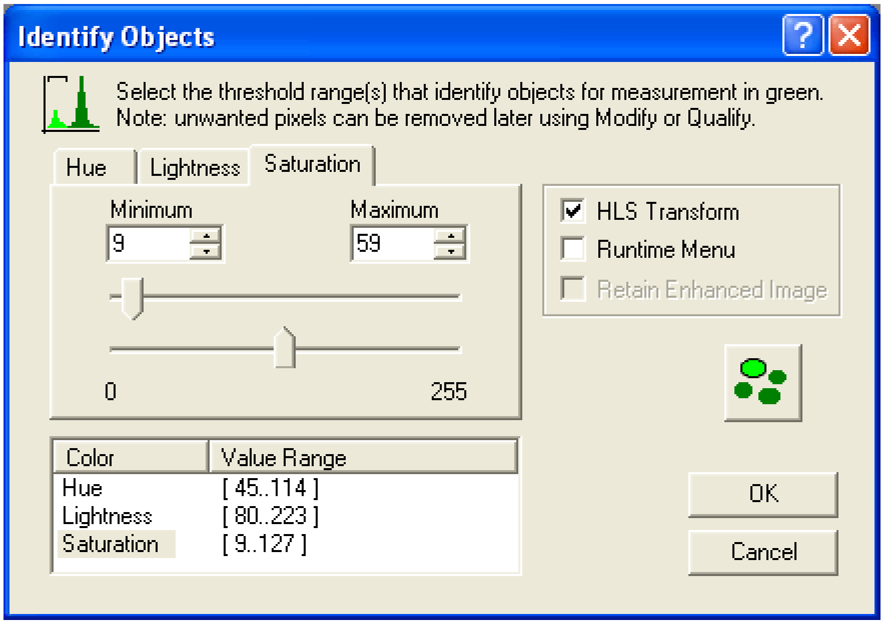
OK

The **OK** button will save the options in the WORKFILE being created or changed, and exit the Dialog.

Cancel

The **Cancel** button will recall the options selected from before this Dialog was opened, canceling any changes, and exit the Dialog.

HLS Transform



When identifying objects in a RGB color image there may be times when the color variation of an object requires such a broad identification range in the RGB mode that unwanted objects or pixels are also identified. This is often the case when lighting is not even across an image, with shadows and highlights causing apparent changes in color of a uniformly colored object, or when a stained thin section has variable thickness making the stained color appear pale in thinly cut areas.

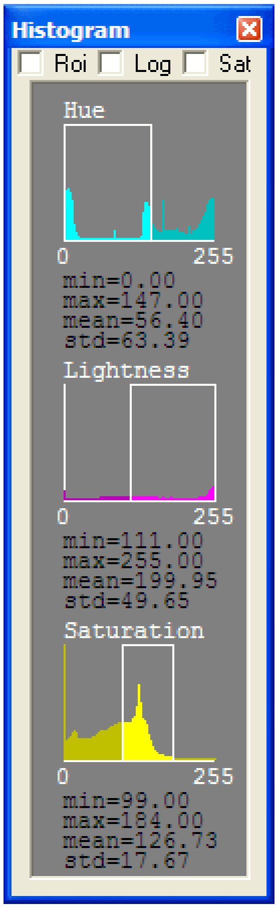
The HLS Transform will create a new image based on the RGB image values where the image has three color components represented by Hue, Lightness and Saturation.

- **Hue** values represent the position of a color along a spectrum of fully saturated (pure) colors.
- **Lightness** values are the average intensity of the RGB values, providing the weighted grey value of the pixel.
- **Saturation** values represent the purity of the color, as white is added to the pixel the Hue stays constant, but the color of the pixel appears paler.

These transformed image components make it possible to target specific color (Hue) detecting the lighter and darker shades. By selecting a narrow range for the Hue, the exact color is specified; applying broad ranges for the Saturation and Lightness coordinates will then allow for the variations in the appearance of the color to be detected without including other pure colors.

In both RGB and HLS color space, it is not obvious to humans what the color threshold values might be for any given color tone or color range. To get a first approximation of these color values use the Training Region button to select a portion of the image containing the target color and sample the intensity ranges present for each color component. The sampled values can then be further adjusted if necessary.

Note: while changing color coordinate systems does not affect the appearance of the image, choosing the appropriate color coordinate system (depending on the image data and the goal in mind) can certainly improve the potential for successfully identifying the desired objects via the specification of threshold limits.

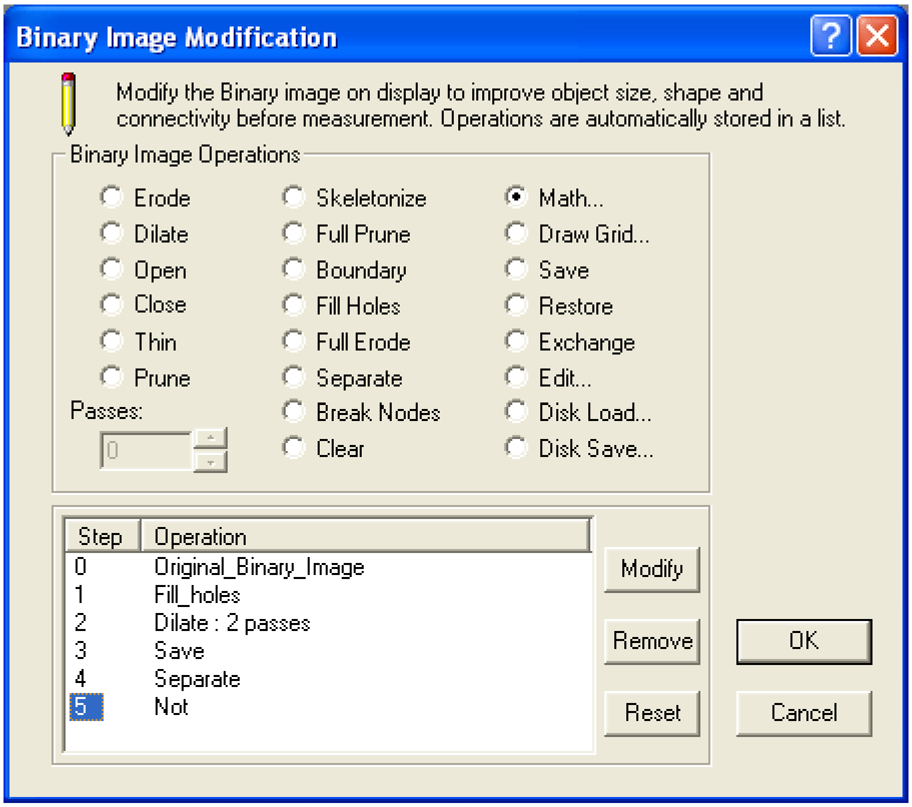


The **Image Histogram Display** shows the current image histogram(s) with a box outlining the current selected MINIMUM and MAXIMUM values. The current values are used for thresholding, and the section of each ranges histogram corresponding to the qualifying pixels are highlighted with brighter colors.

In the case of HLS COLOR images three threshold ranges are defined, one for each of Hue (color wavelength), Saturation (color purity) and Lightness (brightness), and are combined together to determine if each pixel is within all three ranges before it is identified in GREEN in the image display's overlay layer.

Modify Overview

The Image Modification options control the way that Binary Images, loaded from disk or created using the Identify Icon, can be modified using image processing techniques. The aim is typically to improve the size, shape, and connectivity of objects in such a binary image to allow better object measurements.



A combination of image enhancement operations can be applied sequentially to the Binary Image. Each time an operation is applied to the current image, the operation is added to the List. Selecting an operation in the list by clicking on its step number will allow that operation to be removed (via the **Remove** button) or replaced (via the **Modify** button), and will cause clicked operations added from the columns to be inserted immediately following the selected item. The **Reset** button will clear the list.

> **Note:** The top most item in the list represents the original image and cannot be modified or removed.

The modify operations available in the Modify Dialog are listed in the topic Binary Modifications. Most of the Modify operations use digital convolution techniques (sometimes called digital filters). These examine the Binary Image values in the immediate neighborhood of each pixel and replace the center pixel with a value computed from that information. It is not necessary to understand the mechanics of the modifications, but it is useful to know what particular modifications can do in an imaging situation. The Modify Dialog makes it easy to experiment with the different operations available to see what they do.

Multi-pass Modify operations can be re-applied repetitively to make the effects more pronounced. These operations are located in the left-most column of operations in the dialog. At the bottom of the column is a counter labeled "passes". This Pass Counter shows the number of times that the currently selected option has been applied. To re-apply the operation and increase the number of passes use the spin control. Notice that the number of passes applied counts as only one Modify operation.

The center column of options in the Modify Dialog are image processing operations that are not normally applied repetitively, although this is possible by re-selecting the same option more than once.

The right hand column of Modify options are for controlling the storage of a second Binary image in memory, for arithmetic operations between the stored and display images, and for loading and saving disk images.

As a note of caution, the best modification is often no modification. If the image from the Capture Device can be optically optimized for brightness, contrast and focus, and sample preparation can provide high contrast separate objects that are easily identified, this may avoid the need to use any Modify operations, then performance, accuracy and resolution will be maximized. Each Modify operation takes a small time to operate. Applying a long series of operations or multiple-pass operations can cause artifacts to be created that may affect the validity of measurements. Again, experimentation will give knowledge and experience of the operations that are useful in different cases.

Comment field

Note: The comment at the top of the Binary Image Modification dialog, which contains by default:

Modify the Binary image on display to improve object size, shape and connectivity before measurement. Operations are automatically stored in a list.

This comment field is editable, and its contents are written and restored as part of the workfile, providing an opportunity to document the reasoning of the icons added to any particular workfile.

OK

The **Ok** button will save the current Modify options in the Workfile being created or changed, and exit the dialog.

> **Note:** Any Modify operations in the Workfile are executed automatically when the Modify Dialog is opened.

Binary Operations:

Erosion

Erode Shrinks both the interior and exterior boundaries of objects in the displayed binary image by one pixel for each pass applied. Erode can be used to shrink small objects until they disappear so that they will not be measured or counted later. Erode can have multiple passes applied, although the shape of objects after many passes may change. This operation can be combined with Dilate in the Open and Close operators (see below). For Ultimate Erosion to the minimum size of objects before extinction, see Full Erode.

Full Erode Recursively Erodes until the last pass before the object will be extinguished. Full Erode is sometimes called "Ultimate Erosion". Each object is considered separately, and so different sized objects may have a different number of passes applied.

This technique can be used in the separation process to erode all objects to their centers before growing back to the intermediate boundary.

Note that objects may have more than one pixel or group of pixels remaining after Full Erode, depending on the original shape of the object.

Dilation

Dilate Grows the interior and exterior boundaries of objects by one pixel for each pass applied. Dilate can be useful to grow parts of an object together that were separated by incorrect thresholding in Identify. Dilate can have multiple passes applied, although the shape of objects after many passes may change. This operation can be combined with Erode in the Open and Close operators.

Open Performs first Binary image Erosion and then Binary image Dilation on the displayed Binary image. The effect of Open is to enlarge gaps between objects that have incorrect tenuous connections, making them separate. Open can have multiple passes applied, although the shape of objects after many passes may change. An identical number of passes are applied first for Erode and then for Dilate. This encourages the possibility that the Eroded objects will Dilate back to their original size and shape. As the number of passes increases, smaller objects may be completely Eroded, and will not be Dilated back. This method is sometimes used to remove unwanted small objects or noise pixels.

Close = (Dilate&Erode) Cross small gaps between objects that have incorrect breaks, joining them up. Close can have multiple passes applied, although the shape of objects after many passes may change. An identical number of passes are applied first for Dilate and then for Erode. This encourages the possibility that the Dilated objects will Erode back to their original size and shape. As the number of passes increases, smaller holes within objects may be completely Closed. This method is sometimes used to remove unwanted small holes or noise pixels.

Thinning

- **Thin** Performs Binary image Thinning on the displayed Binary image. Can have multiple passes applied, although the shape of objects after many passes may change.
- **Skeletonize** "Ultimate-thin" Recursively performs Binary Image Thinning on the displayed Binary image until the ultimate skeleton of every object in the image is produced. The skeletal shape of objects after many passes may be quite different from the external shape. The skeleton is sometimes referred to as the medial axis of an object.

The effect of the Thin operator is similar to an Erode of the interior and exterior boundary of each object by one pixel for each pass applied, but prevents the objects from disappearing by avoiding further object modifying operations when the object is just one pixel thick. Use on samples which have linear objects.

Pruning

Prune Removes pixels in the image that are End Points i.e. those with only one neighbor. Normally these points are at the end of small branches on objects in the Binary image. This operation can have multiple passes applied, although no change is made when all End points are removed.

Full Prune Recursively prunes the displayed Binary image, until only connected pixels remain.

Pruning is normally applied after Thinning, which create small branches suitable for pruning. If the ultimate pruned skeleton of all objects is required, use Skeletonize, which performs Binary image Thinning until there is no more Thinning possible, and then Full Prune, which Prunes all possible branches, leaving behind a fully connected network of one pixel-thick lines.

Boundary Outlines objects in the displayed Binary image by removing all pixels not located on an interior or exterior Boundary of an object. If only the exterior Boundary is required, use the Fill Holes operation first.

Fill Holes The Fill Holes operator performs filling of objects in the displayed Binary image. The effect of the Fill Holes operator is to Fill any internal holes in the image. Note that some Enhance operations create a border around the very edge of the image, which may be thresholded in Identify, causing the entire image to be filled. Use the ROI set from the Capture Dialog to reduce the ROI so the border will be ignored when Measurements are made.

Separate

Deagglomerates objects in the displayed Binary image. The effect of the Separate operator is highly image dependent.

There are two important steps that must be taken before Separate is selected. First, select Save to store the displayed Binary image of objects, thresholded in the Identify form in memory. Second, the current Binary image on display must contain the objects Eroded by a user-selected number of passes until they are visually separated. Selecting the Separate operator will then grow back the objects until they meet, but DO NOT touch. Then the image will be processed to create a set of outlines of each Separate object, ready for further processing or Measurement. The outlines can be converted into separated objects by selecting the Not and And Binary Math operators.

A typical sequence of steps to separate objects is:

1. SAVE
2. ERODE (say 3 passes) until object group is reduced to separate cores
3. SEPARATE
4. NOT (Binary Math)
5. AND

The separated objects may appear slightly smaller than required for measurement. In this case use the advanced sequence below which uses a copy of the original image, stored temporarily on disk, to match the exact outlines of the original objects:

1. use Image Save to Disk to save the original image using FIXED_NAME "ORIGINAL.MIM"

2. DILATE (1 pass)
3. SAVE
4. ERODE (say 3 passes) until object group is reduced to separate cores
5. SEPARATE
6. NOT (Binary Math)
7. AND (Binary Math)
8. SAVE
9. Use Image Load From Disk to load the original image using FIXED_NAME "ORIGINAL.MIM"
10. EXCHANGE
11. AND (Binary Math)

After Separate, further Modify operations can be applied as necessary.

Break Nodes Pattern matches all objects in the displayed Binary image to remove pixels which are located at the intersection of two or more lines. Break Nodes should be used after the Thinning , Skeletonize and Prune operators, which have produced a network of one pixel thick lines. Break Nodes separates the connected network of lines into individual line fragments for further processing or Measurement. If Break Nodes is selected for a Binary image which is not Skeletonized, then almost all of the pixels have many neighbors, and will be removed as intersection nodes.

Binary Math Combines the Saved and Display Binary images via the logical operators listed below:

- **AND** Includes each pixel which both binary image masks include
- **OR** Includes each pixel which either binary image mask includes
- **XOR** Includes each pixel that only one of the binary image masks includes
- **NAND** Includes all pixels except those included in both binary image masks
- **NOR** Includes all pixels except those included by either binary image mask
- **NOT** Inverts all pixels in the displayed binary mask
- **XNOR** Includes pixels present in either both or neither of the binary image masks

The effect of the Math operation selected is seen on the screen before it is approved. Both the current display Binary image and the Saved Binary image are displayed on the screen in different colors. A prompt indicates which color represent the selected Math operation, and the selection can be approved or canceled. These options are useful for combining the results of modified Binary images with the Saved original image.

Draw Grid

- Vertical Grid evenly spaced vertical lines (specify spacing)
- Horizontal Grid evenly spaced horizontal lines (specify spacing)
- Rectangular Grid can look like graph paper (specify X and Y spacing)
- Radial Lines radiating out from the center (specify angular spacing in degrees)
- Concentric Circles surrounding the center (specify spacing in radial direction)
- Concentric Rectangles surround center (specify x and y spacing)
- Crosshair at center (automatically fits frame)
- Full Frame draws rectangle around entire image
- Full Circle circle which fits in entire image in pixel space
- Filled ROI - all Groups selection using the ROI -shapes layer - all shapes which are filled
- Unfilled ROI - all Groups selection using the ROI -shapes layer - all shapes which are not filled

Specifications for the grids that require a choice of spacings are in terms of pixels.

The grid overlays can be very useful for various object identification and measurement purposes when used in combination with other operations.

Memory I/O

(for Image Math)

Save Makes a duplicate copy from the Binary image currently on display to memory, replacing any image previously Saved by this option

Exchange Swaps the Saved Binary image in memory with the image currently on display

Restore Copies from the Saved Binary image currently in memory to the display, replacing the current image on display

Restore

In summary, the Saved image in the invisible background memory buffer can be used with the Logical Binary operators for arithmetic combination with other images.

Only one image can be saved to memory at one time, if logical operations involving more images is required, use File I/O.

Binary Edit

By default, the toolbars shown at left appear docked alongside the right-edge of the binary edit window:

While the **Accept/Reject toolbar** is visible, there are two exclusive modes of operation:

While either the "**Draw**" or "**Erase**- **Using Current Shape**" buttons are active, the shapes toolbar can be used to edit the object's themselves by drawing or erasing pixels in the binary layer within or between the objects, using the various tools available in the shapes toolbar.

While either the "**Accept**" or "**Reject**" buttons are activated, it is not possible to use the shapes toolbar items until the accept/reject mode is exited (by clicking on the active accept or reject button to release it). Instead, the objects already defined are toggled between being included or rejected from the current set of identified, qualified objects.

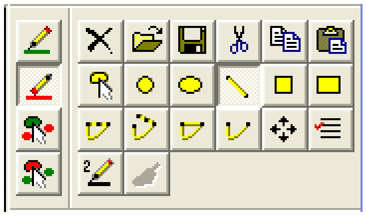
Accept - the first click on an object after activating the Accept mode will de-select all objects *except the clicked object*.

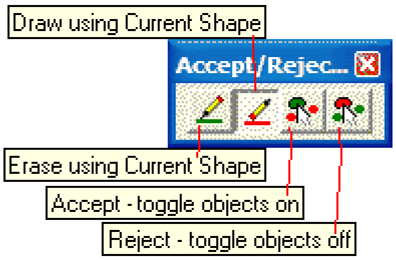
Reject - the first click on an object after activating the Reject mode will select all objects *except the clicked object*.

Subsequent clicks in Accept or Reject modes will toggle each clicked object's acceptance status between inclusion (green) and exclusion (red).

Using the right-click or button command "**Apply Shapes"** while in Apply/Reject mode will remove all red objects from the image. Applying the same command while in Draw or Erase mode will apply the drawn operations.

The image display magnification can be increased for detailed examination and fine changes. This option can be selected in between other modifications.





File I/O

Disk Load... Loads an Image from a disk file into the image currently on display, in **Fixed** or **Query** modes.

Note that image formats will be converted as necessary to fit the target image depth.

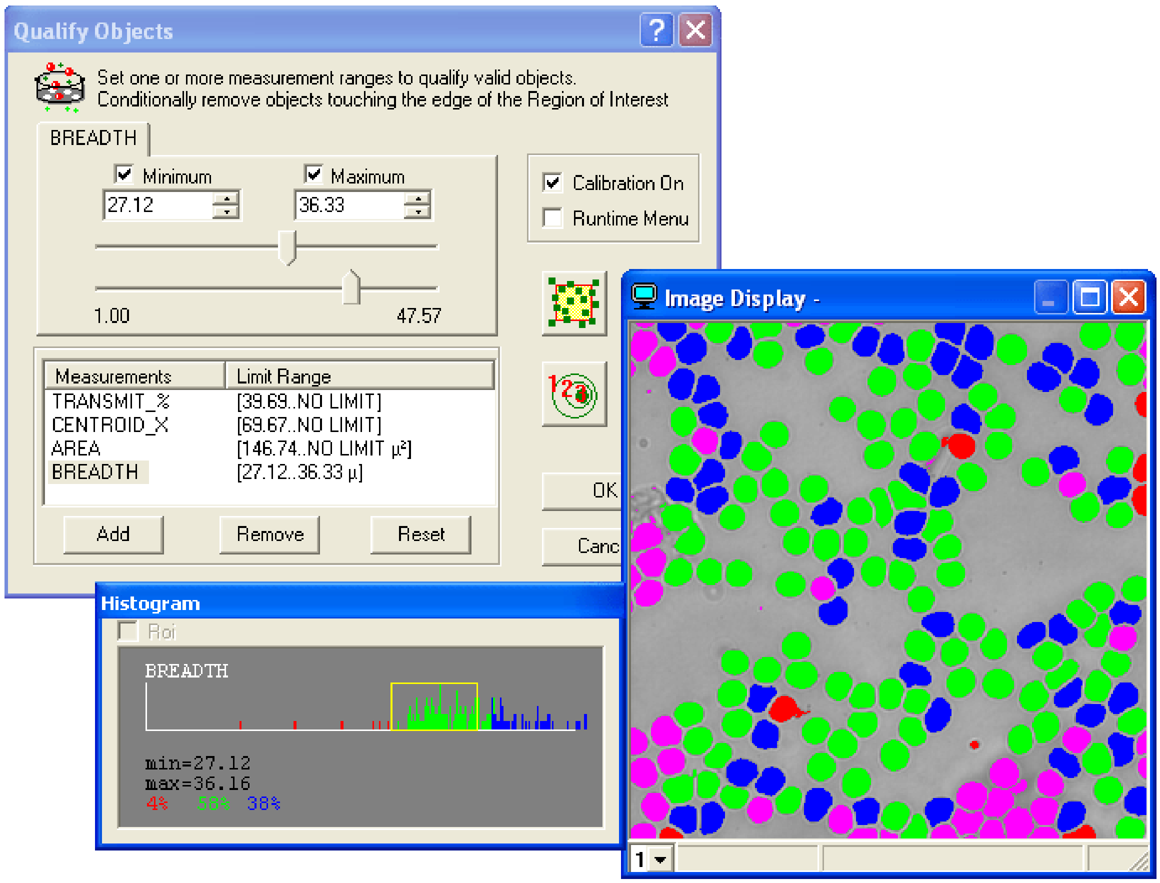
Disk Save... Saves the Image currently on display to a disk file, in **Fixed** or **Query** modes.

Query: allows an interactive Disk Save/Load Dialog to be displayed so any file can be selected

Fixed: stores the filename to load in the Workfile and uses it each time the Workfile is run.

File I/O allows automatic Disk Save/Load operations, and can be used to store temporary images to disk for use in the Workfile process. These operations are necessary if imaging arithmetic needs to involve more than two images.

Binary Image Qualification



Populations of one or more objects in the BINARY image are typically created by using the [Identify Dialog] to threshold pixels from a color or intensity image. Further processing on these BINARY image objects may have been performed using the [Modify] operations.

The **Qualify -** options filters these object populations against one or more qualification criteria as a type of filtering system to reject various objects on the basis of size, shape, intensity, position, color etc. Qualify also allows the convenient selection of **Edge Removal**, where objects that interact with the border of the image or [Region Of Interest] (ROI) can be removed.

Minimum - The MINIMUM measurement value is displayed in the left hand slot in the Qualify Measurement Tab. Object measurement values have to be above or equal to this value in order to qualify and be set to GREEN. The value can be entered or changed using the spin controls or dragging the slider. Only values between 0 and the current MAXIMUM will be accepted.

Maximum - The MAXIMUM measurement value is displayed in the right hand slot in the Qualify Measurement Tab. Object measurement values have to be below or equal to this value in order to qualify and be set to GREEN. The value can be entered or changed using the spin controls or dragging the slider. Values can be entered that are above the range of object measurements in the current image.

Apply Calibration - This checkbox option toggles the display of the Threshold MINIMUM and MAXIMUM measurement range to display in calibrated units, or pixels. Both the Qualify tab and the Image Histogram Display data will reflect this selection.

Note: If **Calibration On** is checked on exit from the Qualify Dialog, the values saved in the Workfile will be saved in calibrated units according to the current scale factor as selected in the Spatial Calibration Dialog. If it is not checked, the Qualify range will be saved in pixels. This makes it possible to use the same Qualify range with the same units, but different magnifications.

One or more Measurements can be selected to the **Qualification Criteria List**, although each measurement can be selected only once.

Select Measurement column

The left-hand column in the list displays the name of each qualification. Click on a measurement name to select it.

While a measurement is selected, its threshold values can be adjusted with the controls above the measurement list.

The image display overlay layer will display object qualification status by color-coding each object.

Color		**Qualification Status**
Red		Object is disqualified (and pending deletion) by the minimum value threshold of the currently selected qualification criteria
Green		Object is not disqualified by any of the listed criteria
Blue		Object is disqualified (and pending deletion) by the maximum value threshold of the currently selected qualification criteria
Magenta		Object is disqualified (and pending deletion) by a threshold of another pending measurement.

Measurement Range column

The right-hand column in the list displays the minimum and maximum limit definitions for each qualification.

When multiple measurements are added to the list, selecting a measurement will reconfigure the display so that the current measurement range will show in red, green or blue for adjustment. The list will re-order itself in order to list the currently selected measurement last, since a given criterion may only be used to eliminate objects not yet eliminated by other criteria, not to re-instate them.

Objects that are disqualified by other measurements in the list are displayed in Magenta.

To add items to the qualification criteria list, click the **Add** button and select the measurement to use for the new criteria. All **Object Measurements** and **Custom Measurements** can be used.

To remove a qualification criteria from the list, select the item in the list and click Remove.

To remove all criteria from the list, click the Reset button.

Qualification Histogram

The **Image Histogram Display** is used to report the current **Qualify Measurement Range** with the corresponding criterion values and the percentages of the total number of objects rejected for failing to meet the minimum limit, accepted, or rejected for exceeding the maximum limit. The histogram uses the color codes for red, green, and blue but only the population remaining after all other criteria have been applied is depicted, so bin-counts corresponding to original objects shown in the display with color-code magenta do not appear in the histogram

Runtime Menu

When this checkbox option is selected and a Workfile is being executed, an interactive Qualify Dialog will pop-up. This allows the operator to check and correct the qualification criteria limiting values for the particular image during execution. The pre-set values are applied and changes to these values are saved to the workfile when the OK button is used to exit the dialog.

This option can be used to start a Scan with interactive supervision of the Qualify values, and then can be checked OFF when it is clear that the values are stable and do not need to be changed anymore.

Edge Removal

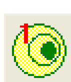
When using a ROI to restrict the processing and analysis area of an image, it is common that objects will be truncated by the edge of the ROI, and therefore incorrectly measured. Pressing this button will open a choice of rules to apply when processing objects on or outside the ROI.

Note: The **Measurement ROI** is set in the **Capture Dialog**.

Rule	**Effect**
No edge applied	This will not affect objects in the image.
Delete all edge objects	This option will remove any objects that touch the Measurement Region Of Interest. This can ensure that no truncated objects are measured, but may decrease the object count per unit area, especially as larger objects may be rejected more frequently than smaller ones.

Cut off objects outside the edge	This option applies the Region Of Interest as a pixel mask. All pixels that fall outside the ROI are rejected, cutting border objects in two. This truncation may reduce the measured size of some objects, but is still useful when counting objects, or for preparing the image for Area Fraction measurements.
Include objects that touch the edge	Where Delete all edge objects (above) will remove any object that touches the ROI, this option includes them but removes all objects that are completely outside of the ROI. Objects that are within the ROI are still included.
Include objects that touch the Right or Bottom edge	This option applies an algorithm to remove objects that touch the top or left side of the ROI and include objects within or touching the bottom and right sides of the ROI. This mechanism is used in Stereology where large areas of a sample are scanned, typically using a motorized XY stage, and where adjacent fields are measured. The bounding rectangle of the ROI is used as the measuring region, and the area outside the ROI is used as a Guard region to ensure that objects can be measured in full without being truncated by the edge of the screen. This exclusion mechanism makes sure that objects are not counted twice. To ensure the success of this method, the guard region outside of the ROI should be the width and height of the largest object to be measured.

Nested/Filled Mode

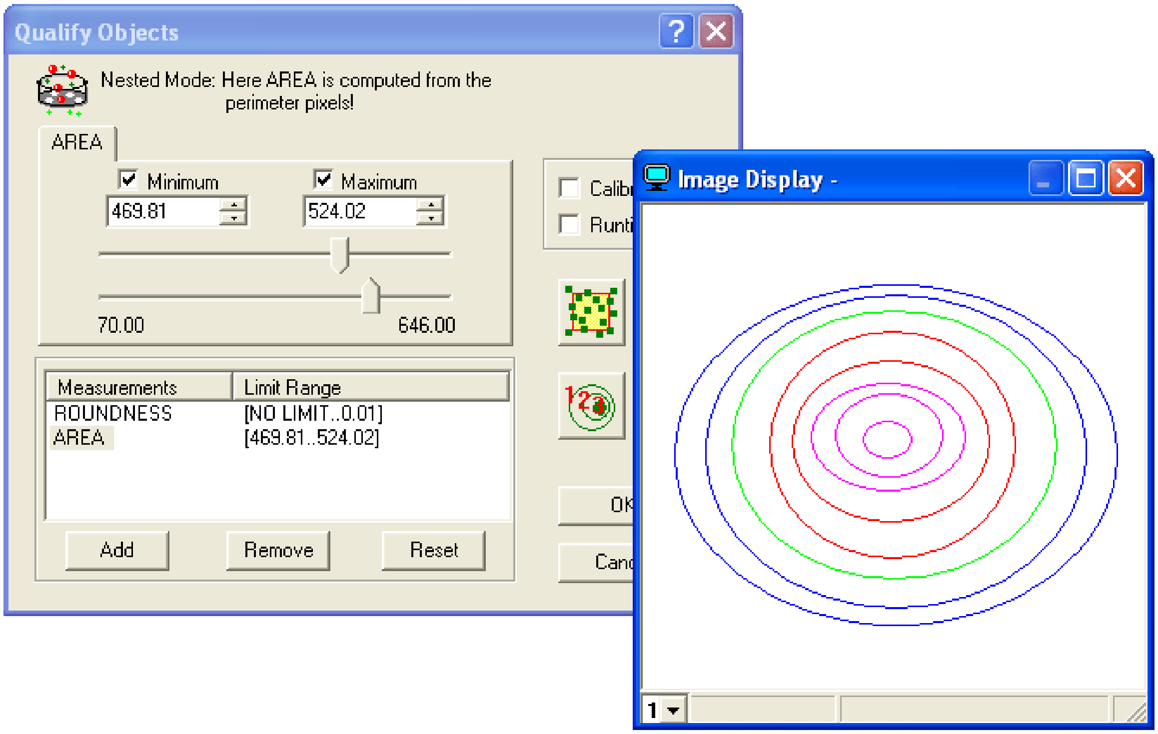
Mode	Icon	Description
Nested		Objects are measured separately even if nested inside one another.
Filled		Each object's area is filled in and conglomerated with any interior objects into a single object prior to making measurements.

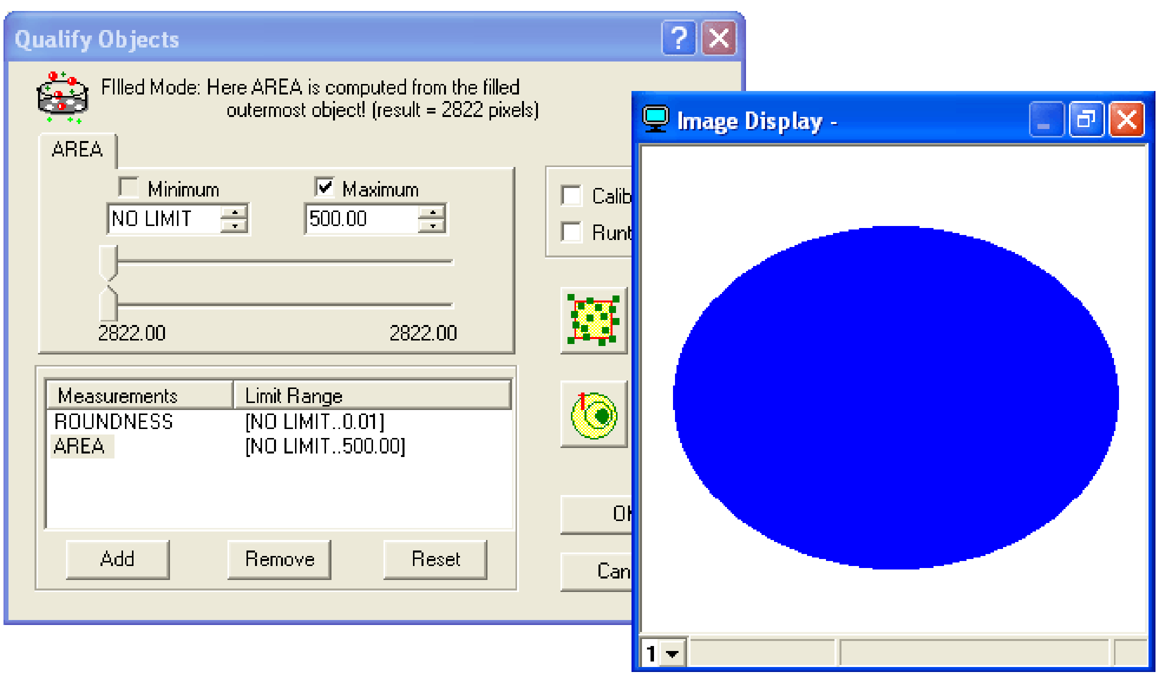
Click *here* to see Qualify Nested/Filled Examples

Exiting the dialog via the **OK** button will not only save the options in the Workfile being created or changed, but also act immediately to delete disqualified objects from memory. Since these objects will no longer exist, they will no longer be available to any of the Workfile icons, including any icons in the Workfile which would execute prior to the just-completed Qualify step while in Workfile Run mode, (and the Qualify dialog itself if re-opened immediately in Edit mode). Thus, completing a qualify step via the OK button and then re-opening the same Qualify icon will display only the green accepted objects, since the previously red, blue, and magenta objects have been deleted.

Therefore, to review the effect of a given qualification step while in Workfile edit mode, it is necessary to click on each of the preceding steps and exit them via their OK buttons to recreate those members of the original object population which the Qualify icon already eliminated on the previous execution.

Qualify Nested/Filled Examples





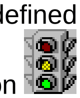
Measure

The **Measure** option allows the selection of which Object and Field measurements should be computed and collected.

Image Document Measure Image functionality:

Within an image document, the **Measure Image** button can be used to open the above dialog to make measurements shapes contained in the image documents ROI Shapes layer. Some of these shapes may have been defined manually using the ROI Shapes Layer's Shapes toolbar drawing tools; others may have been created interactively using threshold identification and if selected, the **Advanced Detection** (Modify and Qualify) **functions**, under operator guidance using that toolbar's **Create Shapes from Overlay** functions.

Note*: It is crucial to understand the ramifications of filled vs. outline shapes on image measurements.*

Within a data document, a workfile's defined measurements are made when in **Collect** mode, activated by the **Start Collecting** icon  in the Data Document's Image Processing Toolbar. The task toolbar then enters its Collect Data mode which allows a Scan to be created for automatic or semi-interactive Capture, Processing and Measurement, where data is recorded into a Data Document for review. If new to measurements using work-files in SimplePCI, please consult the Measuring Overview of the entire procedure used for collecting measurement data.

Select Measurements

The **Select Measurements** Dialog shows two tabs each with a list of measurements that can be selected. Only checked items will be selected for measurement. Use the **View** radio buttons to restrict the displayed list to specific categories.

There are two levels of measurements to select from: Object Measurements and Field Measurements.

Object Measurements are made on a per-object basis, with one set of measurements for each object detected in a field. These measurements are typically of morphological characteristics of the objects detected, and can include Size, Shape, Position, Intensity, Color, Count etc. The Object Measurement data are collected and summarized in **Object Measurement Statistics**, and displayed in the current active **Data Document**. The number of objects per field may be large (in the thousands), and with many parameters per object the data can become large. Because of this the option to save Object Measurements can be selected from the **Record** button in the Collect Data Toolbar. If these data are saved for each field, they can be reviewed in detail and summarized in graphical form after collection has finished. If the data are not recorded only the Statistical summaries may be reviewed. This may be intentional as it can significantly reduce the amount of data for storage or transmission, and a statistical summary may be an acceptable account of the analysis.

Field Measurements are made on a per-field basis, with one set of measurements for each Field analyzed in a Scan. The Field measurements are typically summaries of the Object Measurements, e.g. Total count, Total Area, Area Fraction etc., and also include summaries of the Measurement **Region of Interest** e.g. ROI Mean Intensity, ROI Area etc., and other system parameters such as X,Y,Z stage position. The number of Fields is typically smaller then the number of objects and may number in the tens, or hundreds, although thousands of fields can be analyzed in a Scan. The Field measurement data are also summarized in **Field Summary**

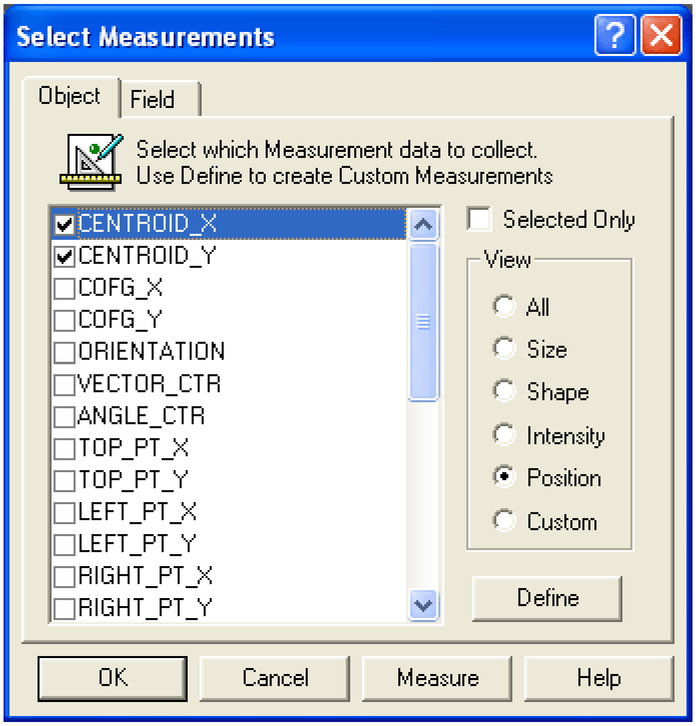
Statistics and are stored in the **Data Document.** As the Field data is of a smaller nature than the Object Data it can be stored in detail with less overhead.

In addition **Custom Measurements** can be created by customizing arithmetical combinations of the available object and field measurements. To create your own measurements, Press **Define**.

These measurement types are summarized in the following sections:

- **Object Measurements**
- **Field Measurements**
- **Custom Measurements**

Select Object Measurements



Clicking the Measure Icon will pop-up a form which has a checklist of selectable measurements.

A Tab-control at the top is used to select the level of measurements, i.e. at the FIELD or OBJECTS level.

A set of radio buttons (grouped under "View") can be used to display all measurements simultaneously or, to make individual measurement selection easier, only a subset of those measurements. If the **Selected Only** checkbox is checked, only measurements which have already been selected will be listed.

The **Define** button is used to open the Custom Measurement Menu for creating arithmetic expressions containing combinations of direct measurements to evaluate more useful results within this application.

These of the following three actions will exit the SELECT MEASUREMENT form :

- **Measure:** Perform the selected measurements on the current data document.

Note: there must already be objects defined (i.e. the result of Identify and if desired, Modify and Qualify operations) to be able to meaningfully conduct any selected object measurements. Use the Image Overlay layer button on the Image Display toolbar to overlay the image display with a bright green highlighted binary image covering all pixels which are considered part of any current objects.

Object Measurements List

OBJECT MEASUREMENTS

The object measurements available for selection are listed below in related groups:

- Size
- Shape
- Intensity/Color
- Position
- Custom object measurements

See also the detailed section on selecting **Object Measurements**.

Object Size Measurements

Measurements relating primarily to object size as measured from a Binary image, typically based on a cross-sectional or plane view.

Area	Count of the Pixels detected in an object not including holes. (Sometimes called Detected Area)
Filled Area	Total area enclosed by the boundary of the object after holes are completely filled
Zone Area	Expanded area of each object, equivalent to its zone of influence, found by growing each object with equal priority until it reaches its neighbors to give an indication of spatial distribution within an image. Sometimes called "nearest neighbor analysis". If the Zone_Area measurements are all similar, the distribution is likely to be uniform, if the Zone_Area measurements are diverse, the objects are likely to be grouped in some areas and spread apart in others. As Zone_Area is measured the image display shows each object in an individual color as it grows until it reaches the extreme of its territory. The Zone_Area measurement process can take a long time if the objects are very spread out. In this case, the measurement may not be a good indicator of spatial distribution, as the edge effects will be pronounced.
Boundary	Total number of pixels located on the edge of the object. i.e. the Area of an object's Boundary. Ignores the direction between neighboring pixels to produce results faster than with the measurement, although less accurately. **Difference between Boundary and Perimeter:** Consider measuring the Boundary and Perimeter of an object with the shape of a magnifying glass which has a handle one pixel thick, and a circular lens ten pixels in diameter. The Perimeter measurement will circumnavigate the object and give a value approximating the circumference of a circle for the glass, plus two times the length of the handle. The Boundary measurement will approximate the circumference of the circle, plus ONE times the length of the handle, as the handle has only one line of pixels.
Breadth	Average thickness of a curvilinear thin object using the approximation Breadth ~ 0.25 * (PERIMETER - (PERIMETER^2 - 16 * AREA)^0.5) *Does NOT yield accurate information when applied to round or rectangular objects with a small aspect ratio (e.g. 1.0 to 2.0).*
Max Breadth	The **Maximum_Breadth** parameter is the maximum projected size of the object at right angles to the Max_Length. This distance is then calibrated into measurement units. The ratio of these two measurements is typically used to compute the Aspect_Ratio shape measurement. Note that the projections either side of the Max_Length may not be in line with each other. To reconstruct Max_Breadth and Max_Length if the image is still on display, use the Object Sample (Eye-Dropper) icon in the Data Document Toolbar, and click on an object.
Diameter	Assumes object is a circle and computes effective diameter using Diameter = 2 * ((AREA / PI) ^ 0.5). See also Hole Diameter.
Filled Diameter	The **Effective Circular Diameter** computed from the Filled_Area measurement. This is used in the case that the outer diameter is required for an object which has one or more holes. Another use is to compare the inner and outer diameters of an object with a single hole. This measurement can be combined with the

	Hole_Diameter by creating a Custom Object Measurement.
Width	Maximum dimension of the object in the X-axis direction (horizontal on-screen). Also called the Horizontal Feret Diameter. Width can be averaged with Height to give an estimate of object Diameter, especially if the objects are circular. The difference between this estimate of Diameter and the Effective Circular Diameter (which is estimated from Area) can be used to give a shape factor index relating to eccentricity while the ratio of these quantities can be used as an estimate of horizontal-to-vertical aspect ratio.
Height	Maximum dimension of the object in the Y-axis direction (vertical on-screen). Also called the Vertical Feret Diameter. Height can be averaged with Width , (see above) to give an estimate of object Diameter, especially if the objects are circular. The difference between this estimate of Diameter and the (which is estimated from Area) can be used to give a shape factor index relating to eccentricity; the ratio of these quantities can be used as an estimate of horizontal-to-vertical aspect ratio.
Hole area %	Total area of holes enclosed by the boundary of the object as a percentage of the Filled_Area.
Hole count	Counts the number of enclosed holes in an object.
Hole diameter	Diameter of a single hole estimated from the Hole Area % measurement assuming object contains a single circular hole. This measurement can be combined with the Filled_Diameter by creating a Custom Object Measurement.
Intercepts (0°,45°,90°,135°)	Number of times that a pixel is "intercepted" in a given direction across the image. Intercepts can be calculated in four directions, which can be later combined to give information regarding shape and size of objects. Note that these measurements are at the object level, but in the Statistics display, the Total values of these measurements are given, to describe the measurements at the Field level.
Length	Longest distance along a curvilinear thin object, using the approximation Length ~ 1/4 (PERIMETER + (PERIMETER^2 - 16*AREA)^0.5) *Does NOT yield accurate information when applied to round or rectangular objects with a small aspect ratio (e.g. 1.0 to 2.0)*
Max length	Longest distance between any two points on the boundary of an object. Sometimes called the maximum diameter, or maximum Feret diameter. This boundary-based measurement: 1. finds the center of the object (see) 2. searches for the furthest boundary point from the Cofg 3. searches for the maximum distance on the boundary away from the furthest point, until the most distant points are defined. This distance is then calibrated into measurement units. To reconstruct Max_Breadth and Max_Length if the image is still on display, use the Object Sample (Eye-Dropper) icon in the Data Document Toolbar, and click on an object.
End point length	Used when measuring elongated objects that have been processed by Skeletonize and Break Nodes in the . The disconnected one-pixel-thick objects are processed to measure the distance between the two ends of the skeleton, and the straight line distance is recorded. This measurement is used to compute Tortuosity. **NOTE**: if the objects in the image are not one pixel thick, the results will be unpredictable. For example, a circular object has no end-points, and will have an End_Point_Length of zero.

Perimeter	The **Perimeter** of an object represents the distance traveled around the boundary of the object. Perimeter can be used to indicate the degree or irregularity of objects. Note that object Perimeter is measured as the total exterior of the object plus the interior perimeter if there are any holes. If only exterior Perimeter is required, and the objects do have holes, use the Fill Holes option in Modify. **Note:** to compare Perimeter between objects in different images, it is important that the images are of similar magnifications. The magnification of an object, especially one with an irregular boundary can significantly change the amount of detail visible. The more detail in the boundary, the longer the distance around its edge, and the larger the Perimeter measurement. Objects in the same image can be directly compared. Perimeter can be used as an estimate of the curvilinear length of lines that are one pixel thick, (after Skeletonize in Modify). The length of the line is measured twice along its length, and so is equal to the Perimeter divided by two. The distance around the edge of an object can be measured in several ways. There are two methods. Perimeter measures the distance from each pixel to the next around the edge of the object using a one pixel increment for straight lines to the pixel neighbors up, right, down and left, and a Root Two increment for diagonal lines to the four neighboring corners. The result is a Perimeter measurement that takes full account of the eight-way connectivity of pixel neighbors. **i.e.: PERIMETER = Horizontal pixels + vertical pixels + 1.4142 * diagonal pixels** In practice a weighting factor is necessary to eliminate some of the inaccuracy from aliasing effects when computing the PERIMETER. Perimeter is computed using this expression: **i.e.: PERIMETER = Horizontal pixels*0.948 + Vertical pixels*0.948 + Diagonal pixels*1.340** The component Perimeter measurements are available separately as Hz_Perimeter, Vt_Perimeter and Dg_Perimeter, described below, allowing the user to modify this formula by creating a Custom Measurement as described in the Analysis chapter. The second method for estimating Perimeter is the Boundary measurement.
Hz perimeter	Counts the number of **horizontal** pixels around the boundary of an object.
Vt perimeter	Counts the number of **vertical** pixels around the boundary of an object.
Dg perimeter	Counts the number of **diagonal** pixels around the boundary of an object.
Min Radius	Shortest distance from the center of an object to the boundary of the object. See Cofg (center of gravity).
Max Radius	Longest distance from the center of an object to the boundary of the object. A comparison of the Min_Radius and Max_Radius can give an indication of circularity for objects with a smooth boundary - they are equal for a true circle. See Cofg (center of gravity) for details on the determination of the center of the object.
Volume	The Equivalent Spherical Volume of an object, assumed to be a sphere is computed from Volume = 4/3 * pi * (DIAMETER / 2) ^3

Object Shape Measurements

Aspect ratio	Ratio of the Max_Length to Max_Breadth. Gives a value close to 1.0 for circular features, and a higher value for elongated objects. Suitable for objects that are not long and thin, for which **Elongation** is more effective.
Elongation	Aspect ratio of an object which is long and thin, computed from the formula Elongation = Length / Breadth
Roundness	Computed from the and via the formula: Roundness = (4 * pi * AREA) / ((PERIMETER) ^ (- 0.5)) As the Perimeter of the object increases in circular proportion to its AREA, the value is stationary around 1.0, if the Perimeter increases more rapidly than Area, e.g. for irregular objects, the value decreases. Due to the digital nature of imaging measurements, the Perimeter of a single pixel is theoretical, and so measurements of very small objects, with say, only a few pixels, may produce Roundness values that are significantly higher than 1.0.
Tortuosity	Degree to which an elongated object curves relative to its axis. The object must have been processed by Skeletonize and Break Nodes in the Modify Menu . The disconnected one-pixel-thick objects are processed to compute the ratio of the curved and the straight line distance between the two end points. A Tortuosity measurement close to 1.0 will indicate that the object was very straight, where the is close to the curvilinear length. A Tortuosity of 0.5 indicates a very curved object, such as a semicircle, or a horseshoe. NOTE: if the objects in the image are not one pixel thick, the results will be unpredictable. For example, a circular object has no end-points, and will have an End_Point_Length of zero, so the Tortuosity will be wrong.

Object Intensity/Color Measurements

For normal Grey or Color images the grey-scale range for intensity-based measurements is 0-255. For images created with grey-scale ranges greater than 0-255 the grey-level measurements will made using intensities from objects in the original 8-16 bit image components.

Absorbance		Computed from the formula ABSORBANCE = Log10(1/Transmittance) and calibrated in Optical Density (O.D.) units
		Absorbance values typically found from TV camera images are between 0 and 1.7, with a maximum of 2.4. To achieve higher Absorbance values, a densitometer may be necessary.
	Absorbance notes:	if using images greater than 8-bit, perhaps from a digital camera input or using Window and Level, the Absorbance values increase accordingly.
Grey-level		Average of the Grey-level intensity of each pixel in the object.
		The Grey-levels in the Grey image follow the convention ([0 ...

		255] ~ [black...white]). An object that is very bright will have a Grey-level larger than an object of the same size that is dark. Even if the objects are of different size, the Grey-level can be used to indicate any difference in average brightness.
	Greylevel Notes	Grey-level intensity measurements can be computed from RGB color images using part of the Hue/Saturation/Intensity HSI transform.
Intensity Statistics	**Min_grey**	minimum occurring grey-level value
	Max_Grey	maximum occurring grey-level value
	Sdev_grey	standard deviation of grey-level values
	Range_grey	range of grey-level values

	Mode_grey	most frequently occurring grey-level value
	Modef_grey	frequency of the most frequently occurring grey-level value
Intensity Statistics Notes:		These statistics describe the distribution of pixel intensity values per object.
Total_Grey		Sum of the Grey levels of each pixel in the object. The Grey levels in the Grey image follow the convention ([0 ... 255] ~ [black...white]). An object that is very bright will have a Total_Grey larger than an object of the same size that is dark. If the objects are of different size and of different brightness, their Total_Grey values may be similar. *The largest* **Total_Grey** *value that can be measured does have a limit, 16777215, which can be reached if a VERY large,*

		VERY bright object is measured. If this is the case, Invert the Grey image in Enhance, and measure the Total_Grey using the opposite intensity.
	Total_Grey Notes:	Total_Grey can be used as an indicator or how optically dense objects are, especially if their size is uniform, or if the size and intensity combination is useful. If it is necessary to relate objects strictly on the basis of average intensity, and they have different sizes, use Greylevel. Greylevel intensity measurements can be computed from RGB color images using part of the Hue/Saturation/Intensity HSI transform.
Mean Color Value	**Mean Red**	By using the component R,G,B values, color indices can be created for other color standards by creating Custom Measurements that combine these values with the appropriate weighting factors.
	Mean Green	
	Mean Blue	

	Mean Hue	These values represent the average color value and purity of an object measured from an RGB image that is first transformed into a HSI image.
	Mean Saturation	
	Mean Color Value Notes:	These measurements represent the average color intensity of an object measured from an RGB image. The measurements are computed as for Gray level, for each color component individually, at intensity levels [0 ... 255]. In the case of Hue, the color value represents the position along a spectrum of pure colors. For saturation, the intensity represents the purity of the color, less pure objects have more white light from poor staining or illumination, representing a lower saturation value.
Mean dose		Average grey-level intensity of each pixel in the object calibrated in the units selected in the Intensity

		Calibration Menu of the Capture dialog.
		The Dose levels in the grey image follow the convention ([0 ... 255] ~ [black...white]), calibrated using the linear or non-linear curve-fit selected via Intensity Calibration.
	Mean Dose Notes:	One typical application of this measurement is comparing the amount of activity in objects *of different sizes*. See Total dose.
Total Color Value	**Total Red**	These values represent the total color intensity of an object measured from an RGB image. The measurements are computed as for Total_GreyMass, above for each color component individually, summing each pixel between [0 ... 255] intensity levels.
	Total Green	
	Total Blue	
	Total Hue	These values represent the total color value and purity of an object measured from an RGB
	Total Saturation	

		image that is first transformed into a HSI image. The measurements are computed as for Total_Grey above for each color component individually, between [0 ... 255] intensity levels.
	Total Color Value Notes:	The measurements are computed as for Total_Grey, above for each color component individually, summing each pixel between [0 ... 255] intensity levels. In the case of Hue, the color value represents the position along a spectrum of pure colors. For saturation, the intensity represents the purity of the color, less pure objects have more white light from poor staining or illumination, representing a lower saturation value.
Total Dose		Total of the grey-level intensities of each pixel in the object

		calibrated in the units selected in the Intensity Calibration Menu in the Capture Menu.
		The Dose levels in the grey image range from 0 to 255, black, to white, calibrated using the linear or non-linear curve-fit selected via Intensity Calibration.
	Total Dose Notes:	One typical application of this measurement is comparing the amount of activity in objects *of similar sizes*. See Mean dose. To compute further object or field grey-level measurements of Dose, use the grey-level statistic measurements in a Custom Measurement combined with the Field measurements of Dose Intercept and Dose Gradient.
Transmittance %		Percentage of light that each object transmitted, assuming that an object with 100

		% transmittance will have an average Grey level of 255 and an object with 0 % transmittance will have a mean Grey Level of zero.
	Transmittance Notes:	Because of the assumptions for this measurement, it is important that the background intensity of an image be at or close to maximum. Use Shade Correction in the Capture Menu to achieve this.

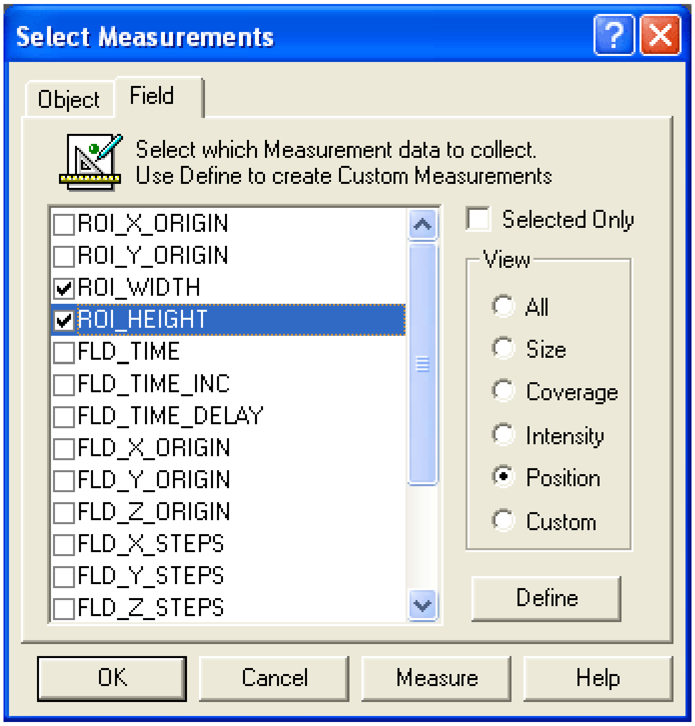
Object Position Measurements

Centroid	**x**	Center horizontal coordinate of bounding box for the object measured relative to the left edge of the image
	y	Center vertical coordinate of the bounding box for the object measured relative to the top of the image
	Centroid notes	Computed more rapidly than Center of Gravity (cofg). Centroid is useful for identifying the position of objects, and can be used for spatial distribution statistics.
Cofg	**x**	Object's center-of-gravity (horizontal coordinate) computed as the average X-coordinate of all pixels on its boundary.
	y	Object's center-of-gravity (vertical coordinate) computed as the average Y-coordinate of all pixels on its boundary.
	Center of Gravity Notes:	The cofg measurements are computed from the each of the boundary coordinates and yield a more accurate location than Centroid.

		To reconstruct the Cofg_X use the Examine icon in the Statistics Object Measurement display, and click on an object.
Orientation		Estimates the direction of the objects principal axis.
	Orientation Notes:	Requires the computation of many other measurements including Cofg_X, Cofg_Y, and Max_Length. Result is measured in degrees clockwise with the top of the image being North or zero degrees.
Vector to center		Distance from the center of the image to the center of each object..
Angle to center		Angle from the center of the image to the center of each object measured in degrees clockwise from a zero degrees vector pointing "north" to the top of the image
	Vector-to-center, Angle-to-center Notes:	One application where this method of recording spatial information about each object is typically useful is the analysis of X-ray crystallography images which display patterns of spots.
Object Coordinates	**Bounding Box**	Coordinates of the intersection of the object with its bounding box These data can be used to selectively qualify objects on one of more edges of the Measurement ROI . **Top_pt_x Top_pt_y** Left_pt_x Left_pt_y Bottom_pt_x Bottom_pt_y Right_pt_x Right_pt_y
	Maximum Dimension	Coordinates of the lines used to measure the Max_length and the Max_Breadth, Length_x1 Length_y1 Length_x2 Length_y2 Breadth_x1 Breadth_x1 Breadth_x2 Breadth_y2
	Object Coordinates Notes:	Measured from the top left corner of the image screen as the origin, (0,0). They are not scaled using the calibration factor.
Object #		This "measurement" records the index of each object in a field, approximating the objects sequential position from left to right, top to bottom in the image. Object numbers should not be used for positional information, as the object number also depends on the size and shape of the object. The **Object_No**. is saved into datafiles by default.

Class #		The **Class_No.** is the number of the current measurement Class being measured. This measurement may be used when data is saved to a file, to allow selection of data based on Class. The Class No. is saved into .CSV Data Documents by default.
Field #		The **Field_No**. is the number of the current field being measured. This measurement may be used when data is saved to a file, to allow selection of data based on Field. The Field_No. is saved into Data Documents by default.
Object #, Class #, Field # Notes:		When exporting **.FCS data files** from Data Documents, it may be useful to include the Object#, Class# and Field# as data fields. Class # and Field # measurements are also available as Field measurements.

Field Measurements



Clicking the Measure Icon will pop-up a form which has a check list of selected measurements, and a set of buttons for selecting measurements. The checkable measurements indicate which options have been selected. The buttons are used to list categories of measurements, and to exit from the dialog. If OKAY is clicked, the current selection of MEASUREMENTS for the WORKFILE is updated, if CANCEL is clicked, the previous selection of MEASUREMENTS is not changed.

The buttons on the right side of the dialog are used to select a subset of the full measurement list to make individual measurement selection easier. The groups are related to the kind of measurements to be made, SIZE, SHAPE, POSITION etc. The Tabs are used to select the level of measurements, i.e. at the FIELD or OBJECTS level.

Field Measurements List

The Field measurements available for selection are listed below in related groups:

- Size
- Coverage
- Intensity/Color
- Position
- Custom Field Measurements

See also the detailed section on selecting **Field Measurements.**

Field Size Measurements

Measurements relating primarily to object size as measured from a Binary image, typically based on a cross-sectional or plane view.

Intercepts	**0°**	Number of intercepts in the vertical directions.
	45°	Number of intercepts in the upper-right and lower-left directions.
	90°	Number of intercepts in the horizontal directions.
	135°	Number of intercepts in the upper-left and lower-right directions.
	Mean	Average of the number of intercepts found along each of the four directions defined above.
	Intercept Notes:	The INTERCEPT measurements compute the number of times that a pixel is "intercepted" in a given direction across the image. Intercepts describe something about the shape and size of objects. Intercept measurements can also be made per individual Object.
Object Area		Sum of the **Area** measurements for each object.
	Object Area Notes:	The Object Area result does not include holes.
ROI Area		Total area considered for measurement analysis

ROI Area Notes:	The ROI was defined in the Region Of Interest dialog from the Capture settings window, and can be any shape. See also: ROI Width, ROI Height.

Field Coverage Measurements

These Field Measurements indicate the Coverage, or Distribution, of objects in a Field.

Name		What is measured:
Density		Ratio of OBJECT_COUNT over ROI_AREA, giving the count density per unit area. Used to compare the frequency of occurrence of objects between images *where the ROI may change*. Complements Object Count.
Fraction (Area)		Ratio of the OBJECT_AREA to the ROI_AREA, which indicates the relative proportion of the ROI detected as objects. Area Fraction is measured as a percentage value, e.g. 52.4%.
Object Count		Number of separate objects found in the image. Used to compare the frequency of occurrence of objects between images *where the ROI does not change*. Complements Density.
Object Identification Phase Status	**PHASE 1**	Percentage of ROI between zero and LOW (inclusive). *Note: zero if the LOW value is zero.*
	PHASE 2	Percentage of ROI between LOW and HIGH levels (inclusive)
	PHASE 3	Percentage of ROI between HIGH and the maximum GREY-level (inclusive). *Note: Zero if HIGH equals maximum possible Grey-level.*

Object Identify Phase Notes:	LOW_ and HIGH_ refer here to the minimum and maximum levels set in the Identification screen, respectively. The maximum possible Grey-level is 255 for an 8-bit image, 16383 for a 16-bit image, ... 2^N - 1 for an N-bit image. Object Identification PHASE status measurements are not valid for RGB Color images.

Intensity/Color Field Measurements

For normal Grey or Color video images the grey scale range for intensity based measurements is 0-255. For images created with digital cameras, when the grey scale range can be greater than 0-255, the intensity measurements will be made using the full grey scale range available in the image.

These measurements are the intensity distribution statistics for the GREY, COLOR and derived COLOR image components at the time of measurement for every pixel in the measurement Region of Interest as defined in the Capture screen.

Intensity Calibration Values	**Dose Gradient**	Slope of the non-linear Intensity-to-dose calibration curve
	Dose Intercept	Y-intercept of the non-linear intensity-to-dose calibration curve
		When creating calibrated intensity measurements using the non-linear curve fitting methods provided in the DENSITY form within the CAPTURE menu, the intensities in the resulting linearized image have a calibration function: Calibrated Dose = (DOSE_ Gradient * Grey level) + DOSE_ Intercept This expression is used to calculate the MEAN_DOSE and TOTAL_DOSE object measurements described under Object Intensity/Color measurements. This expression

		can also be applied to any other Field or Object grey level statistic measured to convert them into calibrated Dose measurements also. Use these parameters in a Field or Object Custom Measurement.
ROI Color Statistics	**Monochrome**	See Grey-Level Intensity
	Red	Average of the red-channel values of each pixel in the ROI.
	Green	Average of the green- channel values of each pixel in the ROI.
	Blue	Average of the blue-channel values of each pixel in the ROI.
	Hue	Average color value of pixels in the ROI after transforming RGB coordinates into HSI coordinates, representing the position of the pixel along a spectrum of pure colors.
	Saturation	Average saturation value of pixels in the ROI after applying a transform to convert to the HSI equivalent representation of the pixel, representing the purity of the color; objects with less pure colors which include more white light (from poor staining or illumination) have a lower saturation value.
	ROI Color Statistics Notes	These measurements are statistics computed from the distribution of pixel intensity values over the entire ROI. By using the component R,G,B or H,S,I values, color indices can be created for other color standards

		by creating Custom Measurements that combine these values via appropriate weighting factors.
ROI Intensity Statistics	**Grey-level**	Average of the Grey-level (Intensity) of each pixel in the ROI using the Intensity component of the pixel after transforming into the HSI (Hue-Saturation-Intensity) equivalent
	ROI Min	minimum occurring grey-level value
	ROI Max	maximum occurring grey-level value
	ROI Sdev	standard deviation of grey-level values
	ROI Range	range of grey-level values
	ROI Mode	most frequently occurring grey-level value
	ROI Modef	frequency of the most frequently occurring grey-level value
	Intensity Statistics Notes:	These statistics describe the distribution of pixel intensity values for every pixel in the measurement Region of Interest as defined in the Capture screen.

Position Field Measurements

The position of each field can be quantified in time, X,Y,Z space and wavelength coordinates, which relate to any Scans that may have been performed.

FLD_Class_No.	Number of the current measurement Class being measured
Class Number Notes:	May be used when data is saved to a file, to allow selection of data based on Class. The FLD_Class No. is saved into .CSV Data Documents by default. For .**FCS data files**, exported from Data Documents, it may be useful to include FLD_Class_No. as a data field. This measurement is also available as an Object Measurement.

FLD_Field_No.	Number of the current field being measured
Field Number Notes:	May be used when data is saved to a file, to allow selection of data based on Field. The Field_No. is saved into Data Documents by default. For **.FCS data files**, exported from Data Documents, it may be useful to include FLD_Field_No. as a data field. This measurement is also available as an Object Measurement.
FLD_Scan_Delay	Target pause between each *series* of images in a **Scan** in seconds, entered in the Scan Wizard.
Field Scan Delay Notes:	*Example:* In the case of an XY SCAN, this delay represents the target time to wait between initiating each SCAN. This may be set to 3600 in order to run an XY SCAN once per hour. If the scan takes only 5 minutes, this delay will be used to pause the system before starting the next scan. If the SCAN takes an hour and a half, the delay will have no effect, the system will not pause, but will continue immediately to process the next SCAN.
FLD_Time	Seconds elapsed at moment field was captured since the first image capture in a Scan's sequence of images.
FLD_Time_Delay	Target pause between each frame of a **Scan** sequence in seconds, entered in the Scan Wizard.
Field Time Delay Notes:	**FLD_Time_Delay** is the target delay, but **FLD_TIME_INC** records the actual delay between fields, as it is possible that the processing may take longer than the target delay. *Example:* In the case of an XY scan where the FIELD DELAY is set to be 5 seconds, and processing each field takes 2 seconds, the FIELD DELAY will be used to pause the system before starting the next field. If the processing takes 10 seconds, the delay will have no effect, the system will not pause, but will continue immediately to process the next image on schedule. (If the processing takes 6 seconds, all the fields will still be captured but while falling progressively farther behind the original

		schedule.)
FLD_Time_Inc		Seconds elapsed between successive image captures in a sequence of images captured using process control.
Field time increment Notes:		**FLD_Time_Inc** can be used with **FLD_TIME_Delay** to compute the time for processing of each image in a sequence.
Field XYZ Origin	**FLD_X_ORIGIN**	First transverse axis motion
	FLD_Y_ORIGIN	Second transverse axis motion
	FLD_Z_ORIGIN	Vertical axis motion
Field XYZ Origin Notes:		These values are the un-calibrated number of steps representing the travel of the motorized X,Y and/or Z controller from the origin as defined in the first image of a sequence. They can be used to compute the absolute coordinate of individual objects in an automatic scan, using the coordinate center of gravity of the object, the scale-factor for calibration, the step size (constant for a given stage) and the current XYZ field position in steps.
Field XYZ Steps	**FLD_X_STEPS**	First transverse axis motion
	FLD_Y_STEPS	Second transverse axis motion
	FLD_Z_STEPS	Vertical axis motion
Field XYZ Steps Notes:		These values are the number of steps to move the automatic stage controller between successive fields of view for analysis, as specified in the CUSTOM RS232 menu for the XYZ stage controller.

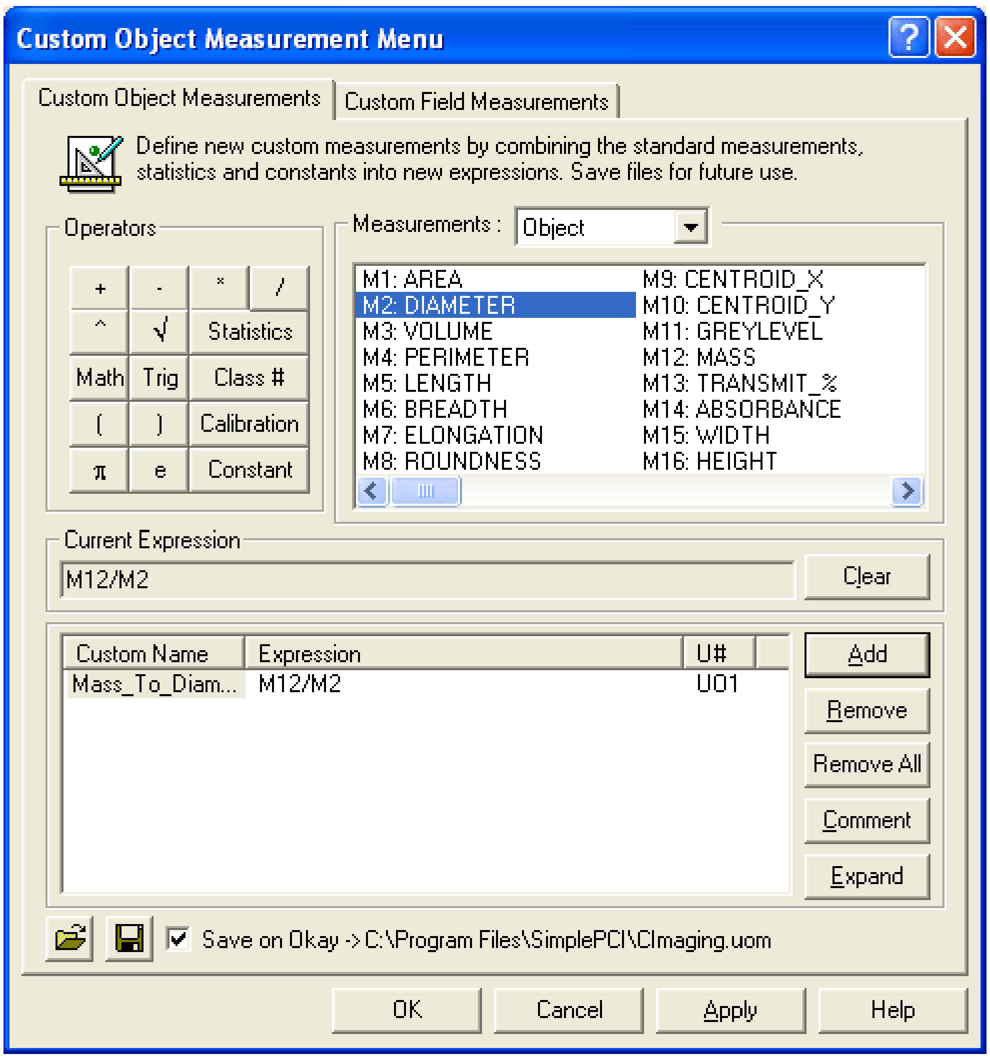
FLD_Wavelength		Transmission wavelength at which a tunable filter was programmed when field was captured.
	Field Wavelength Notes:	It is possible to run a Scan through a wavelength range of a tunable filter to create spectral response data measurements. This parameter provides the peak wavelength value for the current field in Nanometers.
FLD_Wave_Steps		Wavelength step or increment between fields in Nanometers
	Field Wavelength Steps Notes:	Applicable when scanning the wavelength range of a tunable filter to create spectral response data measurements
ROI XY Origin	**ROI_X_Origin**	Horizontal coordinate of origin
	ROI_Y_Origin	Vertical coordinate of origin
	ROI XY Origin notes:	These coordinates define the origin of the Measurement ROI within the full image of the selected camera. This enables the ROI size and position to be part of an expression defined in Custom measurements. For ROI shapes that are not rectangular, these coordinates are from the top left corner of the bounding box that confines the ROI shape.
ROI Size	**Width**	Horizontal size in pixels that the measurement ROI projects in the image
	Height	Vertical size in pixels that the measurement ROI projects in the image
	ROI Size Notes:	For ROI shapes that are not rectangular, these dimensions are from the top left corner to the bottom right corner of the bounding box that confines the ROI shape. The ROI_X_Origin and ROI_Y_Origin coordinates coupled with the ROI_Width and ROI_Height can be used in Qualify to create to exclude objects on the basis of their position in an image. A wide variety of rules can be implemented to exclude

	objects using the object position information of any coordinate from the object's bounding box. This can be used to create a guard region to avoid measuring truncated objects or measuring objects twice. Using this method objects can be deleted if they touch any of the ROI borders. The use of a Guard region is supported by using Edge Removal options as described in the Qualify Menu; however, the use of these ROI parameters in custom measurements increases the flexibility for applying rejection rules to edge objects. Such rejection rules can be based on comparing an object's centroid coordinates, COFG coordinates, or extreme coordinates against the field's ROI values.

Custom Measurements (Summary)

The direct measurements made for both Objects and Fields and their statistics can be arithmetically combined in expressions that are easily customized using the Define button in the Select Measurements Menu to define Custom Measurements. These measurements are evaluated immediately from the measurement data being collected, avoiding the need to go to a spreadsheet to compute a result. The customized measurements can incorporate measurement data, statistics, mathematical operators and other calibrated values providing a flexible and efficient means to solve imaging questions.

The defined Custom Measurements are also available in the Qualify Menu, and so can be used exclusively to reject unwanted objects, or can be used for customized data collection.



All measurements in the Select Measurements Menu can be combined together in algebraic form to create new derived measurements. Custom Measurements can be defined using a combination of Object Measurements, Field Measurements and their statistics, plus mathematical operators, and calibration constants. Objects defined on the Custom Object Measurements or

Custom Field Measurements tabs will appear in the Measurement dialog's Object Measurements and Field Measurements tabs, respectively.

T he Define Custom Measurements dialog is activated from the Define button in the Select Measurements Imaging Icon.

To define a new Custom Measurement:

- Click the Add button and enter a unique name for the new custom measurement. This name will be used to identify the measurement in the Select Measurements list, and will be saved in the data file for identifying the data, so make it a useful and descriptive name.
- Next create an arithmetic expression for the measurement by clicking on each Operator button to display the full custom measurement description. **Expand** field is updated using mnemonics for each measurement. Use the **Current Expression** button or measurement selection until the full expression is entered.
- A Comment can be added to the measurement describing its purpose.

The **Remove** button deletes a measurement from the list. **Remove all** removes the entire list of custom measurements.

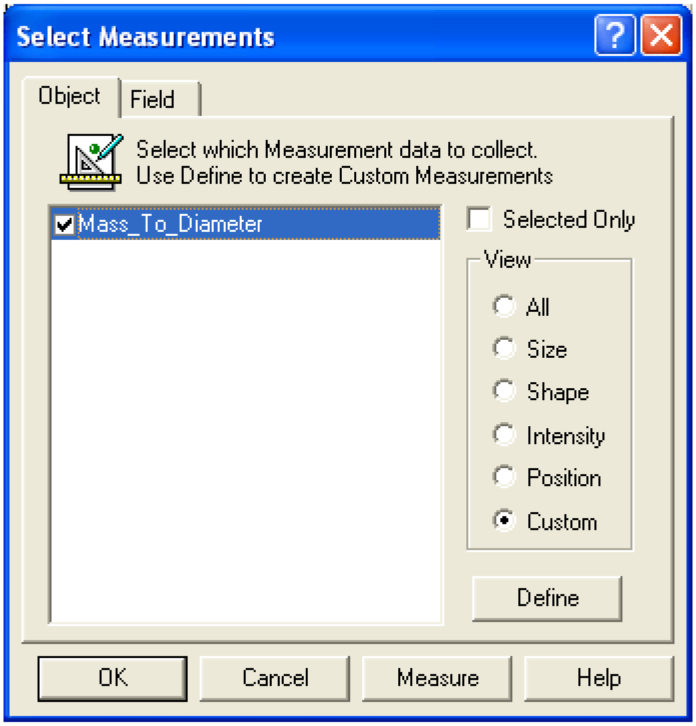
The **file browse** button can be used to choose a file to which to save the collection of custom measurements, which can be loaded and saved allowing multiple groups of custom measurements to be created, i.e. per user or per project. If the **Save On Okay** button is checked, the file will be saved when the OK button is clicked. Otherwise use the **Save** button to avoid losing your changes after exiting the dialog.

There are two separate files, one for Object measurements and one for Field measurements. When using Custom Measurements, make sure that the correct file is loaded by the System Profile.

The **Expand** button can be used to edit all attributes (name, formula, comment) of a custom measurement, and is useful to copy and paste attributes between custom measurements.

When defining a Custom Object Measurement, any parameter all parameters available for that object are displayed in the Object Measurement Tab. Additionally, summary statistics of the current field and other Field and system information parameters are available in the Field Measurements Tab.

Custom Object Measurements will appear on the Object Measurements page and will be stored in a .uom file; Custom Field Measurements will appear on the Field Measurements page and are stored in a .ufm file.



For more information, see Custom Measurement Example.

Custom Measurement Example

A common Custom Object Measurement is to determine a circularity shape factor, where the custom measurement expressions would look like this:

Circularity = (Min_Radius / Max_Radius) * 100

For a perfect circle, the Minimum and maximum radius of an object are identical and their ratio is 1.0, to display this as a percentage, multiple by a Constant of 100.

When defining a Field Custom Measurement, the Class button allows measurements from a previously measured Class in the current Field to be combined. This option is very useful for creating measurements relating one class to another. For example, if two classes of objects are counted and measured, perhaps green cells and red cells, to create a percentage count, the custom measurement expressions would look like this:

Red_Percent = ((Class1(Object_count) / (Class1(object Count)+Class2(Object Count)) * 100

Green_Percent = ((Class2(Object_count) / (Class1(object Count)+Class2(Object Count)) * 100

All of the Field measurements and statistics of Field measurements described above can be combined together in algebraic form to create new derived measurements in the Custom Field Measurements.

Dynamic Intensity Analysis

Use DIA to measure the intensity of cells over time, either during an acquisition sequence (on-line) or within previously captured data (off-line) views suitable for displaying such data visually.

DIA is accessed through the Capture Sequence Scan Wizard.

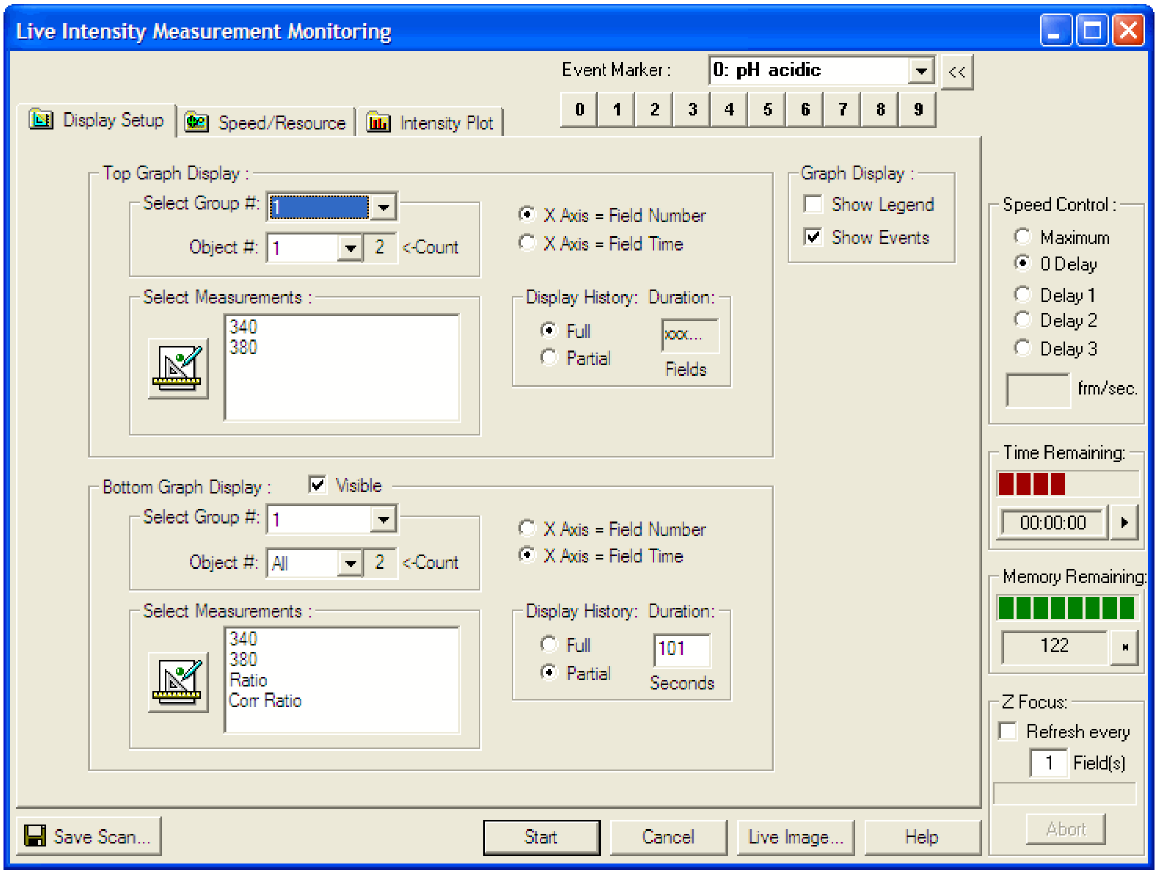
To begin an on-line experiment, select **ScanTime - Intensity Monitor**.

To begin an off-line experiment select **Scan Disk - Intensity Monitor**.

Setup your experiment using the Intensity Monitor Dialog:

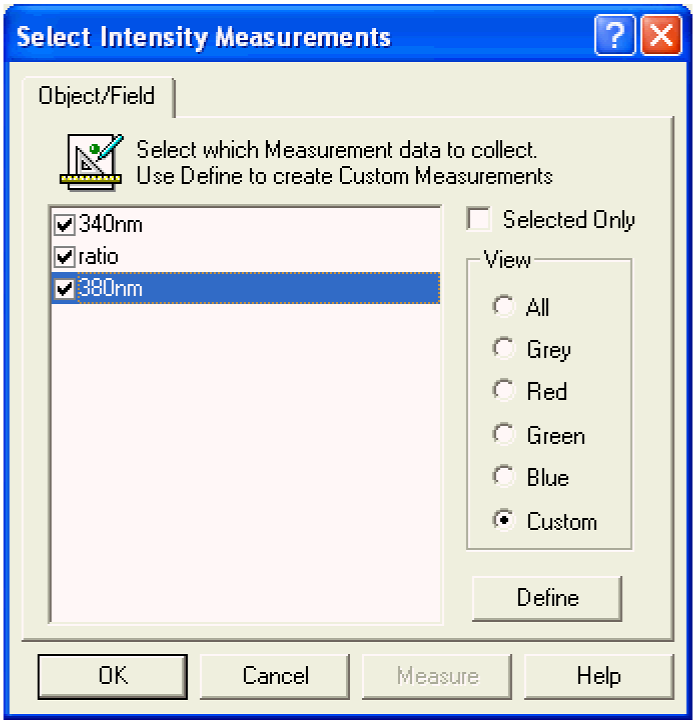
- Display Setup
- Speed / Resources
- Intensity Plot

Display Setup Tab



Target Selection:

Select an individual object, all objects in the group or the average of the objects in the group for analysis. Note that after using Create Shapes from Overlay in the Capture's ROI dialog the objects may be made to correspond to individual cells. (Use Advanced Detection for increased control over the identify results if necessary.)



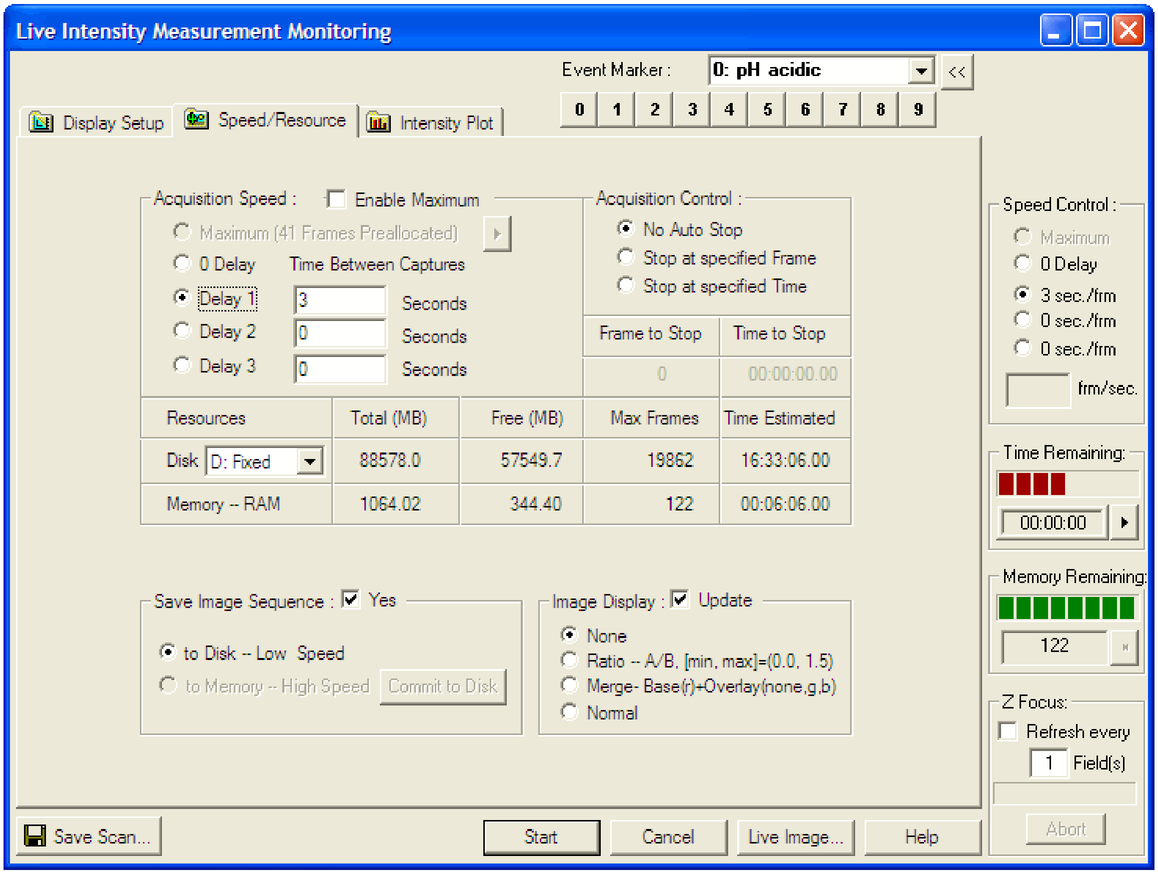
Use the Select Measurements dialog to choose appropriate Object Intensity and Field Intensity measurements to collect dynamically.

Set X-axis plot for time or field number.

Choose one or two graphs for selective data display.

Save the scan settings to a file. This file can be loaded into the capture dialog as the Default Scan to allow for one-click scan setup.

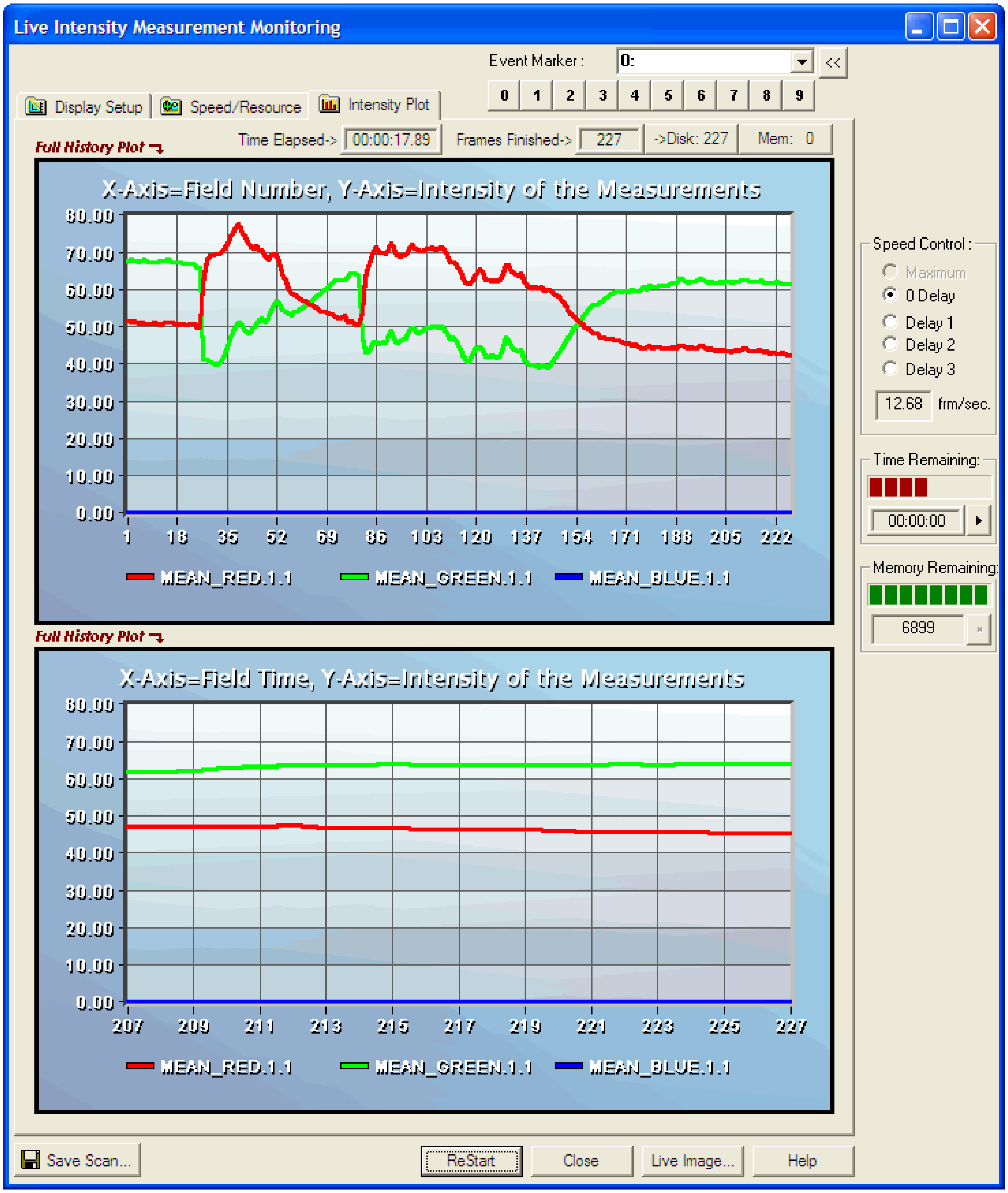
Speed/Resources Tab



What can be done here:

- Define time delays for on-line selection
- Preset end time for completion of image sequence
- Select image save options as None, Disk or Memory
- Specify image display mode for normal, ratio or merge
- Check available storage resources

Intensity Plot Tab



What can be done here:

- Visualize the Intensity Plot of multiple ROIs over time on dual real-time interactive graphs. The zoom features are described below.
- Start, Pause and Resume controls for image sequences
- Move the ROI during the experiment by clicking Pause and then click on the ROI to move it the desired location. Click Resume to resume the experiment.

- Dynamically switch between delay modes
- Monitor number of frames and time progress
- Control the collection of data throughout the course of the experiment

Note: Live mode dynamic intensity analyses uses requires both the DIA and AIC modules.

Event Markers can be added to the data document file during acquisition as notable events occur in the experiment. Both the numbered buttons above and keyboard numbers may be used.

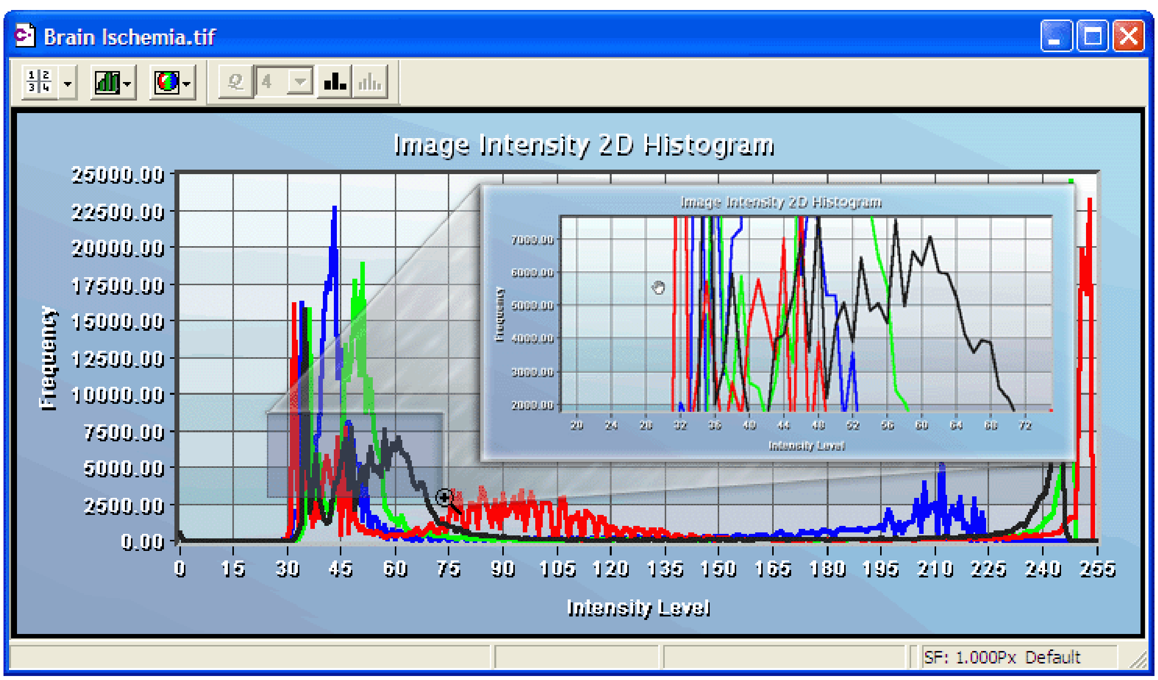
Time Remaining is a reference to delay time. The right arrow button may be clicked to exit the delay.

Acquisition Speed: Must check the Enable Maximum box for Maximum Speed to be an option. During maximum speed, items which slow down acquisition will be ignored (Z-focusing, exposure protection..) Several delays can be entered to allow for fast time switching during experiments.

Save Image Sequence: Choose between high speed memory acquisition or stream directly to disk. If streaming to memory, you may commit those images to disk while paused.

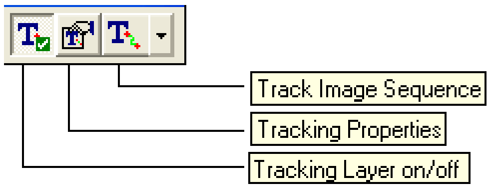
When maximum speed is enabled, you may de-select **Update Image Display** for faster frame rates. When **Enable Maximum** has NOT been checked, you may choose to review previously collected images by selecting **Review Images.**

To zoom in on a particular area click and hold the left mouse button as you drag a box around the area of interest. Release the button and the graph zooms to the size of the box. Click the left mouse button to return to the normal view. While in a zoomed position the user can pan by dragging the mouse in the direction they wish to view. In the example below, a portion of a 2D Histogram has been enlarged.



Motion Tracking Analysis

If the **MTA** module has been activated, the following toolbar icons will be available on the Data Document's Image Processing toolbar.

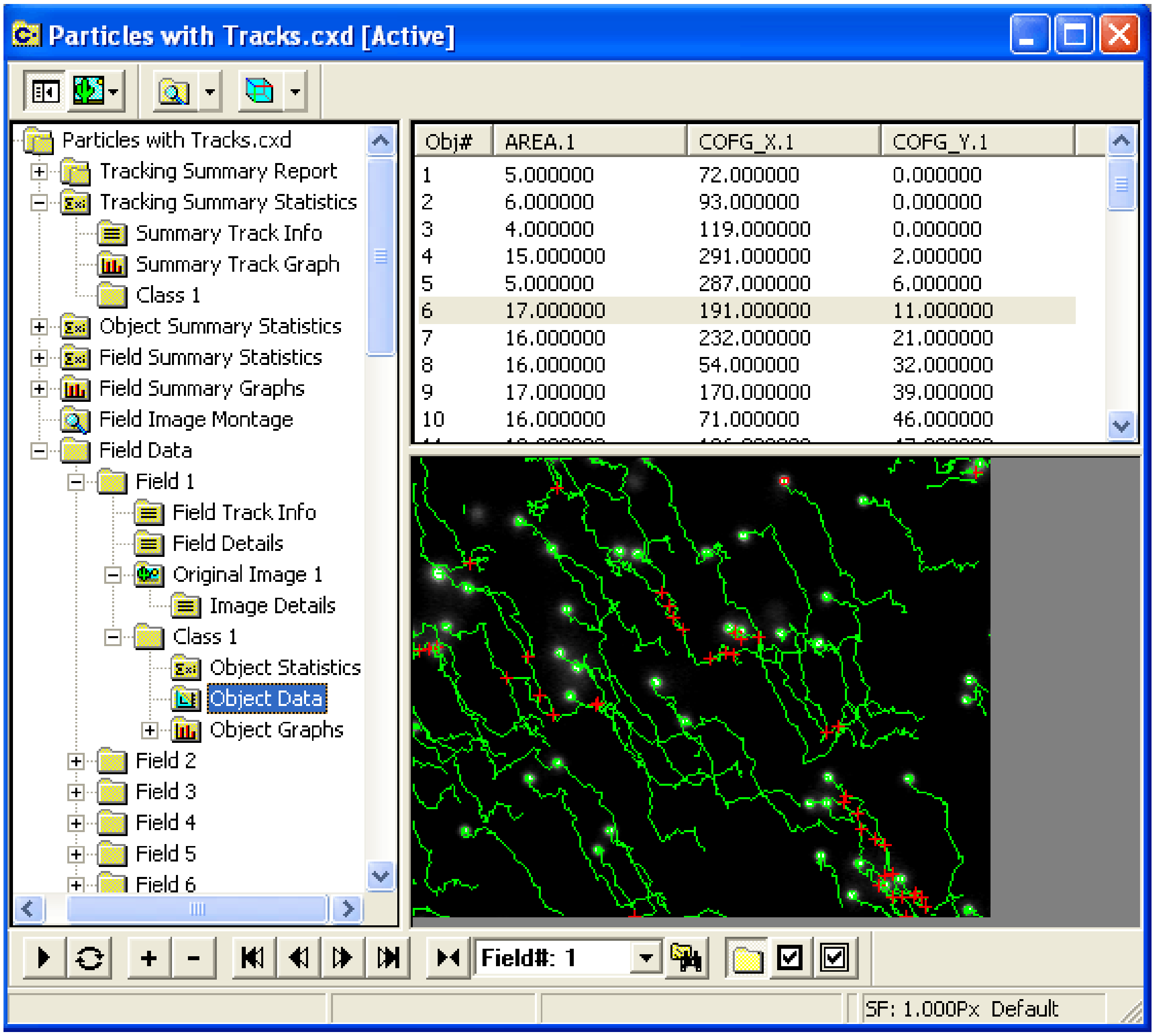


The functionality of the MTA module applies to data in a field of view where moving objects are captured over time. The resultant data document contains a series of images whose tracks of interest can be followed visually.

The fields must be first collected. Using IPA, Objects within each field are identified and then measured for size (Area), position (Center of Gravity - COFG-X and COFG-Y) and time (Field Time).

Once the field measurements are complete, click **Tracking Properties** to adjust the parameters of the Tracking algorithm. Additional functionality is available through the drop-down **Track Menu**.

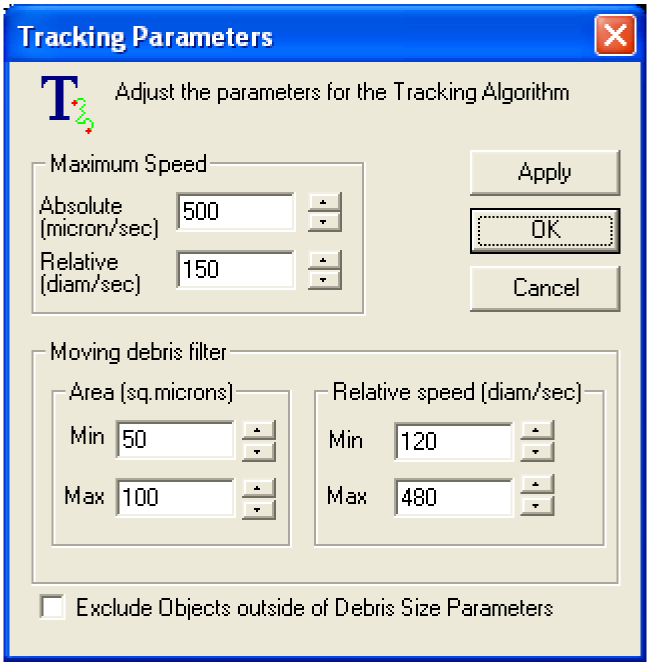
Click **Track Image Sequence** to rapidly track objects. Track reporting includes distance moved with straight and curve line velocity, and all related statistics.



To view the statistics on all of the tracks in the entire sequence, click **Summary Track Info.**

To view the object measurements (X, Y and area) as they make up each track, click **Summary Track Graph**. Then, select Table or Spreadsheet View.

Tracking Parameters



Maximum Speed Values:

- **Absolute Maximum** Speed derives the Upper Distance Limit that objects in Field[n] will be tracked to objects in Field[n+1]

  AbsMaxSpeed = Area (largest object) / time (between frames)

- **Relative Maximum speed** derives the Upper Distance Limit that objects in Field[n] will be tracked to objects in Field[n+1] with respect to object size. Allows smaller objects to be tracked while traveling faster without relaxing the test on larger objects

  RelMaxSpeed = AbsMaxSpeed / sqrt(Area)

It's recommended to start with smallest area and watch the effects on the track as reduce RelMaxSpeed OR start with largest area and watch the effects on the track as increase RelMaxSpeed. Keep in mind that Decreasing these values too much will result in breaks in the tracks.

Moving Debris Filter Values:

- **Minimum Area -** Objects below this number will have more stringent requirements when determining whether a matching object is found. (Two objects will need to be closer in size for a track to occur)

  MinDebrisArea = Area of smallest object

- **Maximum Area -** Objects above this number will most likely be mapped to each other.

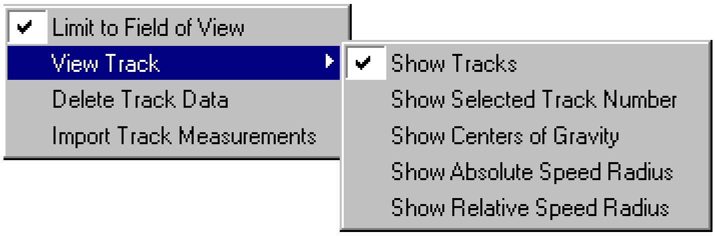
  MaxDebrisArea = Area of largest object.

- **MinDebrisRelSpeed** = AbsMaxSpeed / sqrt(MaxDebrisArea)

- **MaxDebrisRelSpeed** = AbsMaxSpeed / sqrt(MinDebrisArea)

Track Menu

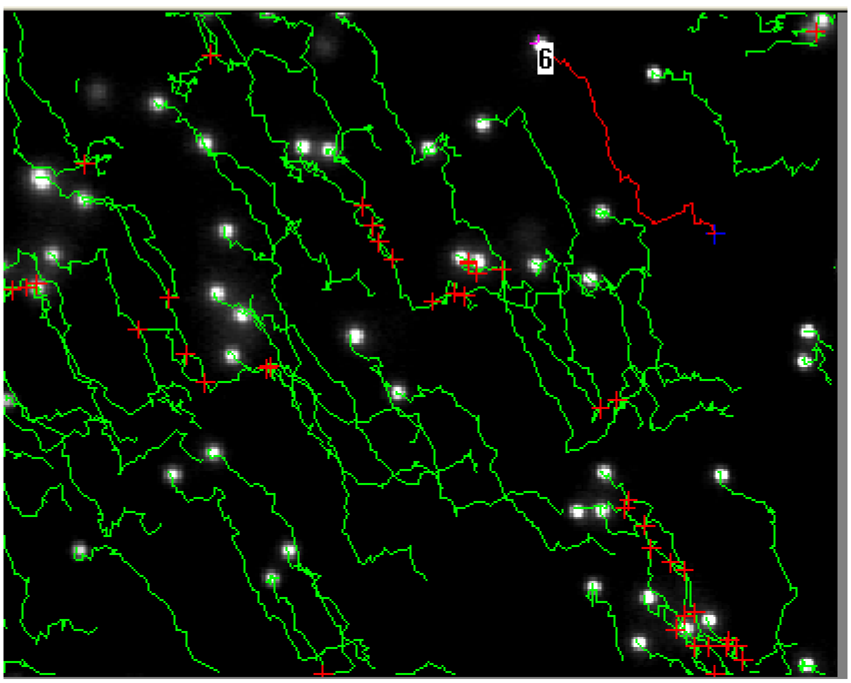
The following tracking functions are available through the **Track Image sequence** drop down menu.



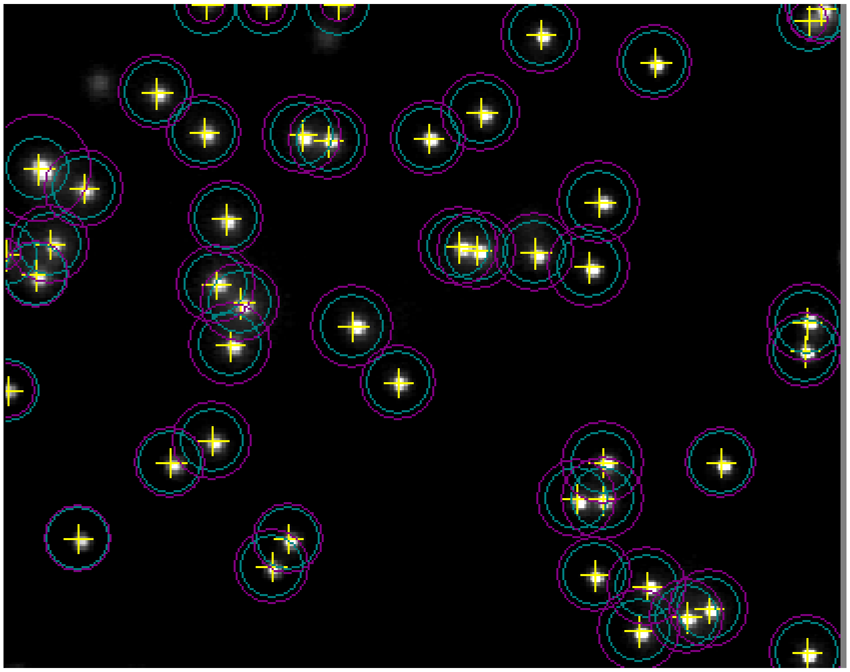
Limit to Field of View

Only Show Tracks existing in the current field.Track Viewing

Show Tracks and Selected Track Number. Selected Track is highlighted in Red. Its start point is designated by a pink cross (+). Its end point is designated by a blue cross (+). Possible Collisions are represented by red crosses (+).

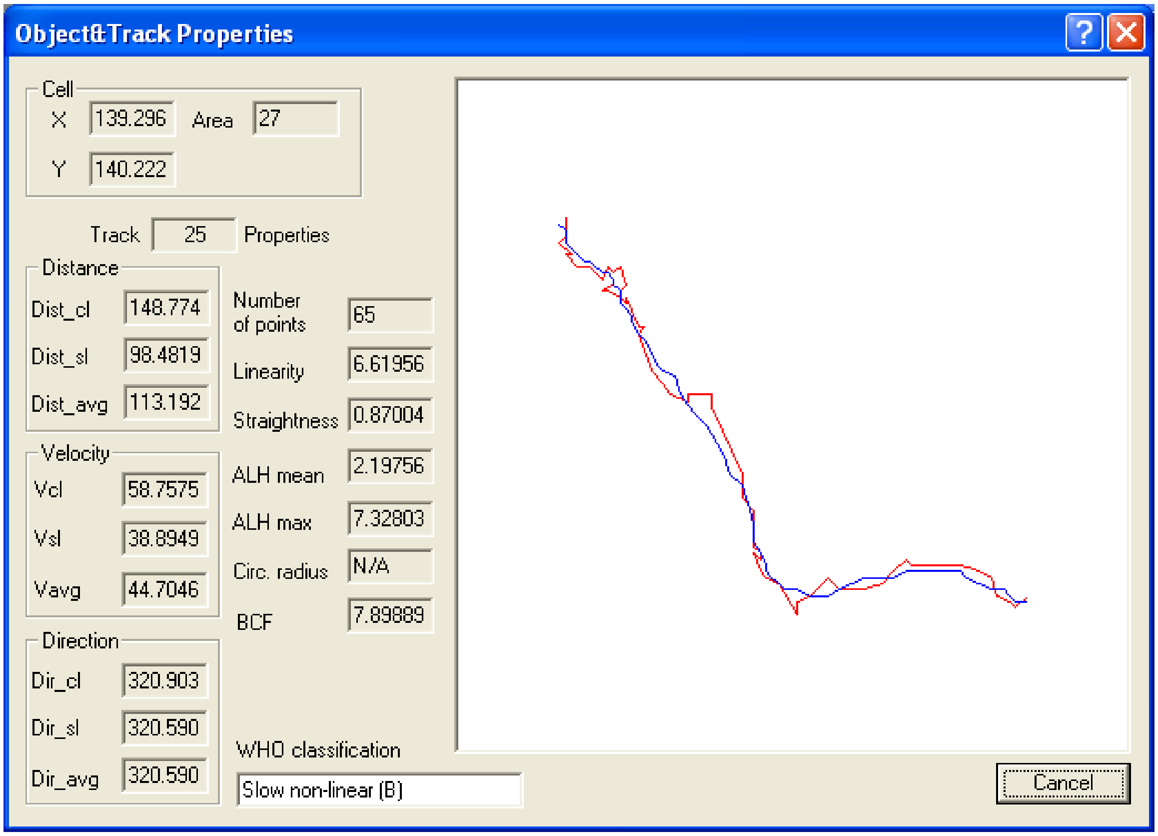


Show **Centers of Gravity**, **Absolute Speed Radius** and **Relative Speed Radius.** Centers of Gravity are designated by yellow crosses (+). Absolute Speed Radius is colored blue. Relative Speed Radius is colored purple.



Track Statistics

Double-Click on a Track to see its individual properties and statistics.



Cell position: X, Y coordinate Cell area: number of pixels

In the Object and Track Properties dialog there are two lines drawn to represent the track. The red line connects the position of the object at each point in the track. Measurements based on this path are called _**cl** or Curvilinear measurements.

The blue line represents a curve fitted through each point on the track to generate a mean path for the track. Measurements based on this path are called _**avg** or mean path measurements.

The start and end point of the track are used to generate a straight line representing the final distance traveled for the object. This line is not drawn. Measurements based on this path are called _**sl** or Straight Line measurements.

Distance	The length of the line in calibrated units (e.g. Microns).
Velocity	The average speed along the track (distance/time taken)
Direction	The angle measured from 0-360 degrees

Number of Points	Number of consecutive images containing the same object used to make up the track
Linearity	**(Dist_sl/Dist_cl)*10.** A factor indicating how close the track is to a straight line. The maximum value is 10 for a perfectly straight line.
Straightness	**(Dist_sl/Dist_avg)** the fractional ratio between the straight line path and the mean path. The Maximum value is 1.0 for a perfectly straight line.
ALH Mean	Mean Amplitude of the transitions either side of the mean path. This calculation is useful for subjects having a regular beating motion (e.g. sperm). A higher value indicates the subject move vigorously away from the center lin.
ALH Max	Maximum Amplitude of the transitions either side of the mean pathfor straight tracks as it tends to infinity. Also the value may not be useful when the direction of curvature of the mean path changes for the object.
Circular Radius	The computed radius constraining the circular motion of a subject. Note this value cannot be computed
Beat Cross Frequency	The **BCF** is calculated from the number of times the track crosses the mean path. It gives another indication of subject motility when applied to samples with a regular beating motion.
WHO Classification	World Heath Organization classification scheme: **Rapid Linear (A)** - subject makes significant direct forward motion. High Vsl and High Straightness. **Slow Non-Linear (B)** - subject makes motion, but moderate Vsl. High Vsl and low straightness. **Non-Progressive Motile (C)** - subject makes some motion, but no progress low Vsl. **Non-Motile (D)** - subject makes insignificant motion low Vavg. **Unclassified (U)** - insufficient information.

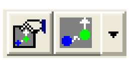
Quantitative Fluorescence Analysis modules: QFA-FRET

The SimplePCI QFA-FRET module adds FRET specific analysis for research into protein-protein interactions. QFA-FRET incorporates selection and customization of algorithms based on published methods for accurate crosstalk correction, FRET measurement and visualization.

SimplePCI offers both the ability to acquire new images for FRET analysis or to perform analysis on previously collected specimens.

To begin your FRET analysis, you will need to open a target FRET image. See FRET Image Acquisition if you have yet to acquire your target image.

SimplePCI installations licensed for the *QFA-FT* module will add the FRET icons to the Image Document Toolbar.



Click the either FRET icon to begin your FRET analysis and proceed to Step 1: Method Selection.

FRET Image Acquisition

The SimplePCI Automated Image Capture module *AIC-CD* provides the automatic control of cameras for the acquisition of image components. The *AIC-SD* module adds control of Standard Devices motorized filter wheels, shutters or monochromators necessary for easy acquisition of images for FRET analysis, and includes and also control of motorized XY and Z stage devices. The *AIC-AD* module adds control of Advanced Devices including automated Microscopes and Macroscopes with motorized filter cube, shutters, and other automated devices.

> Typically images will be acquired with 2 or 3 channels of data representing the fluorescence intensity in both control images and target images. It is required that the acquisition of these images be under carefully monitored identical conditions including illumination, magnification and registration.

FRET images can contain three channels each using specific filter sets as follows:

1. FRET filter set (acceptor excitation, donor emission)
2. Donor filter set (donor excitation, donor emission)
3. Acceptor filter set (acceptor excitation, acceptor emission)

The SimplePCI Capture Dialog allows the specification of capture parameters for each of the three channels required, including camera Exposure, Gain, Offset, Sensitivity (multiplication) Gain and the associated emission, dichroic and excitation filter and shutter combination to provide the correct illumination for each channel. These Capture settings can be saved in the SimplePCI Workfile and reloaded for routine use, allowing the user to maintain the consistent acquisition parameters necessary between image acquisitions. Automated shutter control provides protection for sensitive specimens by minimizing the exposure to excitation illumination. Camera properties including binning, sub-region etc, are also to be kept constant for experimental comparisons.

The image acquisition will generate a three channel image. For consistency the same filter sets should be used with the same channels. SimplePCI will automatically cycle through the acquisition of each channel and merge these three channels together for display in a color image.

The typical order for the capture and display of the image channels is as follows:

1. Red display channel - FRET filter set (acceptor excitation, donor emission)
2. Green display channel - Donor filter set (donor excitation, donor emission)
3. Blue display channel - Acceptor filter set (acceptor excitation, acceptor emission)

Acquisition for FRET analysis requires excitation/emission filter sets for correct spectral separation of the fluorophores involved.

Unavoidable crosstalk or bleed through between channels requires mathematical processing corrections. These algorithms have been published and discussed in the literature. The more complete correction methods require more control images to determine the correction coefficients to use.

Up to three image sets are required, one for each fluorophore, each acquired with up to three filter sets.

The convention for referencing each channel is as follows:

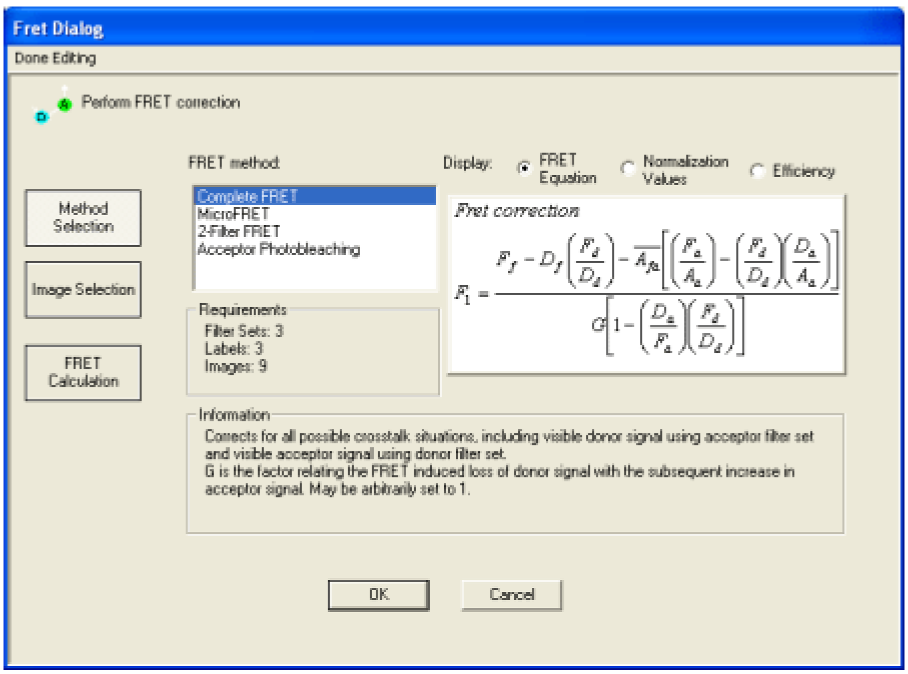
Capital letter = Filter Set	**Lowercase letter = Fluorophore**
F = FRET filter set (acceptor excitation, acceptor emission).	f = donor and acceptor fluorophore
D = Donor filter set	d = donor fluorophore
A = Acceptor filter set	a = acceptor fluorophore

The combination of Upper case and Lower case are combined to indicate the filter set and fluorophore.

e.g. Ff, Df, Af etc.

Step 1: Method Selection

To start your FRET analysis, you first need to choose the FRET correction method. This step determines the algorithm to apply, the filter sets to use and the image combinations required for the calculations.



To choose a FRET method, click on one of the methods in the list. You will be able to display the *FRET Equation*, *Normalization Values* and *Efficiency* Equation for each method by clicking on the radio buttons to the right of the method list.

The available methods are described below.

Complete FRET

This method corrects for all possible crosstalk situations, including visible donor signal using acceptor filter set and visible acceptor signal using donor filter set. G is the factor relating the

FRET induced loss of donor signal with the subsequent increase in acceptor signal. G may be arbitrarily set to 1.

It requires 3 Filter sets and 3 labeled specimens for a total of 9 images.

Fret correction

$$F_1 = \frac{F_f - D_f\left(\frac{F_d}{D_d}\right) - \overline{A_{fa}}\left[\left(\frac{F_a}{A_a}\right) - \left(\frac{F_d}{D_d}\right)\left(\frac{D_a}{A_a}\right)\right]}{G\left[1 - \left(\frac{D_a}{F_a}\right)\left(\frac{F_d}{D_d}\right)\right]}$$

Normalization Values

$$\overline{D_{fa}} = D_f + F_n\left[1 - \left(\frac{D_a}{A_a}\right)\right] - \overline{A_{fa}}\left(\frac{D_a}{A_a}\right)$$

$$\overline{A_{fa}} = \frac{A_f - F_f\left(\frac{A_d}{F_d}\right)}{1 - \left(\frac{F_a}{A_a}\right)\left(\frac{A_d}{F_d}\right)}$$

Fret efficiency

$$E = \frac{F_1}{\overline{D_{fa}}}$$

MicroFRET

This method assumes simplified crosstalk. No signal visible with acceptor labeled fluorophore and donor filter set. (Da = 0) No signal visible with donor labeled fluorophore and acceptor filter set. (Ad = 0)

It requires 3 Filter sets and 3 labeled specimens for a total of 7 images.

Fret correction

$$F_c = F_f - D_f\left(\frac{F_d}{D_d}\right) - A_f\left(\frac{F_a}{A_a}\right)$$

Normalization Values

D_f

A_f

Fret efficiency

$$E = \frac{F_c}{F_c + D_f}$$

2-filter FRET

This method assumes the concentration of Donor and Acceptor are correlated: Acceptor concentration is whole number multiple of the donor concentration. Our calculations assume a 1:1 ratio.

It requires 2 Filter sets and 3 labeled specimens for a total of 6 images.

Fret correction

$$F_2 = \frac{F_f - D_f\left(\dfrac{F_d' + F_a'}{D_d' + D_a'}\right)}{\left[G - \left(\dfrac{F_d}{D_d}\right)\right] + \left[1 - G\left(\dfrac{D_a'}{F_a'}\right)\right]\left(\dfrac{F_d' + F_a'}{D_d' + D_a'}\right)}$$

Normalization Values

$$\overline{D_{fd}} = \frac{D_f + F_2\left[1 - G\left(\dfrac{D_a'}{F_a'}\right)\right]}{1 + \left(\dfrac{D_a'}{D_d'}\right)}$$

Fret efficiency

$$E = \frac{F_2}{D_{fd}}$$

Acceptor Photobleaching

This method measures the donor emission before and after acceptor photobleaching. It provides an alternative method of quantifying the FRET efficiency and can be used to provide a double check on previous FRET methods.

It requires 1 Filter sets and 1 labeled specimen at two time points for a total of 2 images.

Fret correction

$$F_c = I_D - I_{DA}$$

Normalization Value

$$I_{DA}$$

Fret efficiency

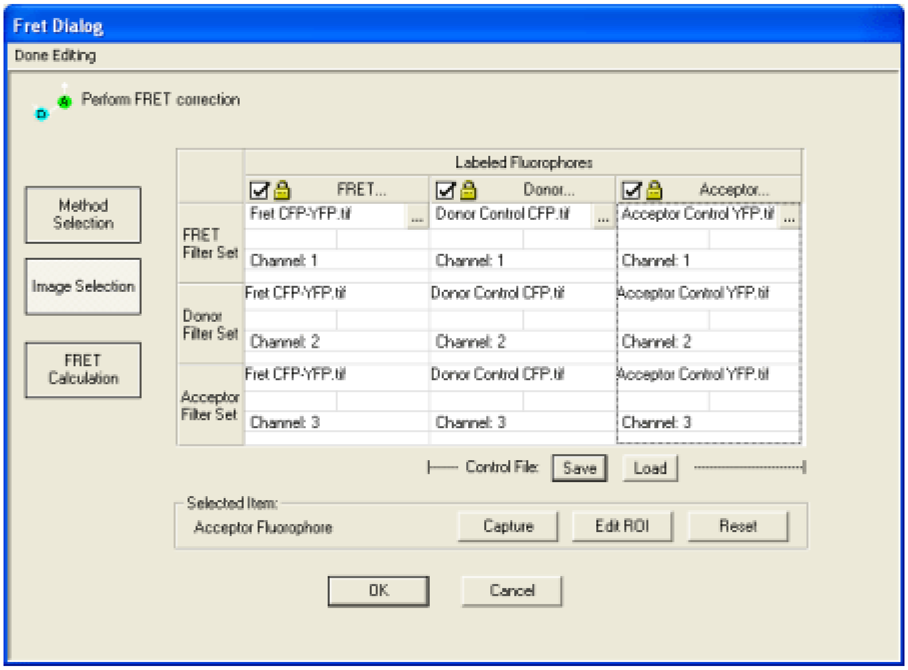
$$E = \frac{I_D - I_{DA}}{I_D}$$

Once you have chosen the ***FRET method***, click ***Image Selection*** to go to the next step.

Step 2: Image Selection

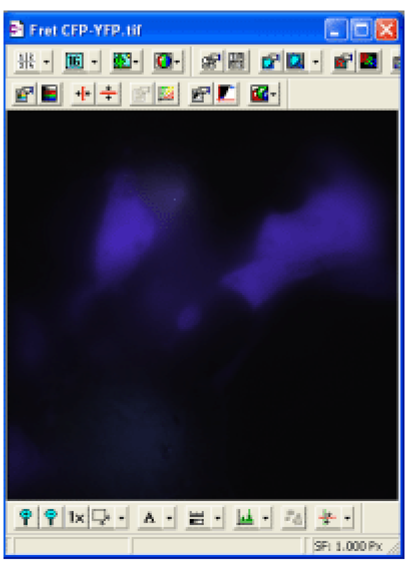
The second step determines the source of the image channels necessary for performing the calculations.

This display shows the 9 images required for a Complete FRET analysis:



Normally, the target FRET image is normally the open document and automatically entered into the image selection list, so only the control images need to be selected. If you are manually entering known *crosstalk coefficients* to calculate your FRET correction, no control images are necessary.

Here is an example FRET image for measurement



Load Images from Disk

FRET images can be selected individually or grouped by fluorophore. You can load three individual monochrome images for the FRET labeled specimen or you can load one three-color image where each component is representative of a filter set. One image can contain three channels

To select an image click on the [...] button 

To load a three-color image one fluorophore, make sure the Locked option is checked for the fluorophore. You will have only one [...] button to select which will load all three components. To load individual monochrome images separately, uncheck the Locked option and there is a [...] button for each component.

A disk image dialog will allow the selection and extraction of any image component from a disk image file or from an image stored in a Data Document.

Capture Images using SimplePCI AIC

The FRET Dialog also has a *Capture* button to allow the convenient capture and saving of images.

The SimplePCI *AIC* module allows images for a fluorophore to be captured with all filter sets at the same time.

Pressing the *Capture* Button will activate the Capture Dialog and allow an image to be captured into the Image Display using the current Workfile settings. Selecting *OK* from the Capture Dialog will prompt for the location to store this image, and the location will automatically be updated in the Image Selection list.

Control Files

For an experiment, the same control images may be used if the capture settings are kept constant. In this case, once the control images are fully loaded, use the *Save* button to save both the Donor and Acceptor control image sets to a single Data Document file which can be easily reloaded later using the *Load* button.

Selecting an Image

Click on the grid to select an image.

The ***Selected Item*** information box in the Dialog indicates which of the filter set/fluorophore combinations are currently selected.

This is also indicated by the highlighted rectangle around the selected image(s)

Clearing your image selections

The *Reset* button will remove the current associated image for the selected item.

Choosing a Region of Interest

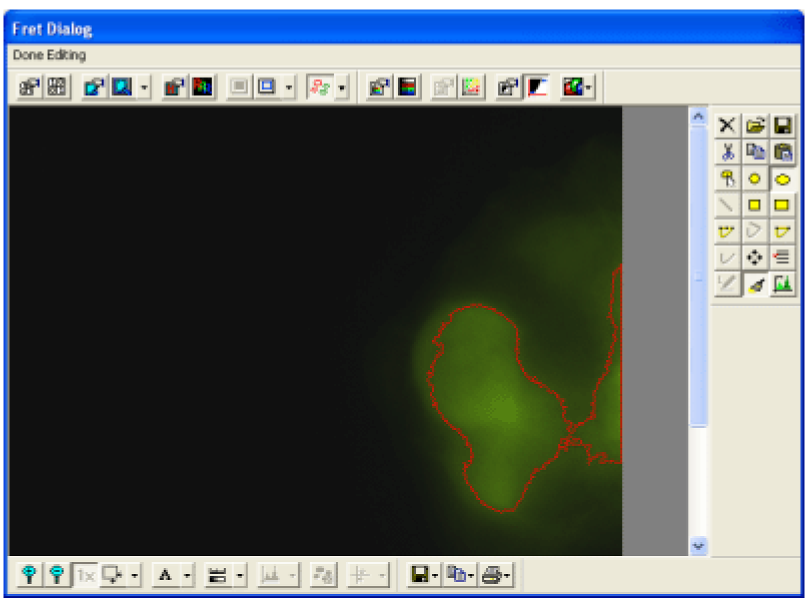
The intensities used for FRET correction may be localized to specific areas within the control images.

The *Edit ROI* button provides a method to identify these areas in the control images from which the control intensities should be measured. This can be used also to avoid any artifacts in the control images.

Select the image of interest. The highlighted rectangle will show the currently selected image to which the regions will be applied. Selecting *Edit ROI* will replace the dialog with a view of the selected image, combined with the Shapes Toolbar, allowing a set of measurement shapes to be defined for the areas of the control image to be used for measurement. The image can be zoomed and contrast adjusted as required to accurately locate the areas for measurement. Areas not included in the measurement shapes will not be used for the correction. The measurement shapes for each of the fluorophore control images are independent. To define the shapes for each fluorophore, lock and select the fluorophore and click the *Edit ROI* button. Define the shapes and then press the *Done Editing* item at the top of the Window.

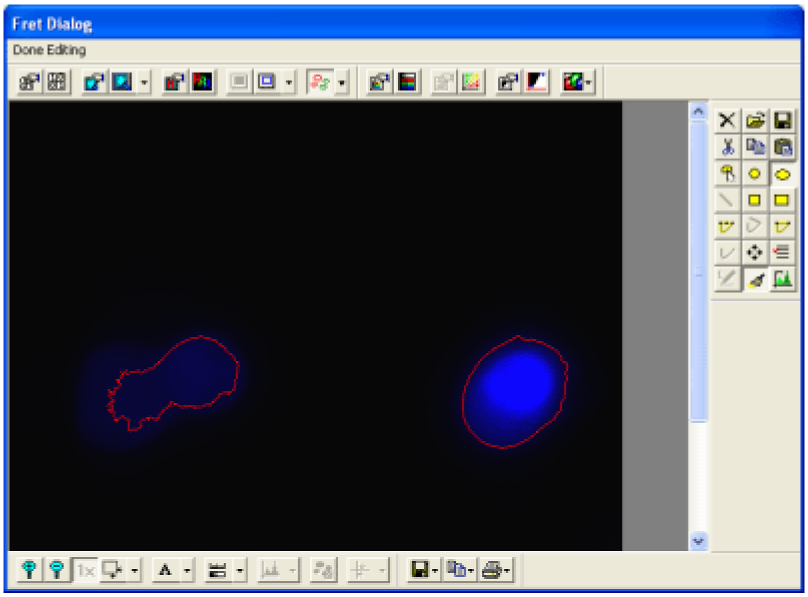
Here we are editing the Donor fluorophore control ROI's:

Notice high intensity in the Donor filter set channel (Green) and low intensities in the Fret and Acceptor channels. This is expected as the Donor labeled specimen reacts when it is excited with the donor excitation and imaged with the donor emission (e.g. CFP/CFP). The FRET channel (e.g. CFP/YFP) and the Acceptor channel (e.g. YFP/YFP) do not show much reaction. This image is used to indicate the crosstalk between the Donor channel and the Fret and Acceptor channels.



Here we are editing the Acceptor fluorophore control ROIs:

Notice high intensity in the Acceptor filter set channel (Blue) and low intensities in the Fret and Donor channels. This is expected as the Acceptor labeled specimen reacts when it is excited with the acceptor excitation and imaged with the acceptor emission (e.g. YFP/YFP). The FRET channel (e.g. CFP/YFP) and the Donor channel (e.g. CFP/CFP) do not show much reaction. This image is used to indicate the crosstalk between the Acceptor channel and the Fret and donor channels.



The Clone tool is useful for replicating the same shape on many targets.

It is convenient to save the control (Acceptor and Donor) image selections into a Data Document. This document also contains the control shapes which are automatically associated when the Load button is used to load a previously created control Data Document. Selecting a fluorophore and Edit ROI allows the shapes to be checked before continuing with the analysis.

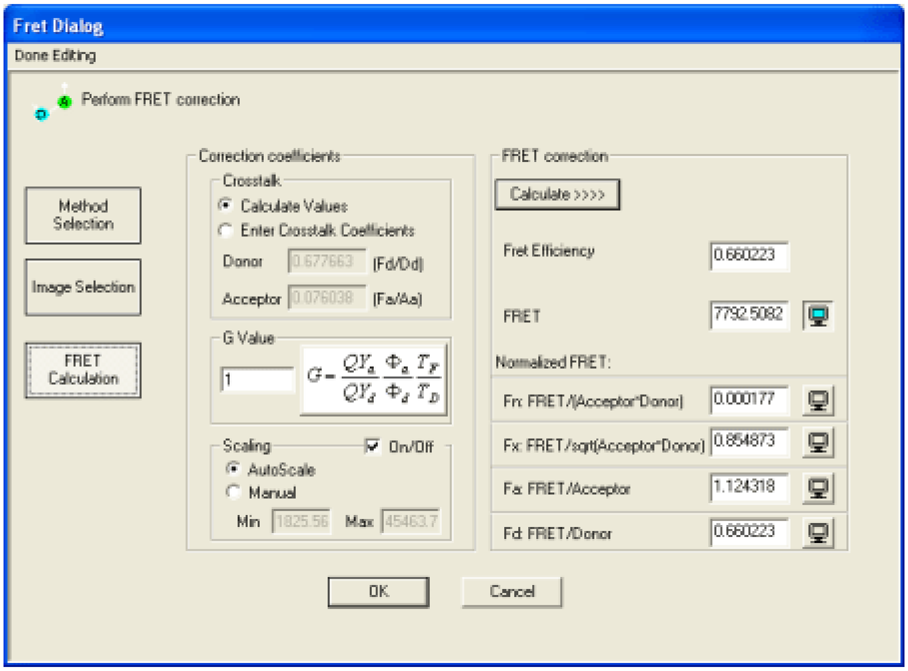
Note: the images for the Donor and Acceptor fluorophore are automatically placed in field 1 and 2 of the Data Document and that the order of channels is expected to be Fret/Acceptor/Donor = 1,2,3.

It is also possible to Edit ROIs for the FRET image. This provides a method for restricting the FRET image display to avoid distracting artifacts or other image details. Areas outside of the ROIs will evaluate to zero during the Calculate FRET operation described below.

Once the images have been selected and any ROIs created, Click *FRET Calculation* to go to the next step.

Step 3: FRET Calculation

The FRET Calculation choice dialog provides tuning of the parameters, summary measurements and a selection of the Normalization Method for the displayed FRET image.



Calculate FRET will process the control images to generate FRET data for each pixel in the FRET image. The processing of control images will automatically calculate crosstalk coefficients which may be used in the future.

Note**:** Known **Crosstalk Coefficients** can be entered manually if correction images are not available, or if the values can be reliably re-used rather than re-calculated. **Calculate FRET** can process these coefficients to generate the FRET information.

FRET values include both Raw and Normalized calculations:

- Raw - Not Normalized
- Normalized using Acceptor/Donor product
- Normalized using Square root of Acceptor/Donor product
- Normalized using Acceptor alone
- Normalized using Donor alone

Data resolution is maintained to provide to high precision per pixel calculations and the resulting data is scaled into the destination image for visualization. **To change the** displayed image, press the ***Image to display*** button next to the chosen FRET value and press ***Calculate FRET***.

The *Min* and *Max* scaling values can be used to control the range of intensities displayed in the visualization image, allowing flexibility in highlighting a specific range of FRET values in the image. You can enter these values manually or select *AutoScale* and these values will be calculated for you.

Once you have applied the FRET calculations, click *OK* to exit the dialog and view the corrected FRET image.

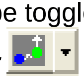
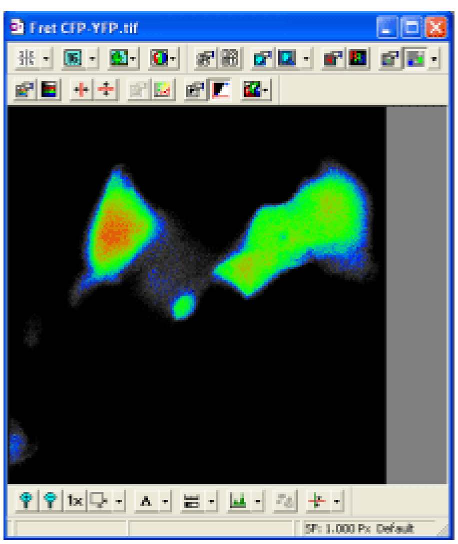
The image can be toggled between the FRET normalization view and the original view by toggling the ***Apply FRET***  button.

Image intensity data can be viewed using the Table and Spreadsheet icons in the Data Document Toolbar.

When the ***Apply FRET*** button is pressed, the Table and Spreadsheet controls display the floating point FRET values without scaling for the highest precision.

This can be combined with the Edit ROI's operation to limit active FRET data in the spreadsheet to specific cellular or sub-cellular regions in the image.

Here is an example Normalized FRET Image:



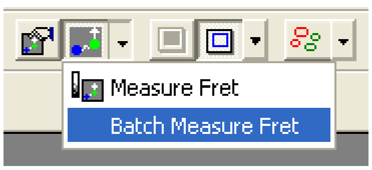
A Display Contrast has been applied using the Pseudocolor Spectrum over a range of intensities to highlight the high FRET values in the image.

Once you have applied the FRET calculations, you will be able to create **specific *FRET measurement*****s** in the next step.

Creating FRET Measurements

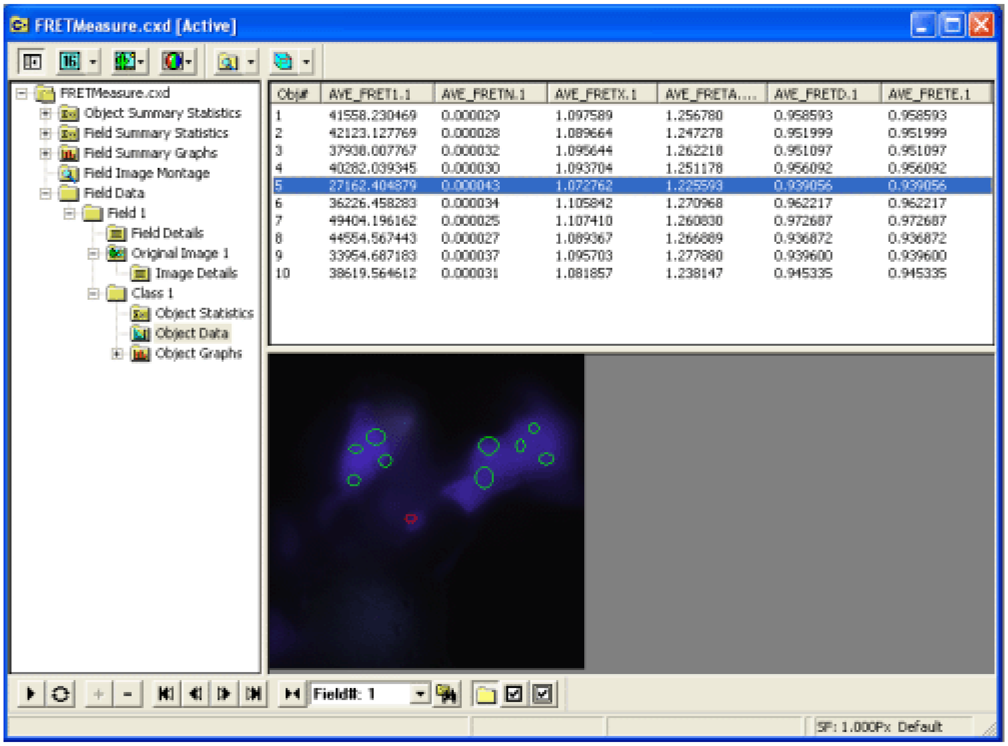
The FRET measurement displayed in the FRET Calculation dialog is a summary FRET measurement, but it is important to get FRET specific measurements for specific regions of the image.

The FRET toolbar items include a drop down menu item ***Measure FRET.*** This item will measure the FRET values for the currently defined shapes in the original image and save them to a Data Document. After selecting ***Measure FRET,*** for any one image in your Data document, you can then select ***Batch Measure FRET.*** Batch Measure FRET will iterate through the entire Data Document, and perform the same calculations, using the same control file for each field in your data document. The result will then be graphed in the object summary graphs.



Like all custom toolbars, the *Measure FRET* item can be promoted from the drop-down toolbar to the main toolbar by pressing the Ctrl key and clicking the item. Demote it to the drop-down menu the same way.

All FRET normalization values and their statistics are measured in one pass and stored in the Data Document file.



FRET1	raw FRET value
FRETN	FRET1/(Acceptor*Donor)
FRETX	FRET1/sqrt(Acceptor*Donor)
FRETA	FRET1/Acceptor
FRETD	FRET1/Donor
FRETE	FRET Efficiency

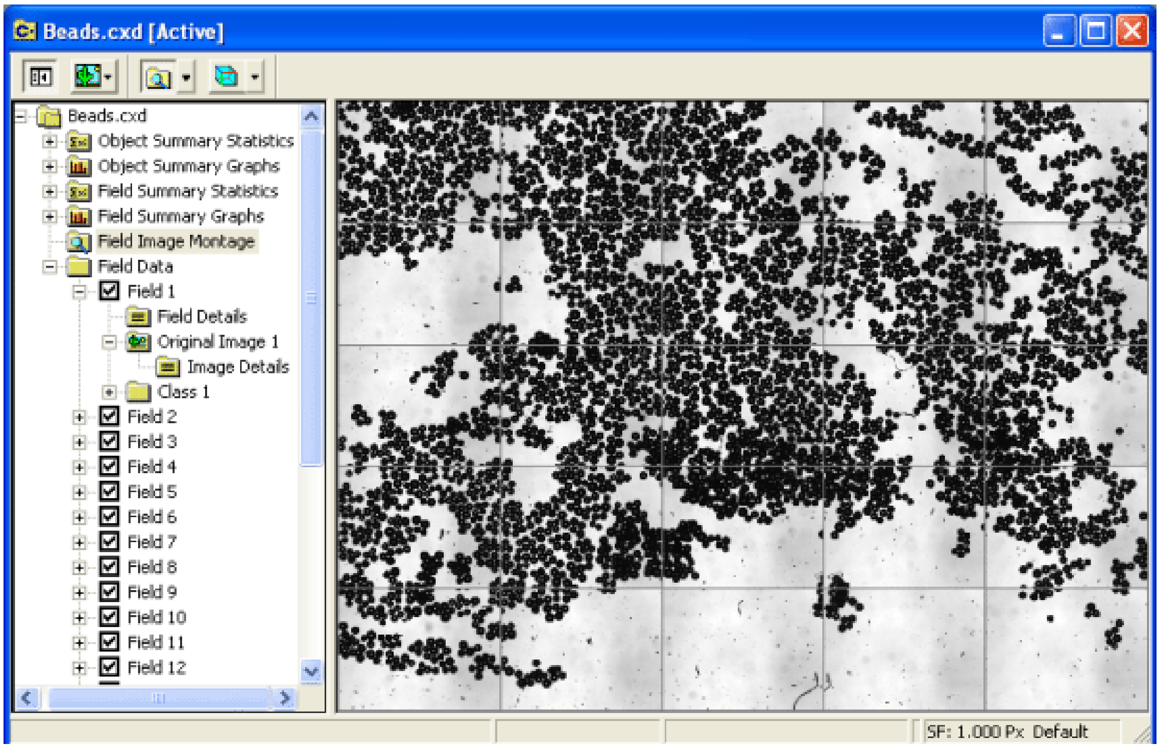
Normal Data Document interaction applies to allow the identification of shapes on the image and the measurements they created.

To analyze multiple images in a Data Document, define the FRET parameters and control images and select OK, then define the shapes on the original image and use the Measure FRET operation to save the measurements to the Data Document for the appropriate field. For another field, redefine the shapes as needed and select the Measure FRET operation.

Image Montage

The **Data Document** provides features to create a **Montage** Display of selected images it contains.

The Montage can be flexibly defined to create 1, 2 or 3 dimensional views of all or selected portions of the image data in the file.



The image Montage controls include the following:

- Montage Menus
- Montage Properties
- Montage Create 3D Movie

Use the Image Document Toolbar control the view and window size.

See also Montage Buffering to potentially speed up Montage creation.

This example Montage shown above uses the following settings:

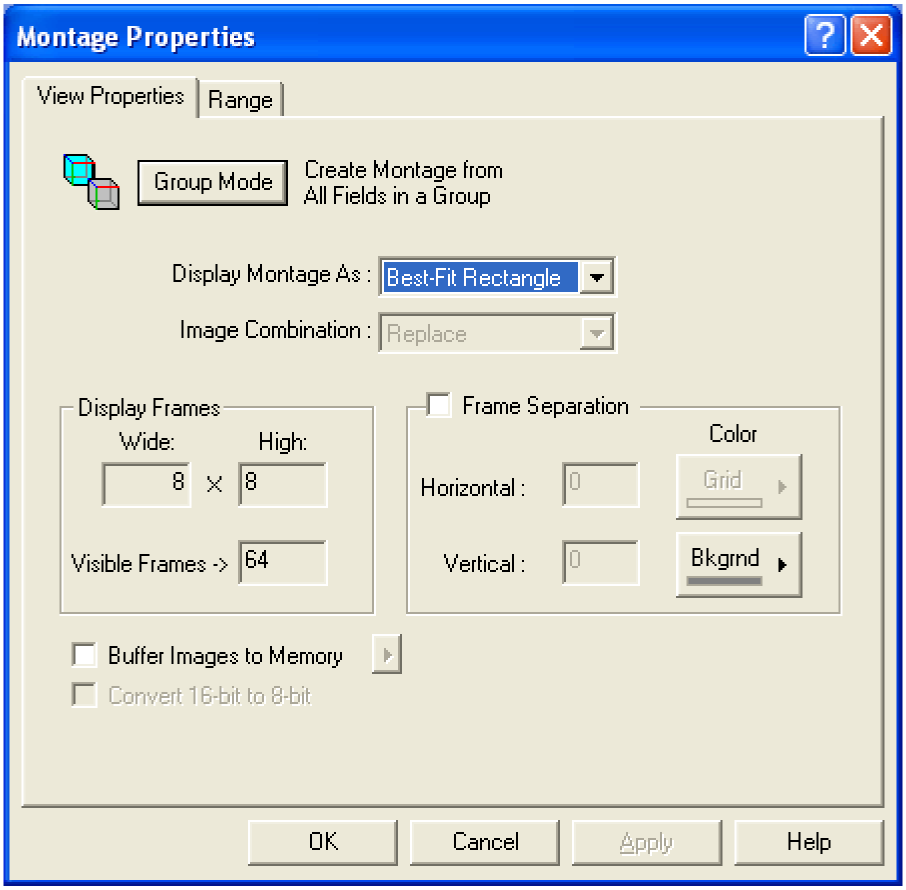
Display Montage As	Best Fit Rectangle
Horizontal Spacing	5

Frame Separation	5
Scaling (Quality)	1/4

When using long image sequences of high resolution or color images, the Montage display images created can easily become very large, so it is useful to first do a check on the size of image that will be created before starting. For instance, a 1024 x 1024 24-bit color image is approximately 3 MB without compression, so a sequence of 900 files will produce a montage requiring 900 MB – highly likely to bring most systems with 256 or 512 MB of physical memory to their knees with virtual memory management. For this reason, some facilities are provided for reducing the size of the Montage image. These include:

- Range Select a subset of the images to add to the montage.
- Bit-depth down-conversion Reduce the final image quality to decrease image size.

Montage Properties



View Properties

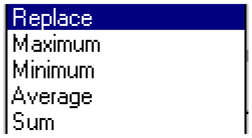
Montage Display Styles

Click the drop-down list next to **Display Montage As:** to display a list of styles:

- **Sequence** displays just one image at a time. If used with Montage Processing, this can create a Maximum or Minimum projection of images.
- **Vertical Strip** positions all images in one column.
- **Horizontal Strip** positions all images in a horizontal row.
- **Best Fit rectangle** (automatic 2D grid of images) creates a rectangular grid of images approximating a square, based on the size and shape of the images regions.
- **Custom grid** (Manual 2D grid of images) creates a rectangular grid of images based on user specified number of frames in the X and Y direction.

Image Combination:

As images from the Data Document are placed into the Montage Image, some Processing can be applied. The processing operations available include:



Replace

This will simply create an image of the last Field in the sequence

Minimum

This will process each image in the Sequence, replacing each pixel with the minimum intensity found for that pixel location in all images. Sometimes called a **Minimum Projection**, this process can be useful for tracking the location of moving cells in a time sequence, or a winding nerve through a sequence of image planes. Use Minimum if the interesting items are dark on a white background, as with normal bright field illumination of stained samples.

Maximum

This will process each image in the Sequence, replacing each pixel with the maximum intensity found for that pixel location in all images. Sometimes called a **Maximum Projection** this can be useful for tracking the location of moving cells in a time sequence, or a winding nerve through a sequence of image planes. Use Maximum if the interesting items are bright on a dark background, as with confocal or fluorescence microscopy.

Average

This will Average the pixel values together for each image in the sequence, creating an average effect. However the Average will be weighted so that the Last image has 50% of the impact on the result. It can create a time averaging effect, or can be used on two images to create a double exposure.

Sum

This Adds the pixel values for each image in the sequence. This can be used to create a summation image over time points.

Frame Separation (Image Spacing)

Each frame can be separated from its neighbor by introducing a colored border. The images will be separated by the grid color and the montage will be surrounded by the background color. The Horizontal Spacing and the Vertical Spacing can be independently specified.

Buffer Images To Memory

Checking this option may stream-line the performance of montage creation operations, depending on the image details and the computer system's hardware configuration.

If checked, images will be loaded from the Data Document as a Montage is created, and cached in physical memory to speed the processing when the montage is re-created. This saves going back to the disk repeatedly, and is especially effective when a Region is applied.

Care should be taken however to avoid using this option if the system is not equipped with enough physical memory to hold all of the images. If there is insufficient physical memory, the images will be loaded and cached in memory. Modern operating systems automatically write portions of their data in memory not immediately required to disk (virtual memory) when more memory is required than is actually available (a process referred to as "memory-swapping" or "paging out".

Therefore if the memory buffering option is utilized on a system with an amount of installed memory which is insufficient to store all the intermediate images in memory, some of the data will end up being swapped out to virtual memory (disk) during the montage creation process; despite the intentions of the memory buffering scheme to speed up the process by avoiding as many such operations as possible (disk-to-memory and memory-to-disk transfers) which defeats the purpose in terms of performance. In addition, the operating system configuration places some limit on how much virtual memory (disk space) may be used for such emergencies and when this option is checked, there is some risk that the system may run out of virtual memory altogether.

Optional Down-Conversion Bit-Depth checkbox

This checkbox will be enabled if completing the specified montage creation will be hindered by insufficient physical memory to reduce the demands of the job.

Montage Range

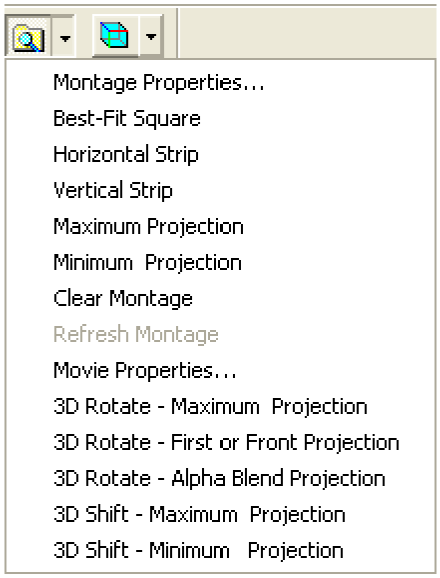
The **Montage Range** setting for the Image Montage allows a partial set of images to be included in the final Montage Image. This can speed performance, reduce memory pressure when dealing with large image volumes, and also can provide a means to sub-sample the images in a sequence for example to show longer time events where neighboring images have little change.

All images		Include all images (other inputs will be disabled)
Range (of images)	**Start**	first image field number to be included in the Montage
	End	last image field number to be included in the Montage
	Increment	# of fields to skip over between Fields to be included in the Montage.
	Count	displays the total number of images that will be included in the Montage.

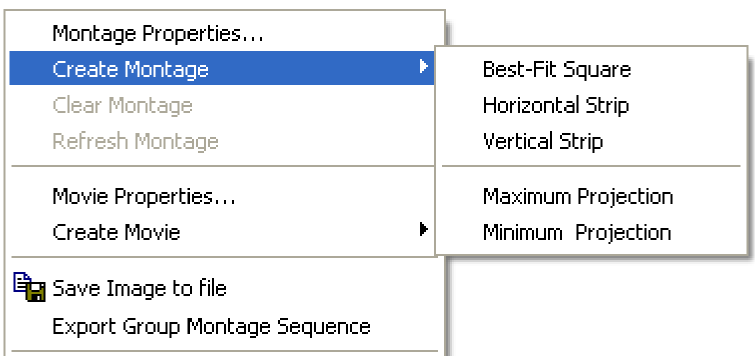
Changing any of the items will re-compute the other items in the display.

Montage Menu

You may access the **Montage** menu via the Drop-down menu on the Montage toolbar



or by right clicking on the image view when the **Field Image Montage** node is selected in the Data Document Tree.



The **Montage Menu** provides access to functions that control and utilize the Montage Image.

Montage Properties... Create a montage setting the characteristics via the Montage Properties dialog.

Quick Montage:

- **Best-Fit Square** (automatic 2D grid of images) Create a rectangular grid of images approximating a square, based on the size and shape of the images
- **Horizontal Strip** Create a strip of images where each subsequent image appends to the right edge of the previous image.
- **Vertical Strip** Create a strip of images where each subsequent image appends to the bottom of the previous image.

- **Maximum Projection** Create an image where each visible pixel combines with the previous result.
- **Minimum Projection** Create an image where each each invisible pixel in an image combines with the invisible pixels from the previous result.

Clear Montage Delete the Montage Image from memory.

Refresh Montage Re-assemble the montage with the new inputs.

Movie Properties... Create a new Data Document containing a 3D movie of the Montage.

Quick Movie:

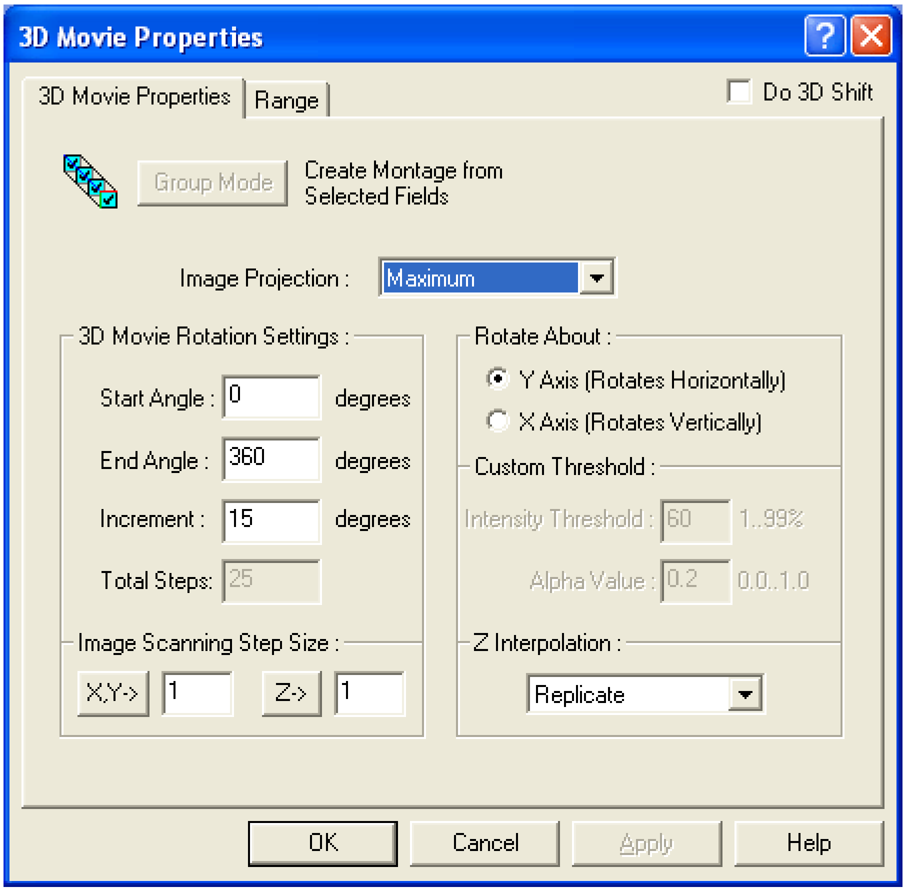
- 3D Rotate - Maximum Projection
- 3D Rotate - First or Front Projection
- 3D Rotate - Alpha Blend Projection
- 3D Shift - Maximum Projection
- 3D Shift - Minimum Projection

Save Image to File Save the montage to an image file.

Export Group Montage Sequence

Montages and 3D Movies

The Image Montage can be used to **Create a 3D Movie**. The Montage can be used in Sequence mode to create an image, which combines all frames into a single view, for instance using the Maximum Projection. To create a Movie, the same Sequence combination is used, but the images can be shifted during the combination to give a perspective appearance to the view. The 3D Movie is created by making a series of combinations and saving the resulting images into a new Data Document which can be played back using the Playback Toolbar .



The 3D Movie is initiated from the right click menu in the Montage View while the **Field Image Montage** node is selected in the data document or from the drop-down menu in the Montage toolbar. See Montage Menu for more details.

A typical application for 3D movies is to render a series of images collected with a time dimension or a Z dimension between each image.

To create a 3D Movie using the Montage controls, you will need to first create a maximum projection montage and then create the 3D Movie. Both procedures are described below.

1. How to create a Maximum Projection Montage
2. How to create a Maximum Projection 3D Movie

How to Create a Maximum Projection Montage

This example details the steps used to create a **Maximum Projection Montage** of a sequence of images. The data set example used here can be found in the example called "Head256.cxd". This data set is contained in a Data Document, with 64 images each registered in the X,Y dimension, and incrementing in the Z dimension. These images originate from an MRI sensor.

1. Open the Data Document using the **Open** command in the **File** Menu.
   This operation will display the Data Document Tree View .

2. Select the **Field Image Montage** item in the Tree.
   The View will display the first image in the Data Document (Field 1).

3. Right-click on the displayed Image to get the Montage Menu and select Properties.
   This will display the Montage Property Sheet with the control Tabs. Set the value in the View Properties page for **Display Montage As Sequence.**

4. Under **Image Combination**, select **Maximum**.
   As the image has bright data on a dark background, we will reconstruct the image emphasizing the maximum intensity (brightest) data.

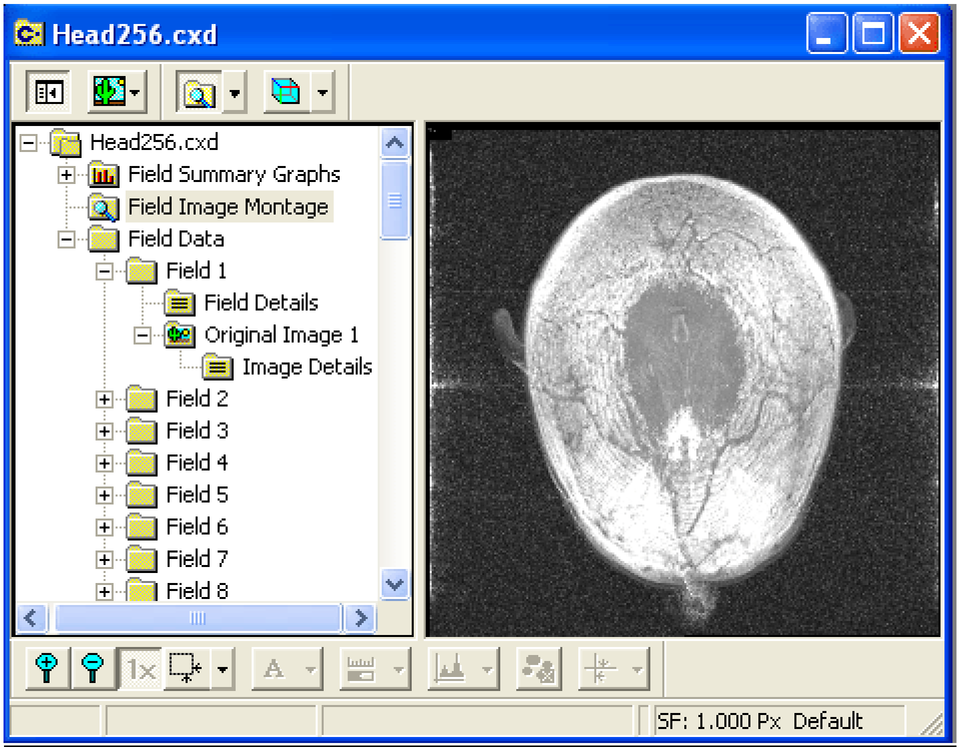
5. Select **Okay** to render a single frame in a **Maximum Projection Montage View**.
   The images will be processed creating a single frame with all images combined together.

   Notice structural information in the image, which is not normally present in any of the simple 2, Dimensional views.

The choice of the Maximum Projection Processing option is dependent on the image data. For example, in the case of fluorescence microscopy where responses are usually brighter than the background a Maximum Projection is the most useful. In the case of bright field microscopy where histological stains are used to identify target cells or tissue, the stain is usually darker than the background, and so a Minimum Projection is more appropriate.

Maximum Projection Montage Result

-

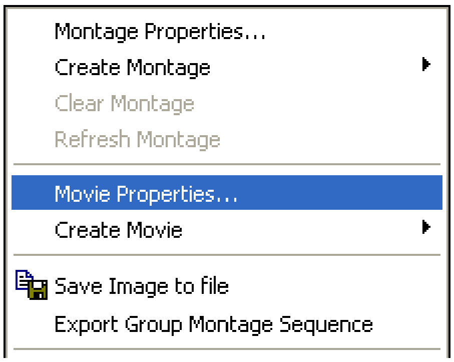


How to Create a Maximum Projection 3D Movie

See also: Movie creation using the new VIS module

Note: This example assumes that the procedure described in the topic How to create a Maximum Projection Montage has already been completed.

1. Right-click on the Montage Image View result and select **Movie Properties...** for complete control over the process. (Do not choose one of the **Create Movie** defaults as they are not optimal for this example).

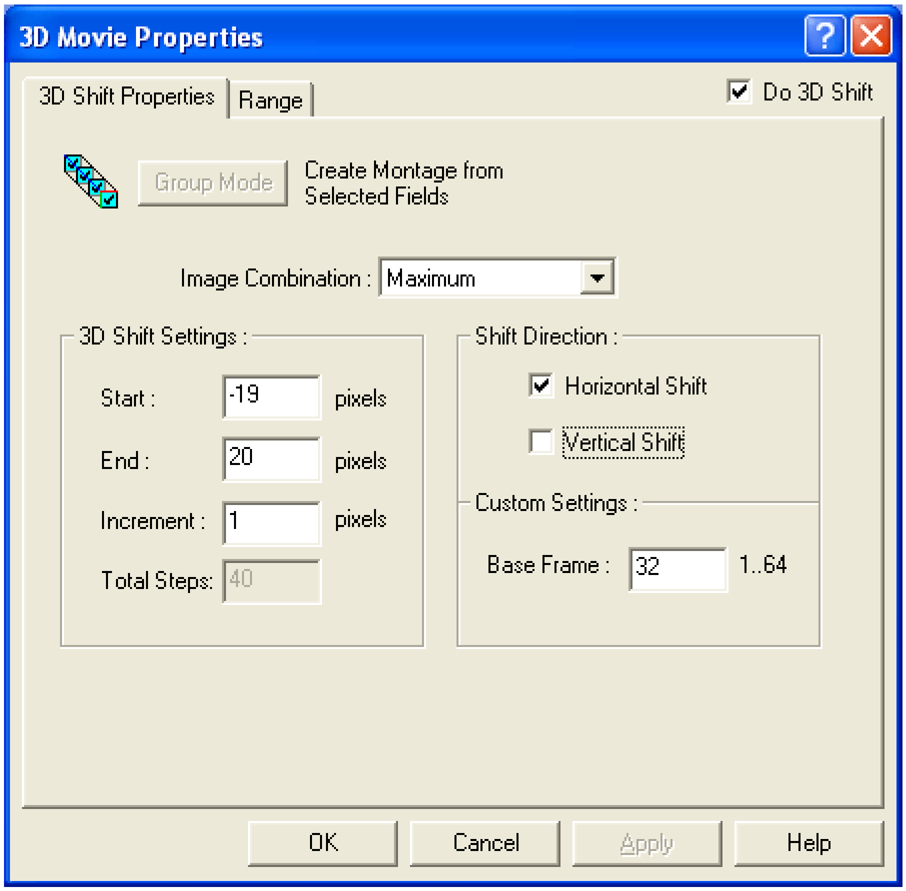


2. Check the **Do 3D Shift** checkbox and set the 3D Shift Properties to the following settings:

Parameter	**Value**	**Effect**
Base Frame	32	Rotate the view about the vertical center of the sequence
Horizontal Shift	checked	Rotate the perspective in the X-Axis
Vertical Shift	un-checked	Do not rotate the perspective in the X-Axis
Minimum Shift	-19	Set the left-most shift
Maximum Shift	20	Set the right-most shift
Increment	1	Increment the shift by one for each new frame

Count	40	Total number of new frames to create

3. Click **OK** to accept the 3D Shift parameters which should now look like this:



4. Specify the **filename** for the data document destination for the resulting 3D Movie's frames in the file dialog which appears and click **OK**.

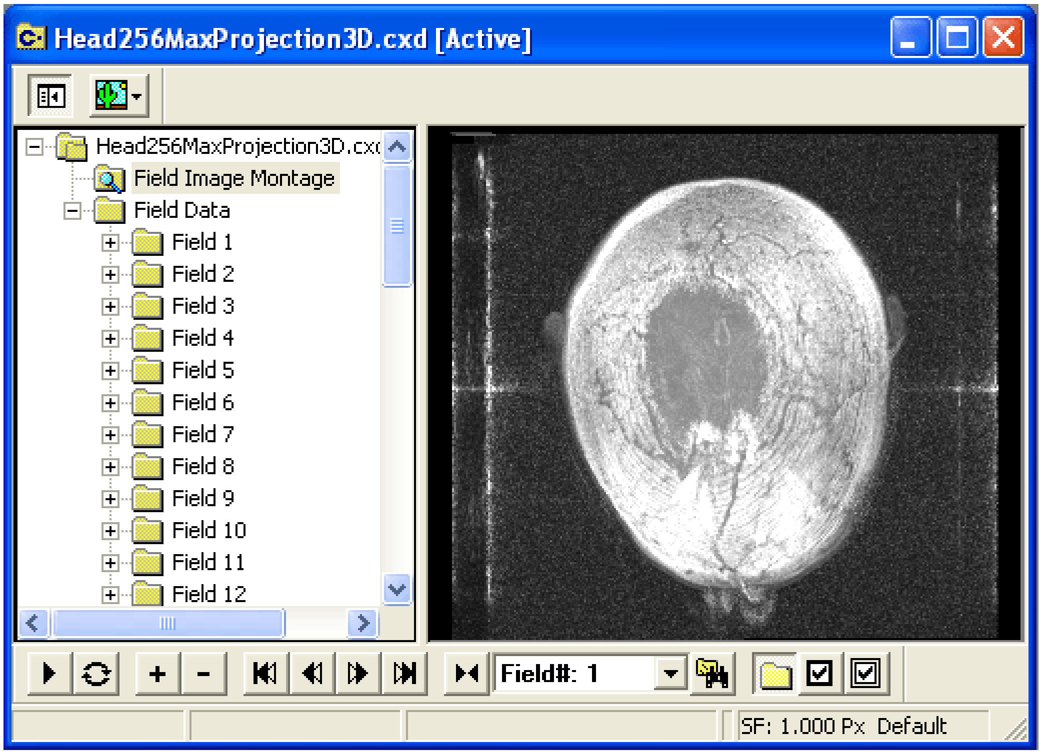
   The images will be processed creating a single frame with all images combined together. Notice structural information in the image, which is not normally present in any of the simple 2D views.

5. Select the first Field Image in the Data Document by clicking on the Original Image node for **Field #1**.

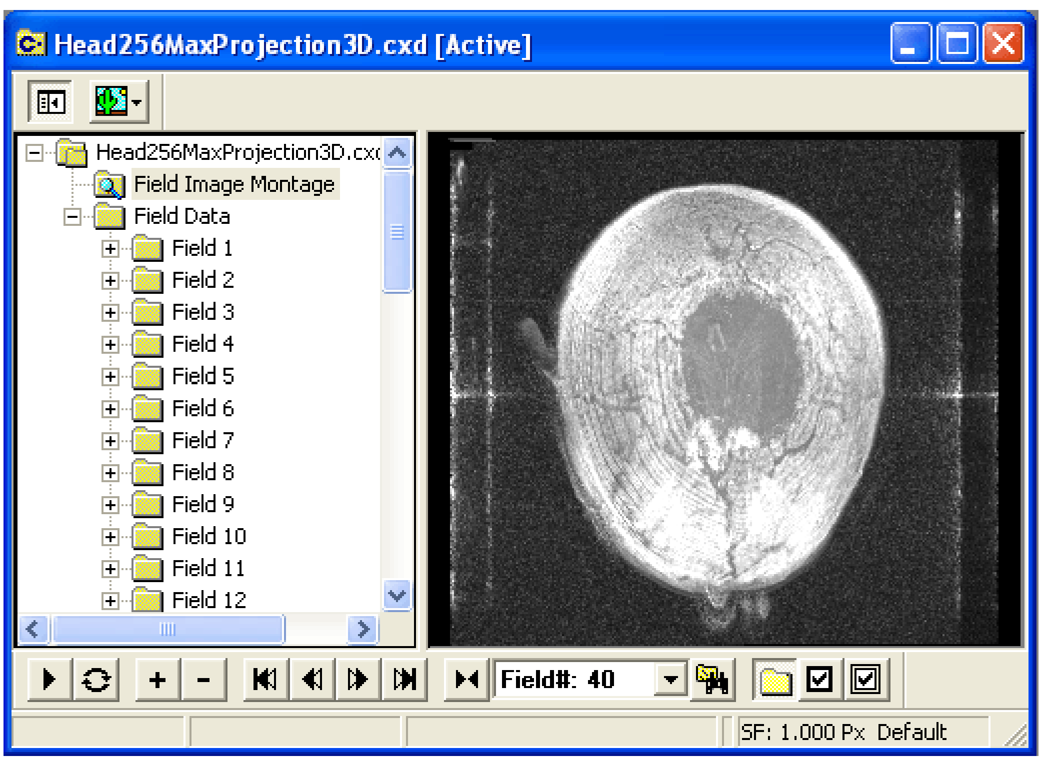
   Each frame of the Data Document is a single perspective view of the combined image sequence.

6. **Play** the Data Document. Using the Playback Toolbar, click the Play button to play the images. Experiment with the Speed and Bounce options.

The screen captures below show the perspective at each extreme end of the 3D shift.



The shift at one extreme is displayed in the first Field (above) and the shift at the other extreme is displayed in the last field (below).



Multi-Dimensional Visualization

The VIS module for SimplePCI provides a mechanism to view and interact with a multi-image data set. A typical image data set would be a series of optical sections through a specimen. Such a data set can be reconstructed and rendered for 3D viewing using the Multi-Dimensional Visualizer as an extension of the Data Document functionality.

Hardware Considerations

The Multi-Dimensional Visualizer renders the data using optimized software and hardware acceleration. It is recommended that an nVidia GeForce 7 series or higher be used for the 3D Visualization.

Following is a non-inclusive list of consumer level video cards which support OpenGL coding:

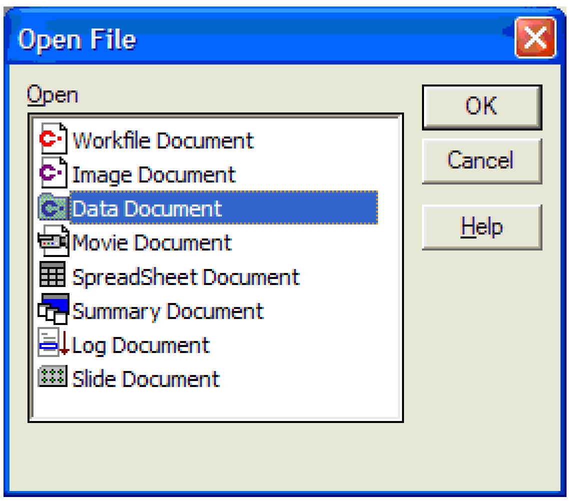
- ATI Radeon 8500, 9000, 9600, 9700, 9800, x600, x700, x800
- nVidia GeForce 6,7,8, GeForce 256, GeForce 2, GeForce 2 MX, GeForce 3, eForce 4, all Quadro lines
- Diamond FireGL1
- 3D Labs Oxygen GVX1 and Wildcat family
- Matrox Parhelia

Starting the Visualizer from a Data Document

The Multi-Dimensional Visualizer capability is activated from the SimplePCI Data Document toolbar using the VIS icon, which is only present when the VIS module license is activated.

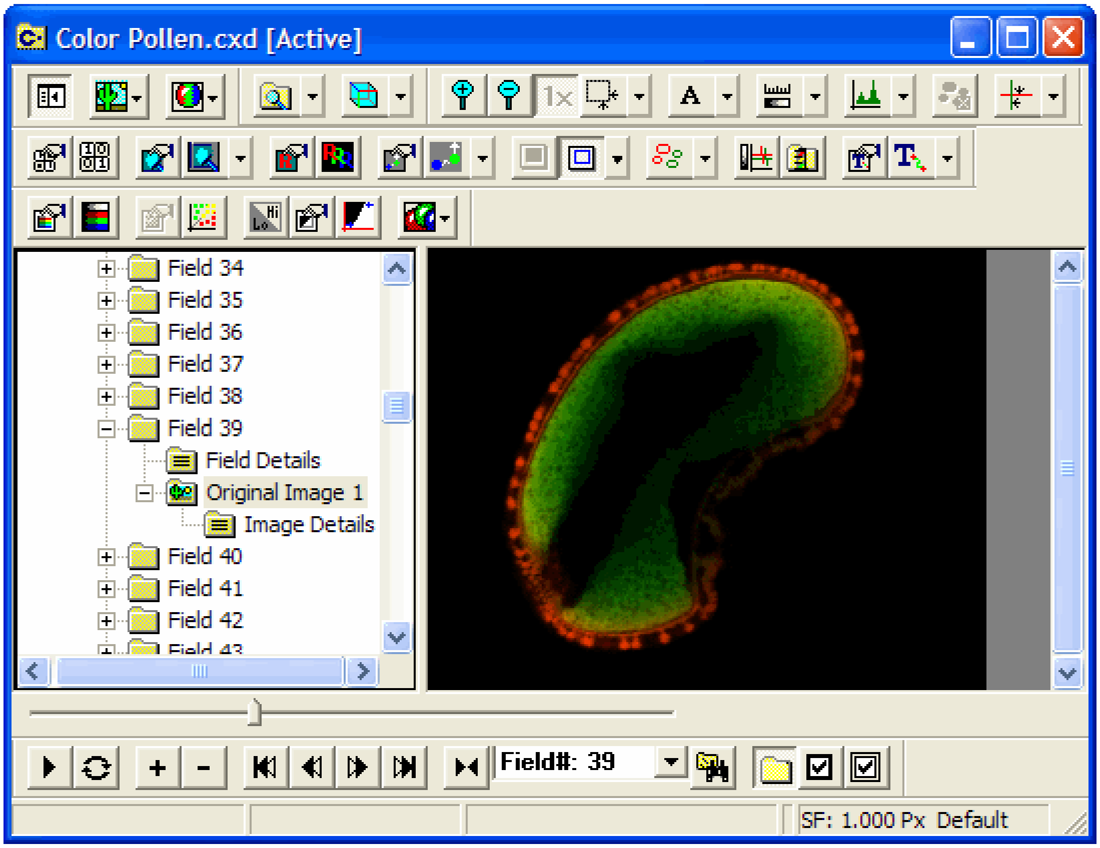
As an example we will introduce the capabilities of the VIS module using a 3D data set found on the SimplePCI Examples CD.

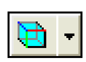
The Data Document is opened using the File Open Menu item, selecting the Data Document and locating the file.



Automatically the first field of the image sequence is displayed.

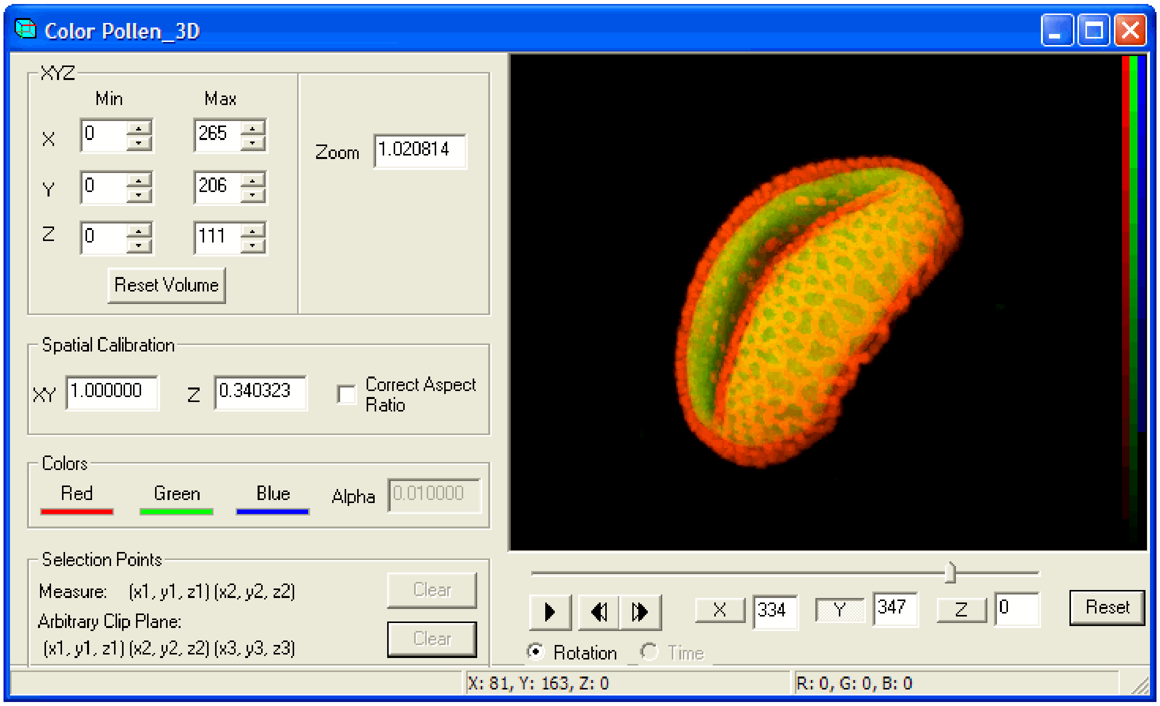
The Playback Toolbar can be used to play through the 2D images.



The **VIS icon** is shown in the toolbar above. 

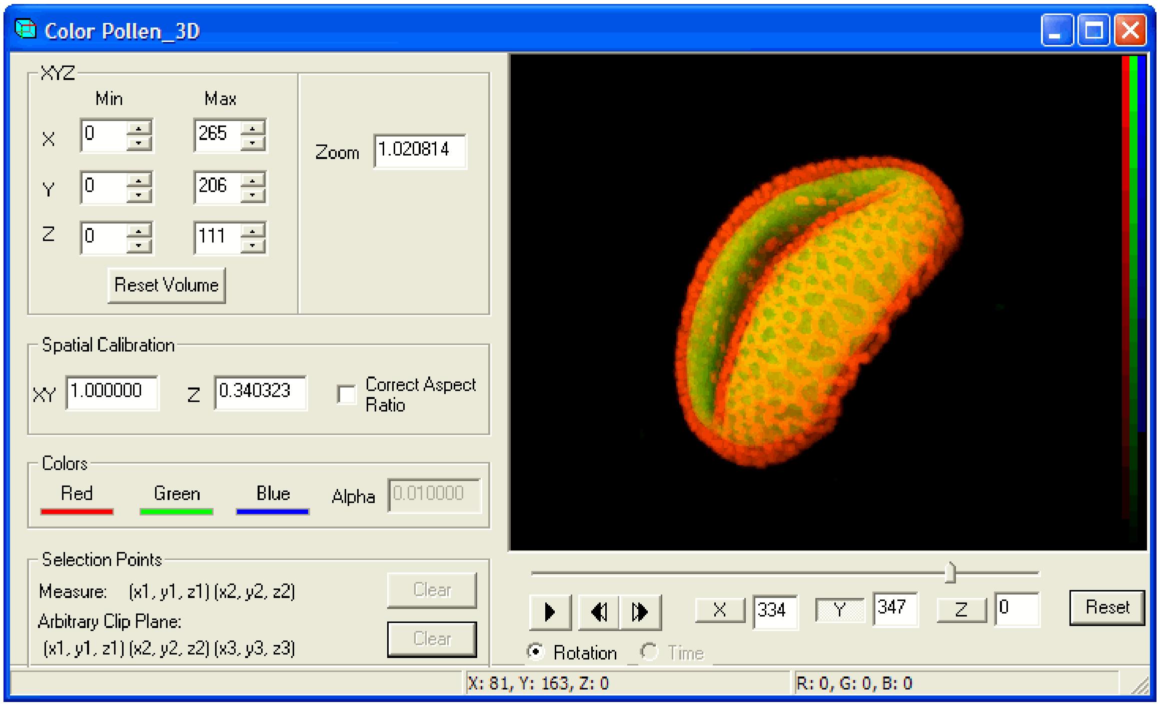
This icon is part of a Custom Menu where icons can be promoted or demoted to the toolbar by pressing the CTRL key when clicking on the menu item.

Pressing the VIS button will open the Multi-Dimensional Visualizer window. Images from the Data Document will be processed into the Visualizer and displayed using the default view properties.



Color Pollen_3D
XYZ
Min
Max
X
0
265
Y
0
206
Z
0
111
Reset Volume
Zoom
1.020814
Spatial Calibration
XY
1.000000
Z
0.340323
Correct Aspect Ratio
Colors
Red
Green
Blue
Alpha
0.010000
Selection Points
Measure: (x1, y1, z1) (x2, y2, z2)
Clear
Arbitrary Clip Plane:
(x1, y1, z1) (x2, y2, z2) (x3, y3, z3)
Clear
X
334
Y
347
Z
0
Reset
Rotation
Time
X: 81, Y: 163, Z: 0
R: 0, G: 0, B: 0

Understanding the MD Visualizer



XYZ Subvolume

This feature allows you to specify the subvolume to be rendered. In the edit boxes (X, Y, Z Min and Max) you may specify the first and last slices that should be included in the subvolume's region. Click the ***Reset*** button to return the Min and Max to their default values.

Zoom

This feature allows you to control the zoom factor for the image by using the scroll wheel on the mouse. Alternatively, you are able to change the zoom by entering the value the zoom field.

Spatial Calibration

XY is the pixel size determined by the calibration factor calculated in the Calibration window of SimplePCI. *Z* is the step size value used during acquisition. The X, Y, and Z values are imported from data documents acquired using SimplePCI. The known Spatial Calibration values may be manually entered if necessary.

Corrected Aspect Ratio

This feature when checked allows the visible dimensions of the object displayed in the 3D Visualizer to be adjusted to take into account the pixel spacing in addition to the number of pixels in the X, Y, and Z dimensions.

Colors

For multichannel data sets this feature allows the user to select a color map based on a standard group of colors as well as fluorescent dyes for each of the three channels. The color drop-down list is imported from the data document and can be customized using the Customized Contrast Tint List in the Display Contrast Dialog.

Alpha Channel

The Alpha channel specifies how a pixel's colors should be merged with another pixel when the two are overlaid, one on top of the other. The alpha channel contains a value ranging from 0 to 1. A value of 0 means that the pixel does not have any coverage information and is fully transparent. A value of 1 means that the pixel is fully opaque.

Selection Points

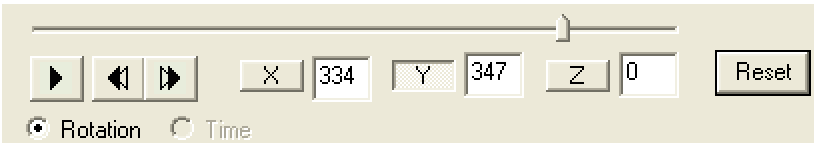
This feature displays the coordinates used in measuring a distance and defining an *Arbitrary Clip Plane*. To measure a distance between two points press and hold the Ctrl button, then left click two points on the object. Click *Measure* and then select *Calculate Distance* to measure the distance between the two points. Click *Clear* to erase the display points and reset the coordinates.

Movie Controls (Rotation and Time)

The default use of the controls is to scroll through a 360 degree rotation in the X, Y or Z plane. To choose the axis of rotation, click on the X, Y, or Z button and click *Play*. The data set will begin to rotate around the selected axis and the degree of rotation is displayed. Use the step next button to rotate the data set +10 degrees around the axis. Click the step previous button to rotate -10 degrees around the axis. Manually enter a value and click Enter and the data set will rotate to entered location. The Scroll Bar may also be used to rotate the data set. Click on the desired axis of rotation and then drag the scroll bar to rotate. The degree of rotation is continuously updated as the data set is being rotated. Click the *Reset* button to return to 0.

Movie Controls

The movie controls are located on the bottom of the 3D Visualizer can have several uses.



The default use of the controls is to scroll through a 360 degree rotation in the X, Y or Z plane. To choose the axis of rotation, click on the X, Y, or Z button and click *Play*. The data set will begin to rotate around the selected axis and the degree of rotation is displayed. Use the step next button to rotate the data set +10 degrees around the axis. Click the step previous button to rotate -10 degrees around the axis. Manually enter a value and click Enter and the data set will rotate to entered location. The Scroll Bar may also be used to rotate the data set. Click on the desired axis of rotation and then drag the scroll bar to rotate. The degree of rotation is continuously updated as the data set is being rotated. Click the *Reset* button to return to 0.

Press the *Play* button in the MD Visualizer toolbar to play the through the current movie sequence.

If the dataset is a time-series from a *Grouped* data document, select the *Time* button to move the dataset through the time points.

Axis

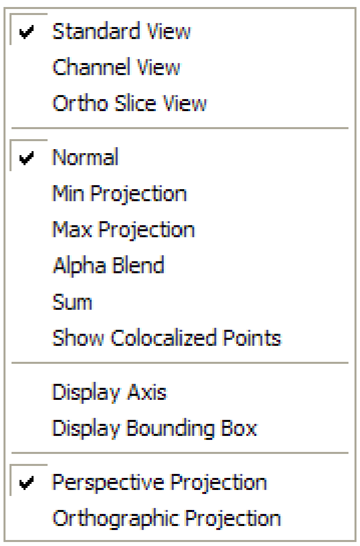
To view the XYZ Axis, select *Display Axis* from the *View Menu*. The red line represents the X axis, the green line represents the Y axis, and the blue line represents the Z axis of the dataset.

Menu Overview

The MD Visualizer Menu provides access to the many options available.

The MD Visualizer displays a graphical representation of the dataset. The different functionalities of the MD Visualizer allow you to create movies, apply different color maps, change projections, adjust and measure subvolume size and rotate the dataset.

View Menu

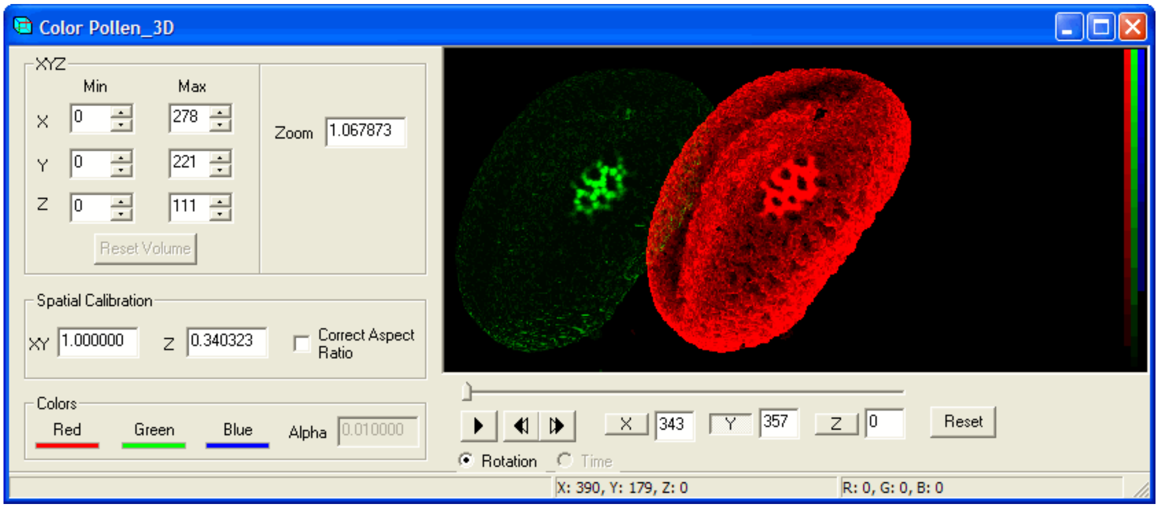


Standard View

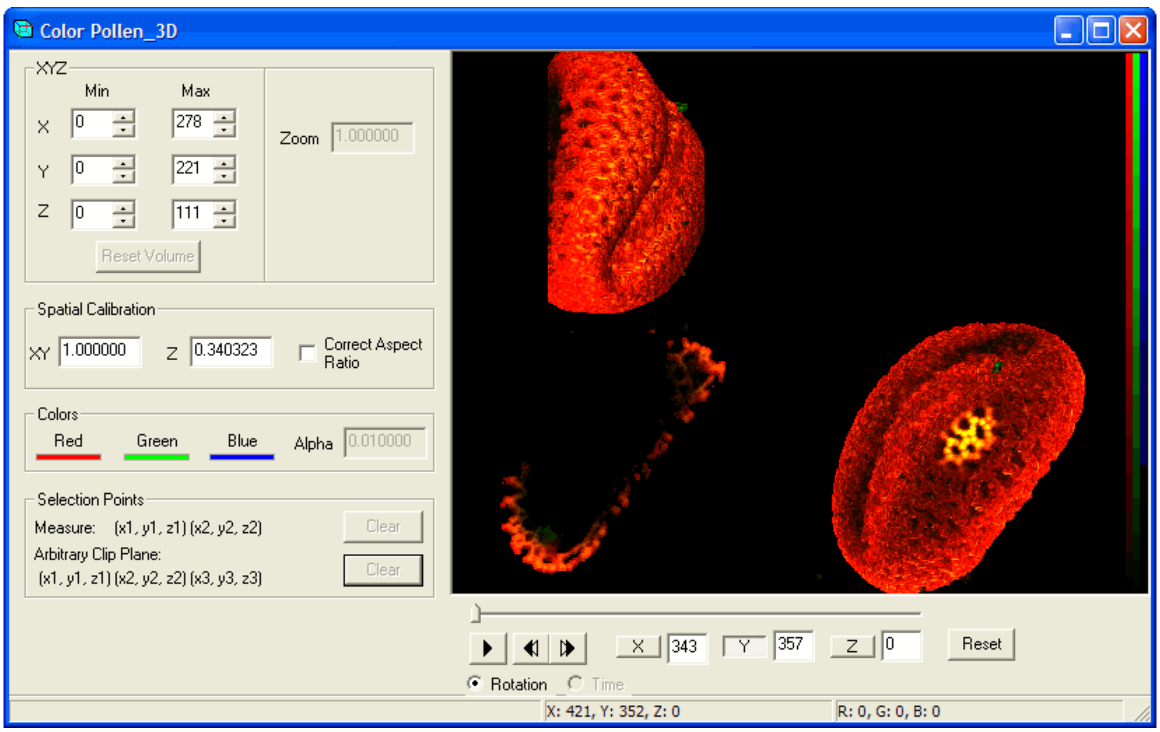
This feature displays a single 2D projection of the 3D volume.

Channel View

This feature displays a single 2D projection of the 3D volume as seen from your perspective for each of the channels captured. Up to three channels are able to be displayed.



Ortho Slice View This feature displays three single orthogonal planes, oriented to the object's XY, XZ, ZY perspectives. These planes may be adjusted changing minimum X, Y and/or Z in the volume XYZ control.



This section of the ***View Menu*** allows you to specify which 2D projection will be shown in the 3D Visualizer**.**

Normal

This feature displays a single 2D projection of the 3D volume as seen from your perspective at the object's selected orientation.

Minimum Projection

This function takes parallel rays, perpendicular to the viewing surface, and casts them through the image. The minimum voxel value encountered along each ray is taken for the projection pixel value and the resulting image is made up of each minimum voxel value.

The Minimum projection provides volumetric representations in which foreground intensities tend to be suppressed. This projection highlights edges and prominent dim features, and is typically used for viewing brightfield images.

Maximum Projection

This function takes parallel rays, perpendicular to the viewing surface, and casts them through the image. The maximum voxel value encountered along each ray is taken for the projection pixel value, and the resulting image is made up of each maximum voxel value. This projection highlights edges and prominent bright features, and is typically used for viewing fluorescence images.

Alpha Blend

The Red, Green, Blue and Alpha Channels are combined to show translucency.

Sum Projection

This function takes all the voxel values along each parallel ray, perpendicular to the viewing surface, and sums their intensity values. This creates a projection of all the summed values. Sum Projections provide volumetric representations of the data
set in which more information from the data set is considered than with the Maximum projection. Background noise will also be included in this type of projection.

Show Colocalized Points

When function is selected only the colocalized points are displayed.

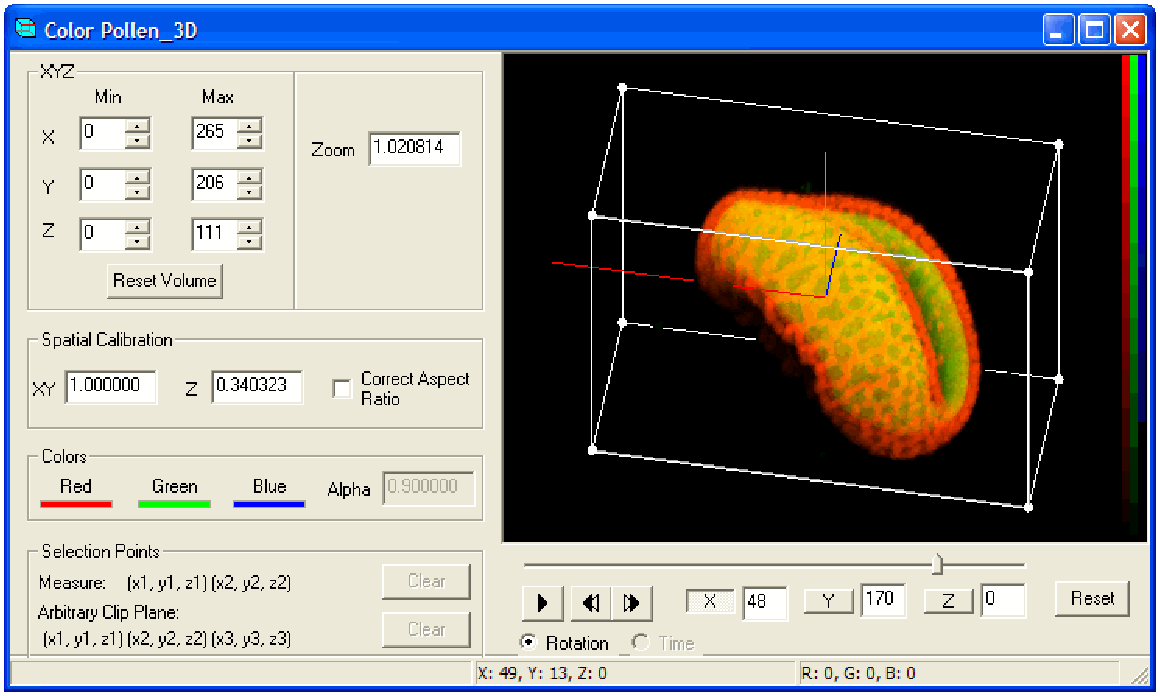
Display Axis

This feature will display colored bars on the axes to make it easier to identify the orientation of the object. The X axis is red, the Y axis is green and the Z axis is blue.

Display Bounding Box

This feature allows you to outline the boundaries of the data set around orthogonal planes, and around subvolume boundaries. This aids in judging the orientation of the dotted line and in judging the relative positions of the planes and the subvolumes.

The image below show a maximum projection of a pollen grain with the display axis and bounding box enabled in a orthogonal projection.



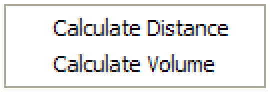
Perspective Projection

Is a 2D representation of a 3D object (the data set) as it is perceived by the eye. The object is displayed in a depth perspective so that closer objects appear larger than distant ones.

Orthogonal Projection

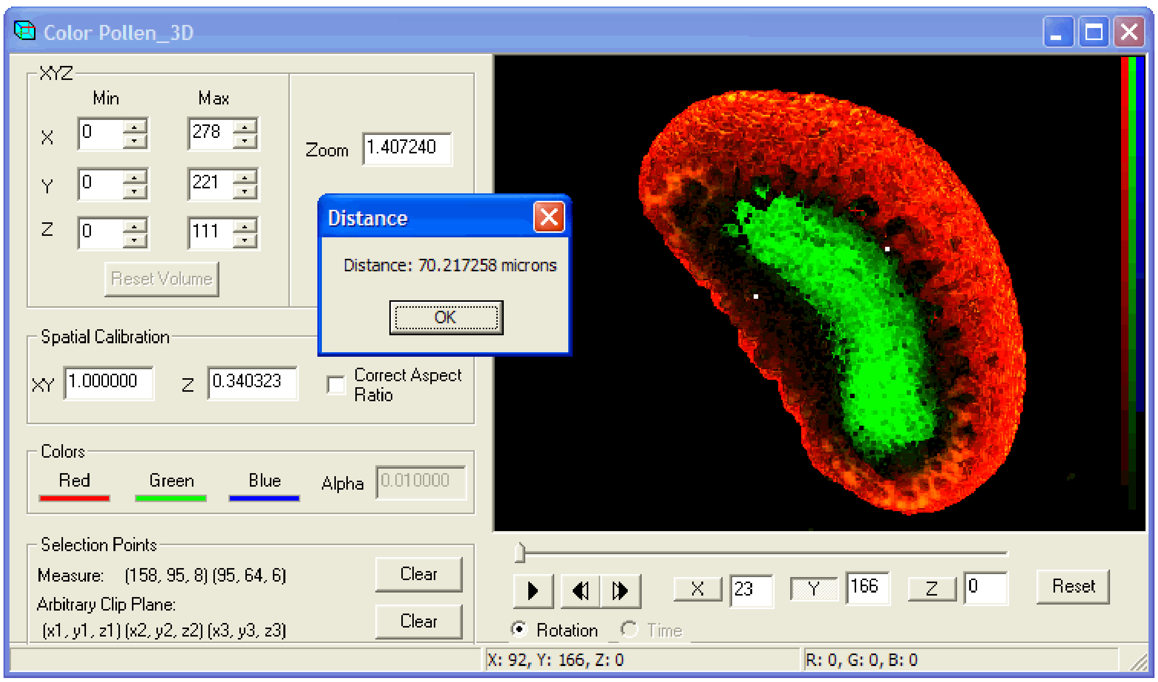
Is a 2D representation of an object formed by the perpendicular intersection of lines drawn from points on the object to a plane of projection.

Measure Menu



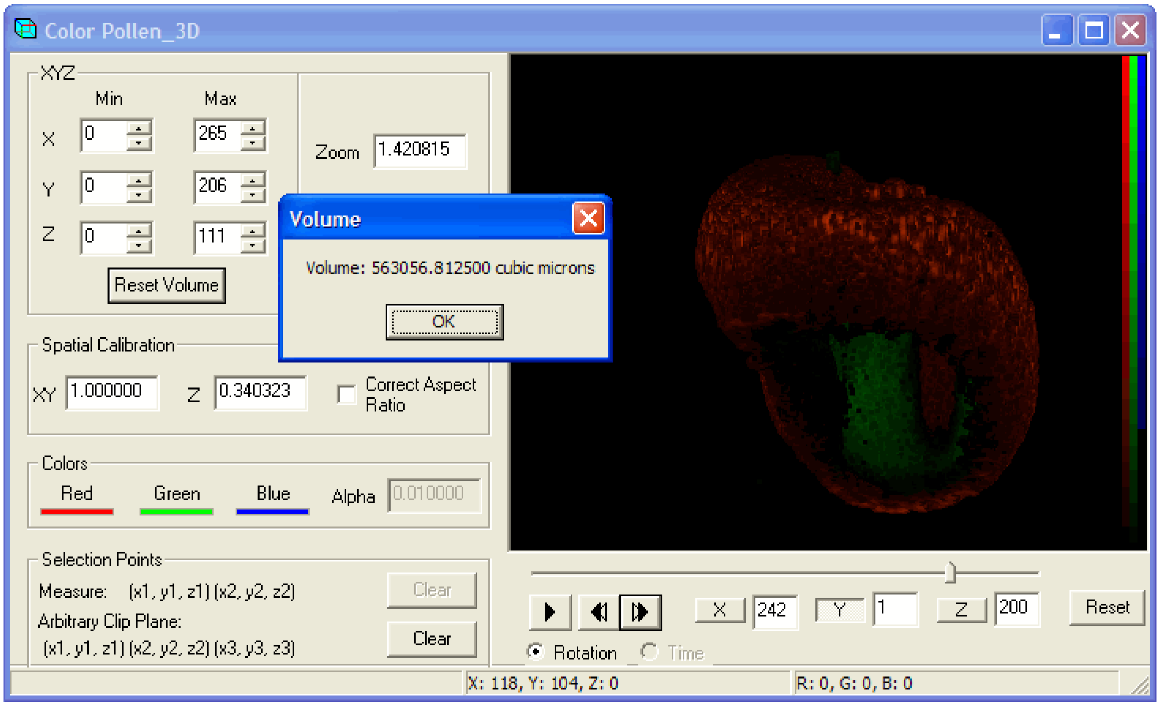
Calculate Distance

The ***Calculate Distance*** feature will allow you to measure the distance between two points within the object. Press and hold the Ctrl button, then left click to create a starting point. Now press and hold the Ctrl button, then left click to create an endpoint. The selected points are represented by white dots in the image and coordinates are displayed in ***Measure*** of the ***Selection Points*** window of the MD Visualizer. Go to the Menu, click ***Measure*** and then select ***Calculate Distance*** to measure the distance between the two points. Click ***Clear*** to erase the display points and reset the coordinates.



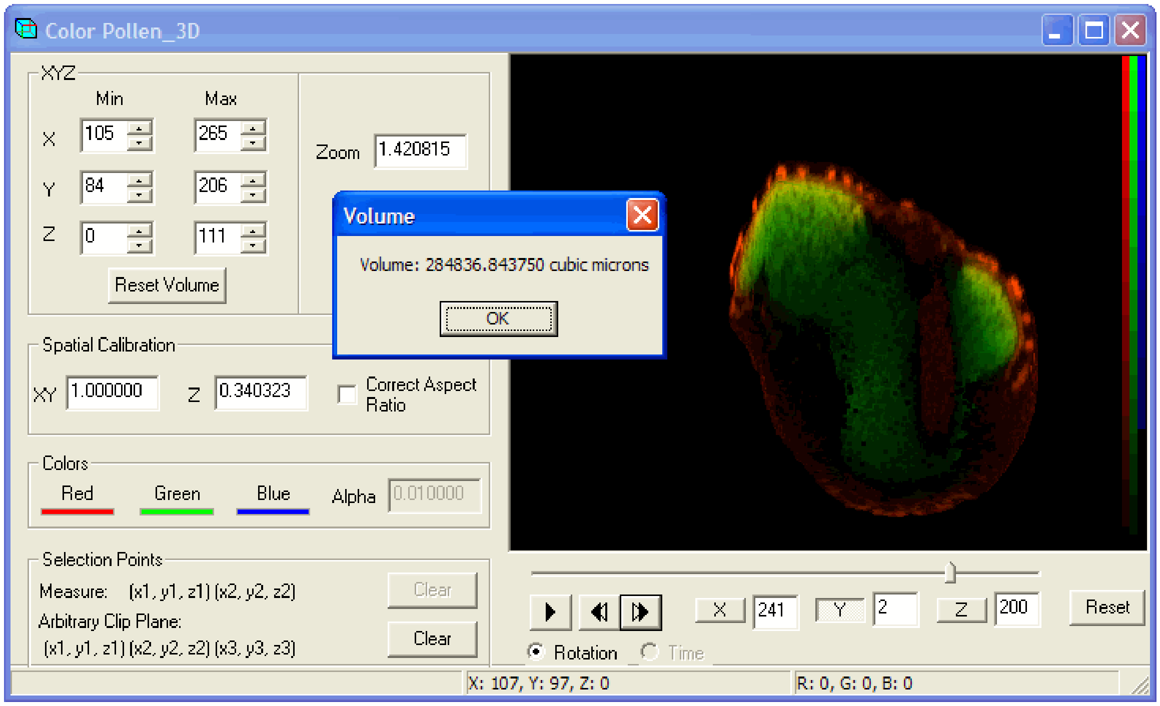
Calculate Visible Volume

This feature allows the user to ***Calculate the Visible Volume*** of an object. Go to the Menu, click Measure and then select ***Calculate Visible Volume***.



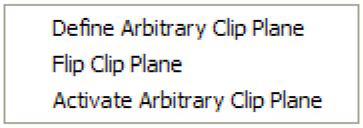
A subvolume may be calculated by enabling the Display Bounding Box, then left-click and drag any plane of the Orthographic Projection. As the bounding box is manipulated, the X, Y and Z plane Min and Max values also change. Any points within the object that are outside of that range are removed. Click on Calculate Visible Volume to get a subvolume of the object.

> This feature allows you to specify the subvolume to be rendered. In the edit boxes (X, Y, Z Min and Max) you may specify the first and last slices that should be included in the subvolume's region. Click the *Reset* button to return the Min and Max to their default values.


Color Pollen_3D
XYZ
Min
Max
X
105
265
Y
84
206
Z
0
111
Zoom
1.420815
Reset Volume
Volume
Volume: 284836.843750 cubic microns
OK
Spatial Calibration
XY
1.000000
Z
0.340323
Correct Aspect Ratio
Colors
Red
Green
Blue
Alpha
0.010000
Selection Points
Measure: (x1, y1, z1) (x2, y2, z2)
Clear
Arbitrary Clip Plane:
(x1, y1, z1) (x2, y2, z2) (x3, y3, z3)
Clear
X
241
Y
2
Z
200
Reset
Rotation
Time
X: 107, Y: 97, Z: 0
R: 0, G: 0, B: 0

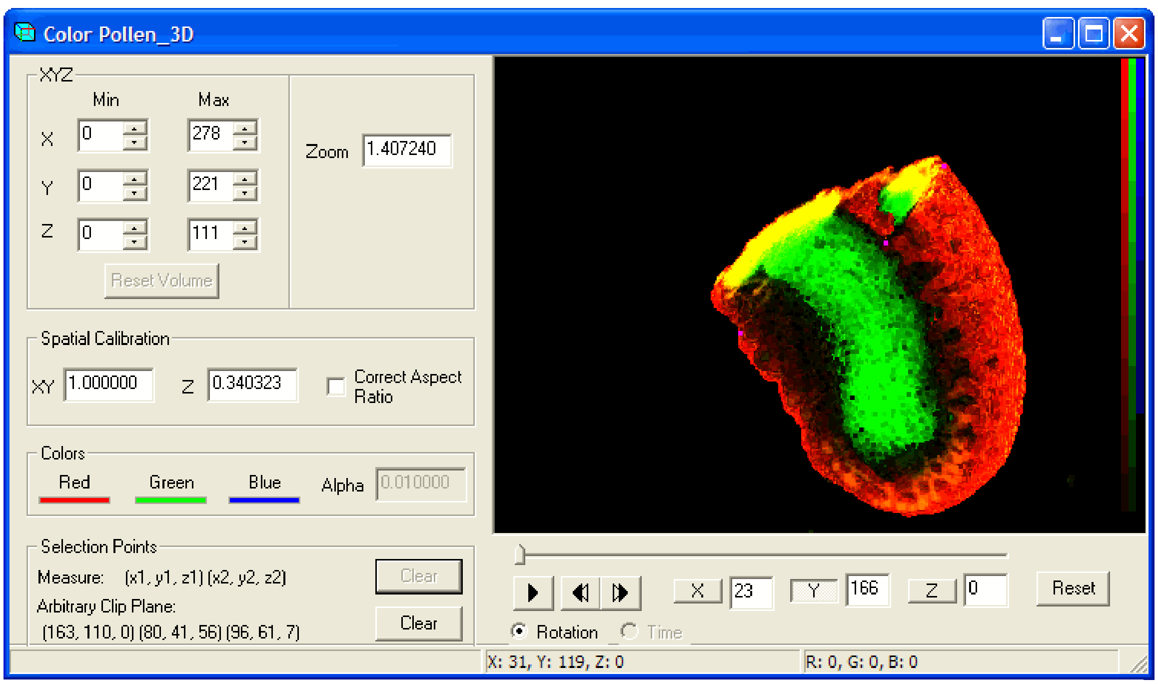
Clip Planes

The Clip Plane feature allows the user to slice the object (volume) along an arbitrary plane and retain everything above or below the slice and then manipulate the rotation, view and projection of the object.



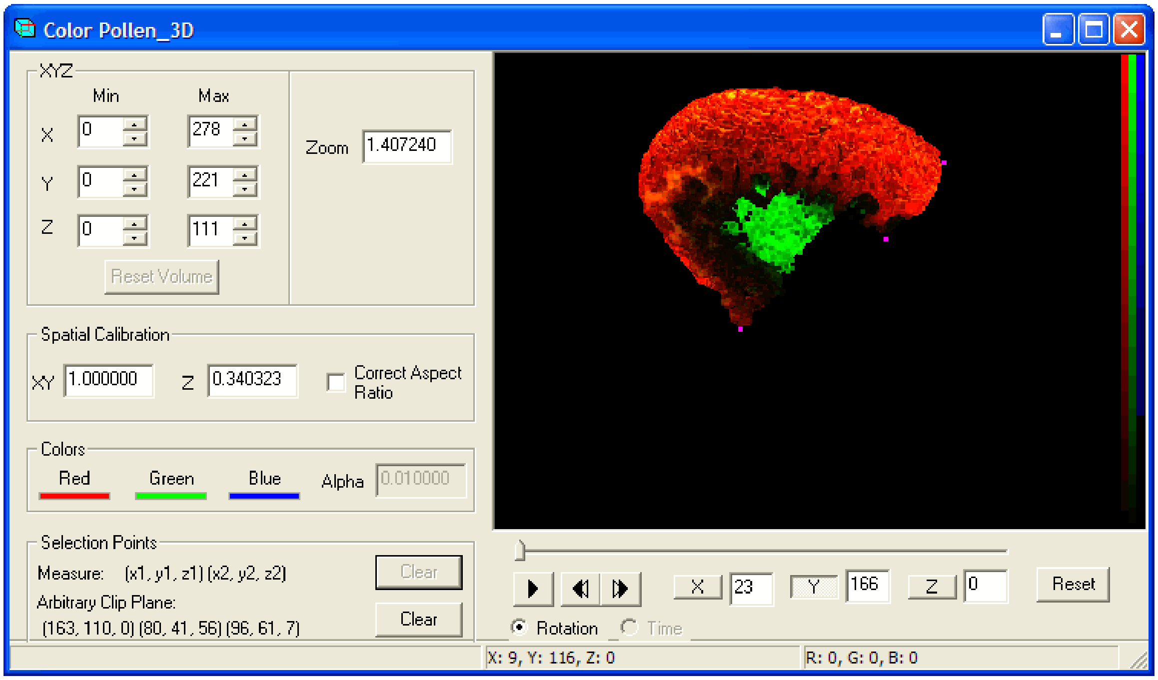
Define Arbitrary Clip Plane

Go to the Menu, click ***Clip Planes*** and then ***Define Arbitrary Clip Plane***. Now press and hold the Ctrl button, then with the mouse left click on three locations to define the Clip Plane. The sliced section of the object retains it's spatial orientation and is able to be rotated around the object's central position. The coordinates of the ***Arbitrary Clip Plane*** are displayed in the Selection Points window of the MD Visualizer. Click ***Clear*** to reset the coordinates.



Flip Clip Plane

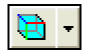
The Flip Clip Plane feature allows you to reverse the direction of the cutting plane so that the visible portion of the object becomes the cut portion and vice versa.



Activate Arbitrary Clip Plane

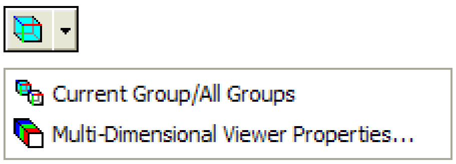
This feature allows the user to view the entire object (whole volume) or the sliced section.

Toolbar and Menu Access

The VIS icon is shown in the toolbar. 

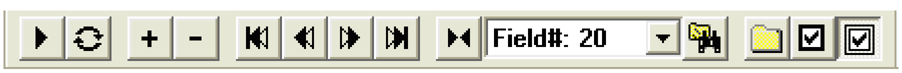
This icon is part of a Custom Menu where icons can be promoted or demoted to the toolbar by pressing the Ctrl key when clicking on the menu item.

This VIS icon has a drop-down menu.



Group Mode on/off Selection

The Data Document Playback Toolbar includes buttons to select between *Normal*, *Selected* and *Group Mode*.



Normal Mode

Each field is indicated by a folder and is included in the Playback, Export operations, etc.

In this mode, all fields will be rendered in a single 3D View

Selected Mode

Each checked field is included in operations

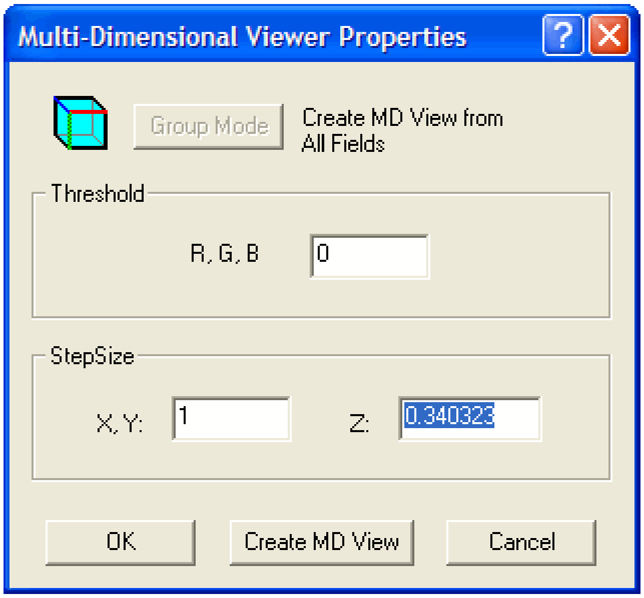
In this mode all checked fields will be rendered in a single 3D view

Group Mode

A defined Group Size determines the fields to be included in the 3D Visualizer.

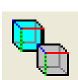
Setting Properties

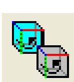
The **Multi-Dimensional Visualizer Properties Dialog** can be activated from the toolbar icon  .

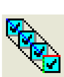


Group Mode

Group Mode has an additional selection in the MD Visualizer Toolbar.

**Current Group -** Create MD View from All Fields in a Single Group. This can be used to render all slices in a single time point.

All Groups - Create MD View from All Fields in All Groups. This can be used to render Z-series over time.

 **Selected Fields-** Create MD View from Selected Fields.

Threshold

If the intensity of Red or Green or Blue is greater then the entered threshold value the point will be accepted. The default threshold is 15.

Step Size

The X,Y Step Size is listed. This is derived from the Data Document Calibration Scale Factor.

The Z Step Size is derived from the Z spacing between fields in the Data Document.

These values can be modified, but changes will not be reflected back to the Data Document.

OK

This will accept the current settings, exit the dialog and refresh the MD View if needed.

Create MD View

This will create the MD View using the settings in the MD Visualizer Properties window.

Cancel

This will restore the previous settings, exit the dialog and refresh the MD View if needed.

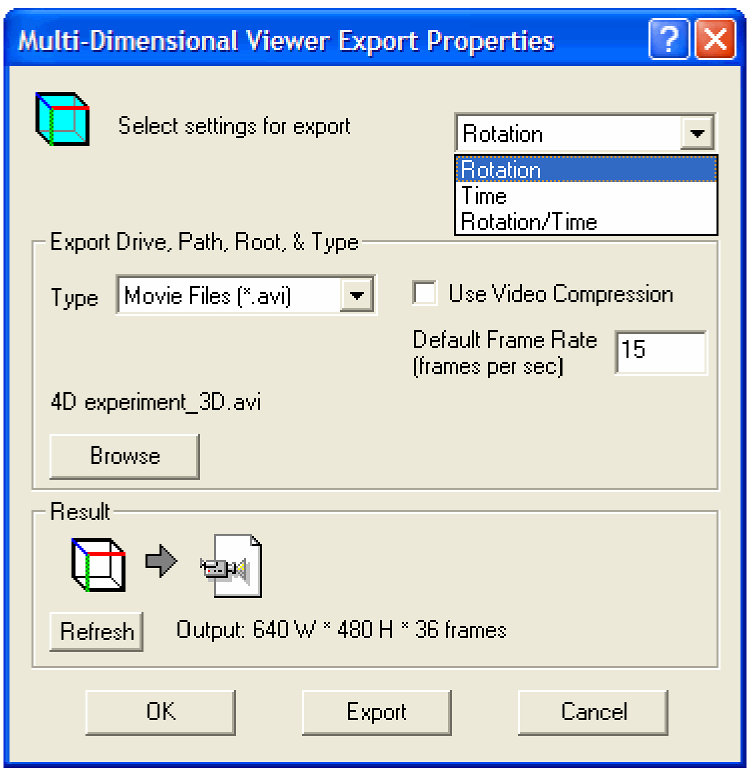
Exporting from the Multi-Dimensional Visualizer

To save images created in the Multi-Dimensional Visualizer, use the items in *File* of the Menu Toolbar.

Copy Multi-Dimensional Visualizer image to Image Document - Images can be copied to a SimplePCI Image Document for annotations etc., and saving to the many image formats supported for single images.

Export from Multi-Dimensional Visualizer

To create a multi-image file, use the Multi-Dimensional Visualizer Export Properties dialog.



This dialog allows the current Multi-Dimensional Visualization and movie settings to be applied to the source data and output as a single file, a movie file or other file sequences.

The rotational settings of the movie depend on the current movie as defined in the Multi-Dimensional Visualizer Properties and the Multi-Dimensional Visualizer.

Settings: Rotation, Time and Rotation/Time. Depending on the available data and the Group Mode selected Rotation may be the only setting available.

Data Document Group Mode:

1. **Normal Mode**

   Normal Mode is indicated by the folder icon. Normal Mode is compatible with single dimension data sets, e.g. a Z scan. The Data Document is viewed as a folder based tree with each field represented by a folder. All images are used in a single rendering.

2. **Selected Mode**

   The Selected Mode can be activated using the checked box icon in the Data Document Toolbar. This converts each Field Folder in the tree to checkable box. Unwanted images can easily be turned off using by unchecking the box.

   To control many fields at once, use the right click menu from any Field icon in the tree to select or deselect a range of Fields at once.

   In Selected Mode only selected (checked) Fields will be processed, so unwanted fields in the document can easily be ignored in the rendering.

3. **Group Mode**

   Group mode allows the Fields in a Data Document to be partitioned into Groups. Each group typically represents a set of Fields related to each other but separated from other groups. This type of data can be created by a Z scan at each point of a time lapse. Each Group has a Group Number. The Group Size is the number of Fields in the scan. Each Field has a Group Index which is the number of the Field within the Group.

   Group Mode is ideal for the Multi-Dimensional Visualizer allowing each group of related fields to be rendered as a group. This is useful for time series sequences of Z scans. Each Z scan is group and is rendered in three dimensions, each group is rendered sequentially giving the capability of 4D movies.

Output Mode (destination)

If more than one group is processed, each group can be exported to the same file (one file name for all groups). See below for examples of the Result Frame which diagrammatically describes the exported results.

Export Drive, Path, Root, and Type

Select the type of destination file (e.g. avi movie file, cxd file or individual image files per frame).

The ***Browse*** button allows the destination file properties to be set, including file path, root name and file type if needed.

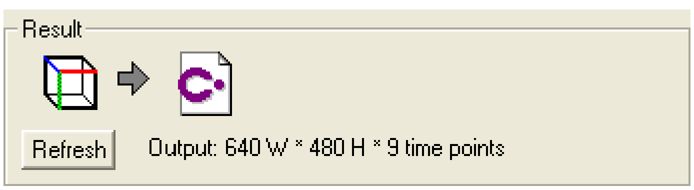
Result

The Result frame graphically shows how the output file will be created, the Output Data File type, and the rendered image width and height.

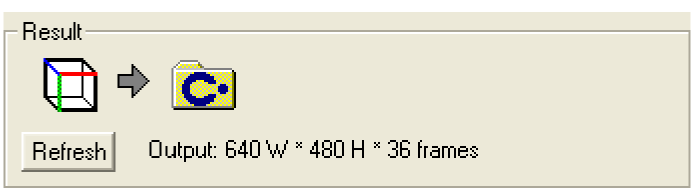
Here are some examples showing the variety of output formats available:

1. Single Image View

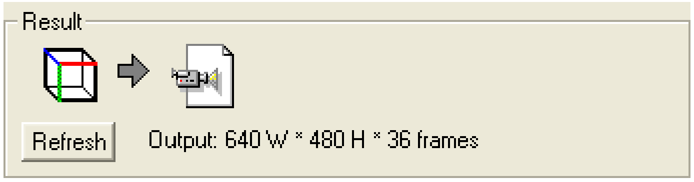
Current View to single image file



Current View Movie to image sequence file (Data Document)



Current View to an AVI movie file format



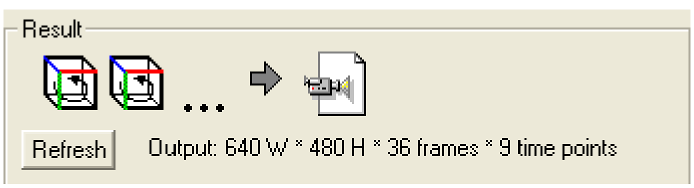
2. Multiple Image View

Multiple Group Movie

One file for All Groups (Data Document file format)

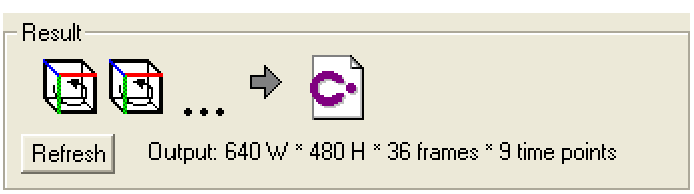
Multiple Group Movie

One file for All Groups (AVI Movie file format)



Multiple Group Movie

One file for All Groups (individual image file format or Multipage TIF)



Use the *Refresh* button to update the result display after making any changes to the settings above.

The *OK* button will accept the settings and exit the dialog.

The *Export* button will exit the dialog and apply the settings to generate the output data files.

The *Cancel* button will ignore changes to the settings and exit the dialog.

Visualizer Shortcut Keys

Below is a list of the available shortcut keys that are able to be used with the Multi-Dimensional Visualizer.

Rotation

UP: Increase X Rotation

DOWN: Decrease X Rotation

RIGHT: Increase Y Rotation

LEFT: Decrease Y Rotation

Subvolume Control

F2 + CTRL: Decrease Min X

F2: Increase Min X

F3 + CTRL: Increase Max X

F3: Decrease Max X

F5 + CTRL: Decrease Min Y

F5: Increase Min Y

F7 + CTRL: Increase Max Y

F7: Decrease Max Y

F8 + CTRL: Decrease Min Z

F8 : Increase Min Z

F9 + CTRL: Increase Max Z

F9: Decrease Max Z

Clip Plane Control

F11 + CTRL: Increase Arbitrary Clip Plane

F11: Decrease Arbitrary Clip Plane

Color Alpha Control

MULTIPLY: Increase Alpha

DIVIDE: Decrease Alpha

Movie Controls

NUMPAD4: Step Previous

NUMPAD6: Step Next

SPACE: Play

Experimental Planner

Welcome to the Experiment Planner™, an innovative and practical tool for managing advanced imaging experiments.

The Experiment Planner™ combines a powerful event processing language with the Active Spreadsheet™ visual environment, providing an efficient solution for automating the process of creation, modification, debugging and execution of complex imaging experiments.

Here is a brief summary of the Experiment Planner features:

- Support for experiments of high complexity level including multi-dimensional and multi-stage experiments, timing diagrams etc.
- Modularity, allowing the incorporation of pre-defined automation components
- Flexible device configuration allowing selection of available hardware to suit the specific experiment requirements
- An extensive set of debugging tools, significantly reducing the possibility of logical errors before the real experiment is started
- Sophisticated interactive data tuning support, allowing to automatically update the Experiment Planner parameters and data in the process of manual hardware manipulation
- A unique set of editing tools allowing the easy manipulation of the experiment structure and data, as well as reducing the possibility of logical and typing errors

The purpose of this document is to provide the information necessary to understand the principles and functionality of the Experiment Planner as well as detailed description of the commands and operations using in the process of automation.

The document has the following structure: the rest of the chapter discusses experiment design in general and how the Experiment Planner™ addresses it.

How it Works explains the principles the system is based upon.

Getting Started describes the visual environment used to create and execute the experiment plan as well as how to work with its components and what the typical development cycle is.

Commands, Devices and Operations reference Manual contains the complete reference on commands and operations used in the Experiment Planner language along with some simple experiment planning problem which will be solved step by step while more devices and operations will be introduced.

The remainder of this document discusses the programming techniques used to solve different general types of the planning problems. A set of examples are included which can be used as a starting point for implementing experiment automation tasks.

The purpose of the experiment planning and how it's reflected in the Experiment Planner(TM)

Let’s suppose we want to carefully design our experiment in such a way that all actions are documented so it will be possible to analyze or reproduce the experiment in future. Let’s suppose also that we the piece of paper (or bunch of paper) is available to accomplish this task.

The typical experiment setup can be described as follows.

First of all, it is necessary to define the set of devices to be controlled, i.e. Stage (x, y, z), camera, camera settings, the order in which the data are recorded etc. So, the careful experiment developer first of all would write down the list of such devices or, even better, the table, which would look something, like this (Fig.1):

Devices						
Stage			Acquisition		Storage	
X	Y	Z	Settings	Capture	File	Image

Figure 1. Table of devices

Next, he would like to write down the sequence of actions performed during the experiment, each action involving the control of some or all of the devices. Each action would represent some important step in the experiment control, which separated, in time or logically, from others. So, taking into account the set of devices previously defined some simple experiment description (or experiment log) would look like this (Fig.2):

Actions	Devices						
	Stage			Acquisition		Storage	
	X	Y	Z	Settings	Capture	File	Image
Select the file into which the images to be stored						"Experiment"	
Move stage to the position first position	X1	Y1	Z1				
Select visible light source				Switch to visible			
Acquire the image and save it as an Image #1					Acquire		Image #1
Move stage to the second position	X2	Y2	Z2				
Change light source to fluorescent				Switch to fluorescent			
Acquire the image and save it as an Image #2					Acquire		Image #2

Figure 2. Experiment log

For more complex experiment, it quickly becomes apparent that one-level list of actions is just not adequate. For example, taking an image of the field of interest would involve moving the stage to <X,Y> position, then finding the optimum focus <Z> then switching the lamp to fluorescent mode, adjusting the camera gain and exposure, taking the image and finally storing it etc. For multi-site time lapse experiment, each group would involve the same set of routine operations and our experiment log will be quickly overloaded with details hiding the big picture. The situation will get even worse if we consider the multi-stage experiment when some agent is added to the cell colonies at the beginning of each stage and then all fields are observed in the order described above.

There is a well-known way to have deal with this problem: the plain list needs to be transformed into the hierarchical one, where the sequence of low-level operations constitutes a macro-operation with higher-level meaning. The set of such operations would constitute even higher-level macro-operation and so on. The resulting log list would look similarly to the content of the book that has chapters, subchapters, paragraphs and so on.

In our hypothetic experiment 3 levels of hierarchy can be easily identified:

- Experiment stage related (Stage 1, Stage 2 etc.)
- Field of interest related (Field 1, Field 2 etc…)
- Operations on the Field (Op.1, Op. 2 etc.)

So, the final experiment plan would look like this (Fig.3):

Actions	Devices						
	Stage			Acquisition		Storage	
	X	Y	Z	Settings	Capture	File	Image
Stage 1						"Experiment"	
Field 1	X1	Y1	Z1				
Operation 1				Switch to visible			
Operation 2					Acquire		Image #1
Operation 3	X2	Y2	Z2				
Operation 4				Switch to fluorescent			
Operation 5					Acquire		Image #2
Field 2							
Operation 1							
...							
Field ?							
...							
Stage 2 (add reagent)				...			
Field 1							
...							
Stage 3 (add 2nd reagent)							
...							

Figure 3. Hierarchical experiment log

Note: that even if the experiment is controlled "by hand" it is essential to keep the record so it can be reviewed or reproduced at any time later. Therefore, even the printed experiment log is an important part of any experiment.

The Experiment Planner™ follows exactly the same common sense approach. It takes 3 key components of experiment planning:

- The hierarchical action tree,
- The set of devices to be controlled
- Device-related commands and data

and combines them into the Active Spreadsheet™ (see Fig. 4) automating the process of the creation, modification, execution and debugging of the experiment.

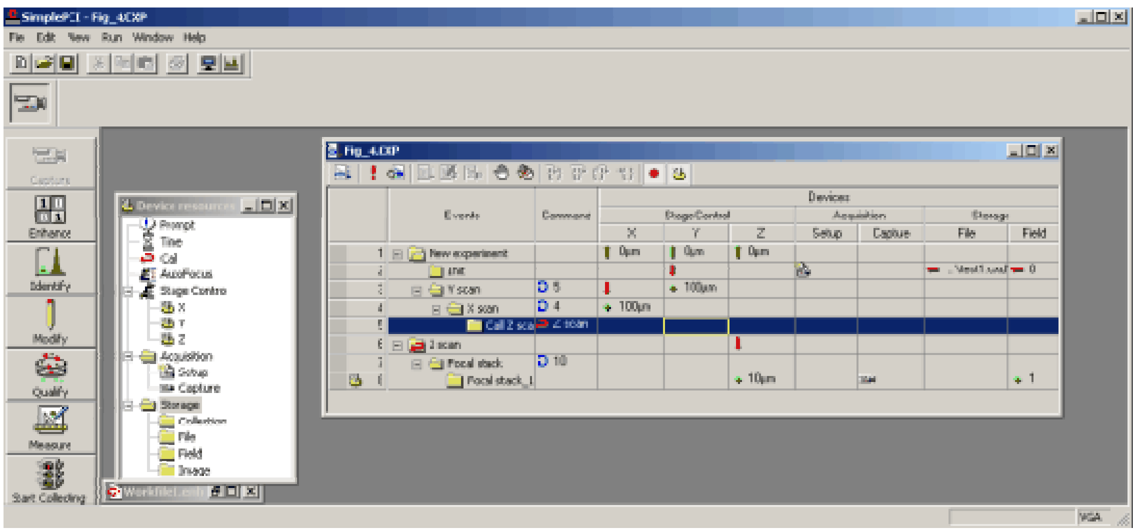


Figure 4. Experiment Planner™ screenshot

A brief definition of the Experiment Planner(TM)

The Experiment Planner is a high-level tree processing language with Artificial Intelligence (AI) elements coupled with Rapid Application Development (RAD) visual environment and tuned for automated experiment planning, debugging and execution. Its visual environment, the Active Spreadsheet, incorporates 3 key components:

1. The hierarchical tree representing logical actions or Events performed during the experiment and defining the flow of operation coupled with command altering if necessary the general flow.
2. The hierarchical header containing the devices to be controlled during the experiment
3. The grid of cells each containing the command to be performed on the particular device and data to be exchanged with device during the command execution.

Here is a functionality summary the experiment planner provides (EP™ document is referred as an Event Log):

- Automatic execution of the Event Log
- Creation and modification of the list of devices to be controlled
- Combining multiple Event Logs into one composite Event Log using either pre-created Event Log templates or calling external Event Logs during the execution
- Debugging the Event Log using Break Point/Pause/Step Mode functionality
- Switching between simulation and physical device control using the Physical Action Marker
- Enabling/disabling the portion of the Event Tree or Device List to use the pre-created Event Log template in different physical environments
- Adjusting the device data of some or all physical devices for the particular node of the event tree (the Feedback mode)
- Saving and retrieving of the previously created Event Log

Advantages over conventional dialog-based or wizard-based experiment control environment

In recent years the complexity of modern biological experiments has grown enormously. No matter how carefully the dialog or wizard based control environment is developed, it is always designed around the assumption that software vendor is able to cover all possible situations the user will run into. Unfortunately, it is not the case in the modern biological experiment: there will always be a situation where some of the components of the experiment will not be covered by the scenarios the dialog based environment provides. If this happens, the user does not have much choice: he either have to ask the vendor to add the needed functionality into the system (which is an extremely time-consuming process and is not always possible) or he will be forced to turn to another vendor who has the functionality needed and possibly drop some features the first vendor provides. Time, effort and money will be wasted if this happens.

The Experiment Planner provides an elegant solution to this problem: instead of covering the all possible experiment scenarios it provides the visual programming high-level language with the same functionality as any other programming language such as C/C++, Delphi, Basic and so on. In fact, the EP syntax is very similar to the Lisp one (the language used in AI systems development). This makes it more likely that any experiment user can think of can be effectively implemented in EP environment.

The question is: can the EP be as effective in the case of standard scenarios where dialog-based environment is so natural and easy to use? The answer is definitely YES!

The Active Spreadsheet™ is designed around the concept of using standard pre-developed Event Log documents (or templates) covering some particular experiment scenario, that can be incorporated into the final Event Log through simple drag-and drop operations or an external call. An extensive library of such templates is provided, which can be expanded any time without changing the application itself.

Advantages over conventional programming or macro-language

In spite of the fact the conventional programming language provides the unlimited flexibility as well, it has few fundamental flaws especially when it comes to the experiment planning environment.

First of all, the creation and editing of a conventional program uses text-based editing. The user types and edits the text that will be compiled and executed later. It means that he has to remember hundreds of keywords, function names and has to type very accurately to eliminate syntax errors. The text he is typing is a plain text that hides the hierarchical relationships typical in an experiment plan. This means that user cannot get a "big picture" hiding the routine details of the implementation unless he is a very experienced programmer. There are also no much possibilities to use drag and drop technique to drag a "code template" into the program as it is very easy to drag the portion of the text into the wrong place, say into the middle of the word, and not realize that a mistake was made.

On the other hand, in the Experiment Planner™ environment the user deals not with text but with self-contained objects instead. When he starts to edit the cell of the Active Spreadsheet the system provides the list of operations available for this particular cell and device only so he does not have to remember hundreds of operations for all devices available in the system. When he types-in the data, the system performs real-time boundary and spelling check so he will never be able to enter "10 seconds" into the cell corresponding to the X position of the stage. Sometimes cell commands and data would represent very complex operations and can be adequately represented only through a dialog. The Active Spreadsheet recognizes this situation and provides the appropriate dialog. It would take many dozens line of code to perform such an operation using the conventional programming language.

Further, the Experiment Planner™ environment is specifically tuned to provide as much flexibility as possible when it comes to the Event Tree manipulation.

The tree nodes can be collapsed or expanded on demand so the user can chose the view that is most adequate to the "level of abstraction" he is on recently.

The user can easily move or copy the portions of the tree from one place to another or from one Event Log document to another. Nodes can be duplicated, reshuffled or moved to different levels of the hierarchy without the risk to damage the integrity of the Experiment Log. The ability to adjust the data using real-time feedback from the physical devices is beyond the scope of the conventional programming language.

In summary, the Experiment Planner significantly shortens the learning curve, eliminates syntax errors, reduces the development cycle and provides more adequate visual representation in comparison with the conventional programming language.

Other Benefits

The Experiment Planner is essentially an object-based language, where each object represents either a logical step in the experiment implementation or a specific device operation. It means that as unique devices or some new operation for an existing device is required, this new functionality can be provided by modifying some particular set of operations or developing a completely new device without touching the rest of the system. So, another feature of the Experiment Planner is an expandability that allows the user to minimize the waiting time in case he wishes to add the new equipment into his experiment setup.

Conclusion

The Experiment Planner™ system combines "the best of two worlds" and provides the optimal compromise between error protection and ease of use of dialog based environment and the unlimited flexibility of the conventional programming language.

Introduction

Let’s take a look at the screen shot of the Event Log document (see Fig.5).

As was mentioned before, it has 3 main components:

- The Event Tree, defining the flow of operation with optional commands altering the general flow if necessary
- The hierarchical device list, defining the physical equipment to be controlled
- The body of the spreadsheet containing the data and operations which the physical device will perform when the given spreadsheet cell is activated during the execution

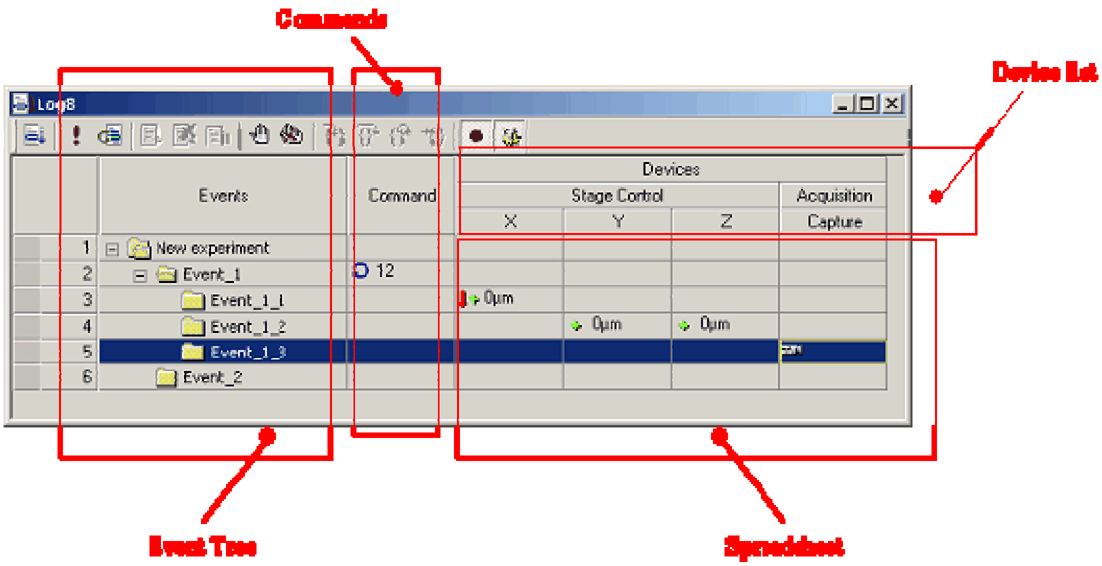


Figure 5. Sample Event Log

General

The execution of the Event Log and the interaction between its main components can be described as follows:

The system starts the execution from the topmost node of the Event Tree going from top to bottom and executing all child sub-nodes of the given node of the given level N before it starts the execution of the next node at the same level N.

In our example screen shot (Fig.5) the order of execution will be:

Root->Event_1->Event_1_1->Event_1_2->Event_1_3->Event_2

Simply speaking the child nodes can be thought about as an integral part of the parent node or as the details of the parent node implementation. When the system executes some particular node, it does the following:

1. It decodes the ***Command*** field and uses it to alter the flow of the operation according to it’s content (see "In Depth" chapter below).

Note: By default, if the **Command** field of the node is empty, the system interprets this as "do not alter the general flow" directive.

2. Next, it detects all non-empty cells in the given line of the spreadsheet, and executes them from left to right according to the operation and data each cell contains.
3. When executing a particular cell in the spreadsheet, the system figures out which device the cell belongs to (according to the column the cell is placed), detects the operation and executes, it transferring the data between the device, the cell and system internal buffers if applicable

There can be more than one top-most node in the Event Tree but only the first one (the Main tree) is used for execution during the normal flow of operation. The others serve the role of independent modules (or sub-modules) and executed through the **Call** command (see below) from the Main tree. They are used to perform some routine set of operations that is frequently invoked from different places in the Main tree eliminating unnecessary repetitions and effectively reducing the size of the Event Log. After finishing the execution of the given sub-module the system does not switch to the execution of the next one but instead returns to the Main Tree.

Commands as flow of operation modifiers

It is often necessary to alter the general flow of operations described above in order to satisfy the nature of the experiment. Below there is a list of some common examples typical for biological experiment planning that illustrates this subject:

Repetitive tasks

In case of Z focal stack creation (see Fig.6) the system needs to move stage with dZ increment and then perform some standard set of operations for every Z position. In order not to create a huge log containing the same actions for every Z position it is reasonable to use the ***Loop N*** command which causes the system to repeat the execution of the given Even Tree node N times (including underlying child nodes). In this case the parent node would just contain the Loop N command and the operation incrementing the Z position.

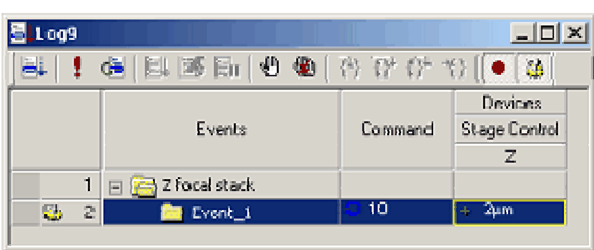


Figure 6. Focal stack creation

Scheduled tasks

Let suppose that we work with micro-well plate and observe the behavior of cell colonies adding different growth factors into the different groups of wells. The colonies in each well group would proliferate faster or slower depending on the growth factor. The faster the colony changes, the more frequent observations would be needed to monitor the cell activities. This means that different wells would need to be visited with different frequencies. To provide such functionality, it is reasonable to introduce the command ***Schedule N, M, L,…*** which when used within a loop executes the first child of the node containing the Schedule command at the first N passes, the second child – at the next M passes, the third child at the L next passes etc. repeating the process when it reaches the end of the list. If each child node will represent some action necessary to observe the particular group of wells, the problem of different frequency observing will be solved (see Fig. 7).

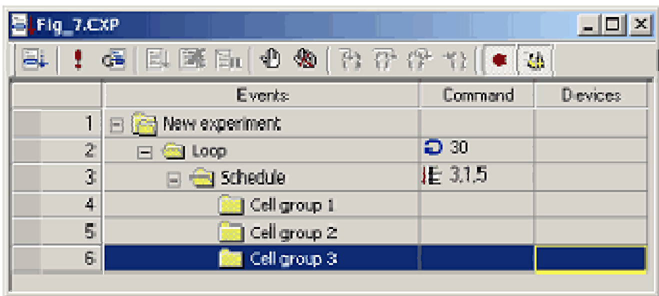


Figure 7. Observing groups of wells with different frequencies

Invoking the same set of operations from different parts of the Event Log

Let's suppose we have an irregular list of <X,Y> positions on the slide and we want to invoke the same set of operations (perhaps a complicated one) for each of these positions. The situation is somehow opposite to the repetitive task example above but the goal remains the same: we want to eliminate the repetition of the same set of operations. In order to do this we can introduce the ***Call Name*** operation that causes the system to jump to the node "Name", execute it (and all its child nodes) and then return back to the node from which it was called.

This approach can be extended in such a way that system can make calls to other event log documents using the command ***Ext. Call Name*** where "Name" is a name of another Event Log document (see Fig. 8).

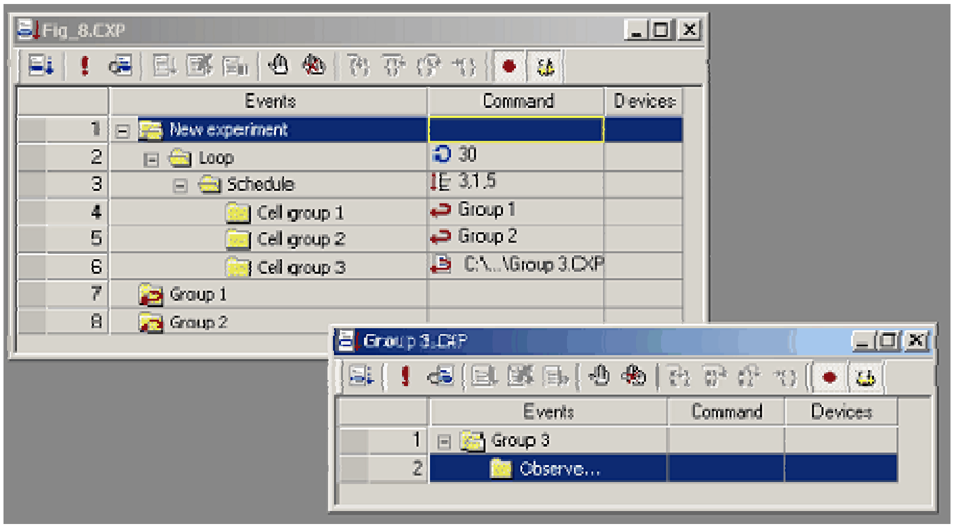


Figure 8. Calling a sub-module or an external log

Conception of virtual device and physical action

There are many types of cameras, stages, lamps, filters etc. available from different manufacturers. In order to deal with them in a uniform manner the system internally supports the conception of the "virtual device" which represents the most common features of the device of a particular type and provides an additional layer which separates the logical operations from the physical ones. For example, the Stage virtual device has 3 sub-devices (X, Y, Z) each of which contains coordinates for the motor of a particular axis. The interaction between a cell of the spreadsheet, the virtual device and the particular physical device is represented on the Fig.9a. When the operation is executed the cell exchanges the data with the virtual device X and not with the physical device itself. In order for the physical device (in our case the X motor controller) to move the stage or read the current position the additional "commit" directive needs to be sent. By default, the system will not perform this committing action unless user marks the corresponding line in the spreadsheet with special "Physical Action" marker (column on the left of the Event Tree, see Fig.9b)

The reason for such a separation is following: consider the situation when it is necessary to perform a few steps of computations involving changing data in the register of the logical device before sending the data to the physical device. If the additional committing step is not used, the stage, for example, would erratically move each time the system changes the data.

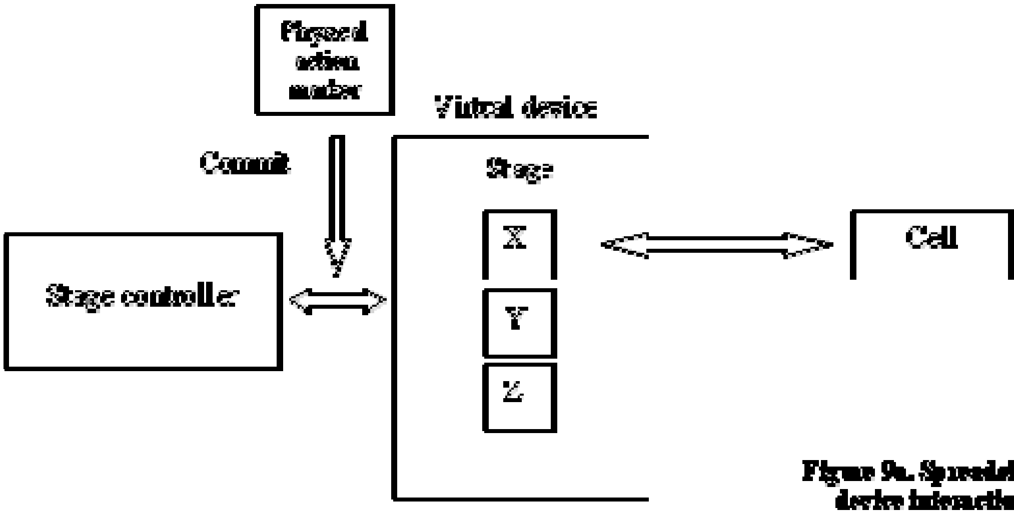


Figure 9a. Spreadsheet-device interaction

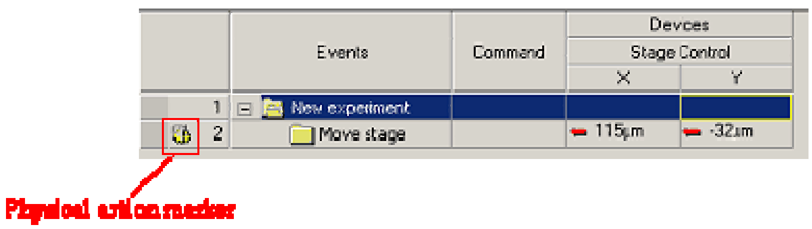


Figure 9b. Physical Action marker

Cell data and the Parameters Buffer

Consider the situation when it is beneficial to reuse some portion of the Event Tree through the Call or Ext. Call command. In this case it would be reasonable to provide some level of flexibility allowing the system to alter the parameters in the data cells of a reusable sub-tree at the moment of the call. For example, consider the sub-tree performing the <X, Y> field montage for different positions of the origin. In this case it is necessary to tell the system what the coordinates of the origin are when it processes this sub-tree. In order to achieve this goal the system supports the internal Parameters Buffer and the data cell can Save/Restore the data to/from the buffer using the appropriate command. In fact, the system dynamically creates a separate Parameters Buffer for each level of the Event Tree in the process of traversing and execution. When the system switches to the child level of the given node or to the sub-module it promotes all the parameters saved at the higher level to the current level of hierarchy. The content of the current Parameters Buffer can be changed during the execution of the given level of hierarchy but it does not affect the higher levels as the system switches to previous Parameters Buffer when it finishes the execution of underlying child nodes.

Generic classification of the Experiment Planner device operations

In spite of the fact that there are hundreds of devices with different styles of control available the Experiment Planner effectively reduces the set of operations necessary to control this huge variety in such a way that each operation always has to deal only with three entities mentioned above: the Virtual Device, the Cell Data and the Parameters Buffer (see Fig.10).

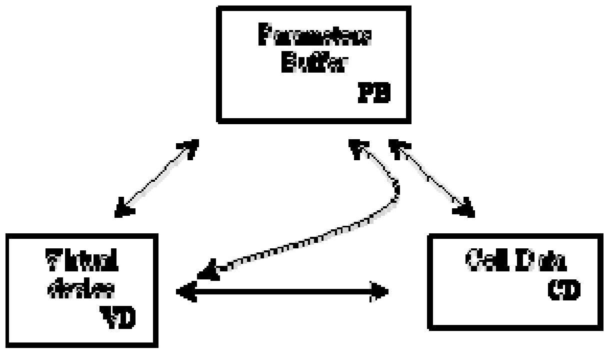


Figure 10. Data interaction diagram

Denoting these entities as VD, CD and PB correspondingly it can seen that all Experiment Planner operations fall into three categories:

- Own operations of type VD->VD or CD->CD (an example: the device Reset operation)
- Unary operations of type CD->VD or PB->CD
- Binary operations of type (BP*CD)->VD

The operations of the particular device can have very special names (for mnemonic purposes) but their underlying meaning will always satisfy the classification above.

Getting Started

Naming conventions

The following names will be frequently used in the following writing:

- Experiment Planner™ - the entire system supporting the experiment planning and automation
- Event Log – sequence of commands and device operations defining the execution of the experiment
- Event Log document – document containing the Event Log
- Rapid Application Development (RAD) environment – visual environment supporting fast and robust creation of the Event Log document
- Event Tree – the hierarchical tree of nodes with associated commands and device operations defining the flow of operations during the Event Log execution
- Sub-Tree - part of the event tree
- Event Tree node (or simply the node) - the single element of the tree.
- Event – the tree node with all associated command and device operations

Active Spreadsheet™ - the window in which the Event Log created, modified and executed

Introduction

The Fig.11 shows the main components of the visual environment that are typically used while working with the Experiment Planner™. The first and the most important component is the ***EP Document*** in the form of ***Active Spreadsheet Window*** that user creates, edits, saves, debugs and executes in order to control the experiment environment. The second one is a ***Device Resources Window*** that serves as a repository for devices available for control. User places the devices needed to perform the experiment into the header of the EP Active Spreadsheet Window using drag and drop (or copy and paste) operations. The other EP documents shown on the Fig.11 are previously created EP documents that represent the standard solutions for typical experiment problems that can be used if necessary as:

Templates:

User can drag/drop or copy/paste some part(s) of the Event Tree (or the entire tree) from the template document(s) into the main Experiment Log if such a part represents the ready solution of the particular problem.

External EP documents:

Can be used through the "Ext. Call" command (see How It Works/In Depth chapter) to invoke some standard procedures during the execution. The purpose of this approach is to unload the main Experiment Log from technical details. They do not have to be opened explicitly as on Fig.11 as the system will load and execute them automatically if they are not loaded yet.

Note, that templates and external documents are just the same EP documents: they have the identical format and come from standard EP library provided or the user can create his own ones in the same way he creates the main Event Log. And of course they can be used only if it is necessary.

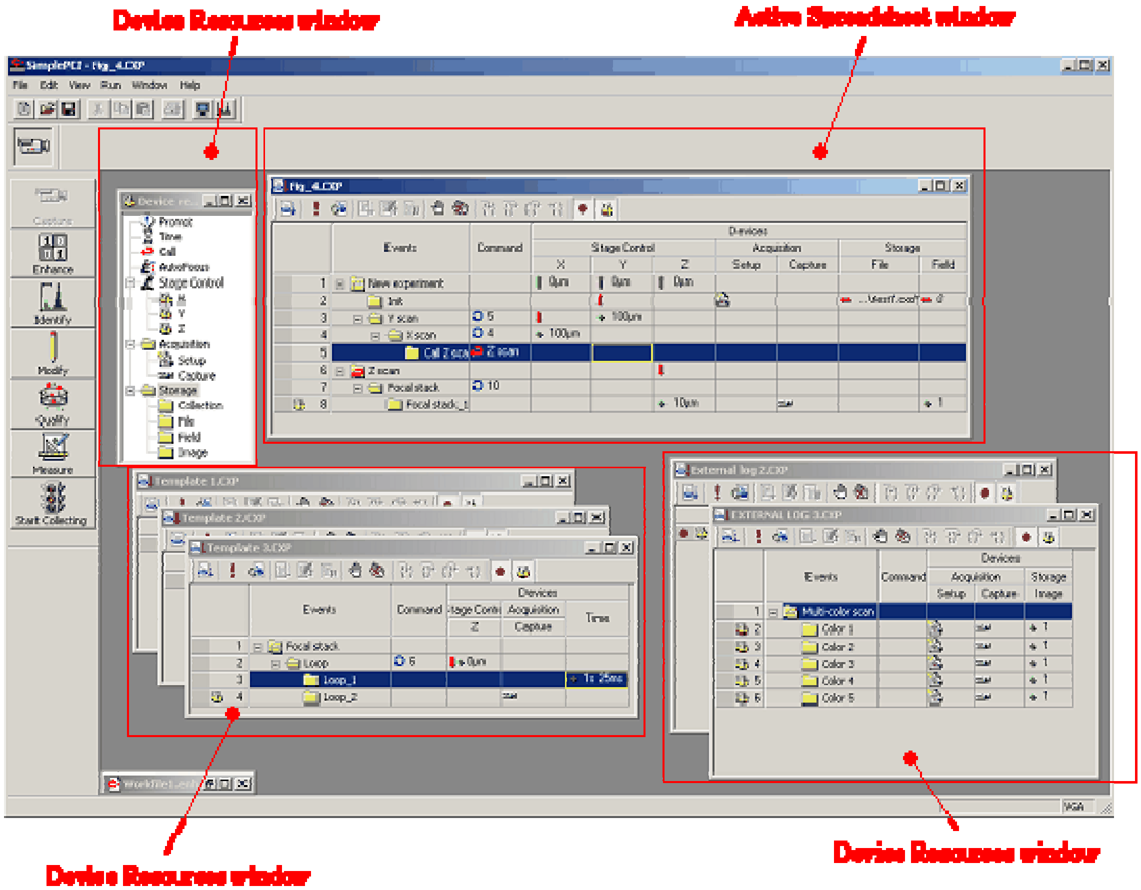


Figure 11. Visual environment components

The main tools typically used are (see Fig 12 a, b and c):

- **The Main Menu** – allows user to Create, Save and Open EP document, create new Window for the same document, run and debug the Event Log etc (Fig. 12a).

- **The EP Toolbar** – provides user the most convenient way to change some aspects of visual appearance, debug and run the Event Log. Typically the functionality of each button in the toolbar can be invoked through the Main Menu/Run sub-menu (Fig.12a).

- **Right-click menu** – when user clicks on any node of the event tree, on the header of the spreadsheet or on the cell in the spreadsheet the system will popup context-sensitive menu listing the operations that can be performed (Fig. 12b).

- **The click on previously selected item** – when user does this on the Event Tree node or non-empty spreadsheet cell the system will start the node name or cell data editing. When the second-click on the empty cell of the spreadsheet is performed, the system activates the cell and displays it's default content (Fig. 12c).

- **Drag and Drop** – used to copy or move the portions of Event Tree (including the whole command line) from one EP document to another or within the same EP document. It is also used to add the device or group of devices to be controlled from the Device Resources Window or from another EP document.

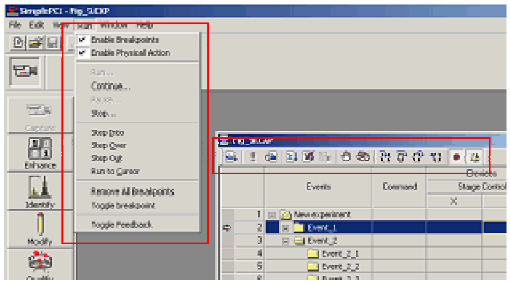


Figure 12a. Main menu and EP toolbar

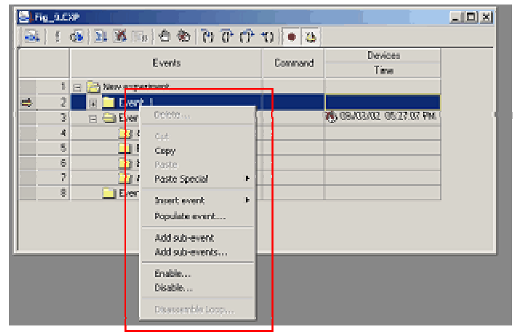


Fig. 12b. Right-click menu

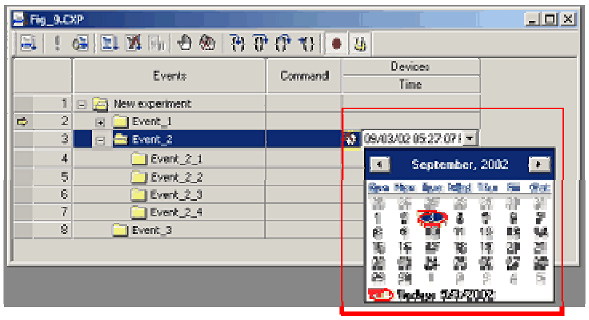


Figure 12c Click on previously selected item

The Typical Development Cycle

During the development and working with the Event Log it will be necessary to perform some typical steps listed below.

Note, that the order of steps is not mandatory and depends on the situation and taste of developer. Some steps can be omitted or repeated if necessary.

Create of the new experiment log

Use File/New/Log Document command from Main Menu

Add events and/or sub-modules to the Event Tree

Use Add Event(s) or /Sub-module(s) commands from the right-click menu on the existing tree node. A second click on a previously selected item, allows renaming of the node so that the names can illustrate the logic of the Event Log execution.

Useful Event Tree portions can be copied from template EP documents if necessary.

Add necessary devices from the Device Resources window

Open the Device Resource Window using View/ Device Resources command from Main Menu.

Activate and select appropriate commands and device operations and data

Using click on the previously selected cell activate the cells in the ***Commands*** and ***Devices*** columns and then select the command and data (see Cell Editing below)

Mark the lines of the spreadsheet where physical actions will be performed

The meaning of the physical action marker was discussed in How It Works/In Depth section above. Place the marker into the appropriate line by clicking on the Active Spreadsheet column left from the Event Tree.

Adjust the device data using the feedback mode

The feedback mode (see description in the ***Feedback Mode*** section below) allows user to adjust the device cell data.

The user selects the line he needs the data to be adjusted and the presses the Feedback button from the toolbar. After that user can manipulate the particular device by hand (for example, move the stage using the joystick and the system will update the data in the selected line accordingly.

Debug the completed Event Log

This is the one of the most important steps in the Event Log preparation. It does not matter how experienced the user is the logic of the Event Log he has created may contain flaws or the devices may not work as expected, so it is highly recommend to pay as much attention as possible to this step.

Put breakpoints on the appropriate lines of the Event Log by clicking on the cells of the leftmost column of the Active Spreadsheet. When all breakpoints have been set, press the Run button on the Toolbar. The system will start the execution until it reaches the first breakpoint. The system stops the execution before it executes the line with breakpoint and switches to the step mode in which user can execute the command lines one by one (see the description of the Tool Bar/Debug Group in section below). User can resume the execution any time or abort it if he is not satisfied with results. If this happens, modify the Event Log and then try again until everything works as expected. Save the created EP document using File/Save or File/Save As command.

The ability to interrupt, make changes and continue provide an additional flexibility over script based methods, which would typically need to be recompiled, or restarted from the beginning.

Execute the Event Log

When everything is working as expected remove (or disable, see Toolbar Breakpoint Group in section below) all the breakpoints and press the ***Run*** button. The system will start the execution and from this moment the Event Log can be re-run whenever it is needed by loading the previously saved EP document using ***File/Load*** command from the Main Menu.

Working with the Event Tree

Overview

All Event Tree manipulation (creation, deletion, insertion, moving parts of the tree around etc) can be accomplished using 4 basic techniques described above:

- Selection of the particular node or group of nodes
- Invoking the right-click menu on selected node(s) (see Fig 12b For Event Tree right-click menu).
- Clicking on the previously selected node
- Using drag and Drop operation

There are few basic principles the system follows while manipulating the Event Tree:

Uniformity

All manipulation steps described below can be performed on any node of any level and as many times as necessary.

Default naming convention

When creating/inserting the new events the systems assigns these events the default names using the format <***ParentName_N***> where "ParentName" is a name of a parent node and N is a number of the given event. For example, if the parent node name was "Montage" then the default sub-node names would be Montage_1, Montage_2... etc. User can modify these default names any time if necessary. There is one exclusion from this rule: the default name of the root node is "New Experiment" and the default name of it's sub-node is "Event_N".

Context-dependent menu

The set of commands available in the right-click menu is context-sensitive. For example, some operations available on a single node may not be legal for group node selection and will be automatically disabled. The set of operations available differs between the top-most node and all other nodes etc. The user does not have to worry about this as the system provides all the necessary adjustments automatically. The Event Tree node right-click menu is shown on Fig. 12b.

Creation

- **Adding new sub-event(s)**

When the new Event Log document is created it initially contains only one node named "New Experiment" which is a root node of the Experiment Log. To add the sub-node(s) to the new Event Tree select the root node and then invoke the right-click menu on it and choose "Add Sub-Event" or "Add Sub-Events" command. In the first case the system will add sub-node named Event_1 and in the second case it will open the dialog, prompting user to enter the number of sub-nodes he wants to add. After entering the number and pressing the OK button the system will add N sub-nodes named Event_1, Event_2,..., Event_N where N is a number entered. This operation can be repeated on any existing node of the tree as many times as necessary thus creating the desired hierarchical structure of the Experiment Log.

- **Inserting a new sub-event**

Sometimes it is necessary to insert a node before or after a particular node at the same level of hierarchy. In this case use the commands ***Insert Event/Before*** or ***Insert Event/After*** from the right-click menu. The system will insert the new node before or after the node selected and assign it the appropriate default name. Note, that in this case the system will re-name all default names at the given hierarchy level to keep the ascending order of enumeration.

- **Populating the event**

Sometimes it is necessary to create a list of events that perform the same operations on the same devices but with slightly different parameters. To minimize the amount of typing the following technique is suggested. Create the new node and activate all the device related cells in the spreadsheet choosing the appropriate operations.(see Working with Spreadsheet Cells chapter below). Invoke the right-click menu on the node and choose "Populate Event" command. The corresponding dialog pops up prompting the user to enter the number N of times the event to be populated. After pressing "OK" the system will make N-1 copies of the selected event, assign them the default names and place them immediately after the source node so the total number of copies will be N. After that user can edit the cell data or adjust them using the feedback mode (see Feedback Mode chapter below).

Modification

- **Renaming the event.**

It is often reasonable to rename the nodes of the Event Tree in such a way they reflect the logical sense of operations performed. For example, the parent node of the sub-tree performing the XY scan can be renamed as "XY Montage", the sub-tree responsible for moving the stage over X axis can have a name "X scan" etc. The node names can be used as comments to the Experiment Log flow of operations. In order to rename a node, select it using the mouse and then click on it again. The system will highlight the name and switch into the editing mode. After that the user can type in any name or a whole phrase reflecting the logical sense of the given event. In order to commit changes made press "Enter" or just select the another node. To disregard the changes press "Esc".

- **Deleting the event(s).**

To remove the event(s) that are not needed anymore select the node or group of nodes and invoke the "Delete" command from the right-click menu.

Note: during this operation the content of all active cells in the corresponding line(s) of the spreadsheet will be deleted as well.

Sub-tree manipulation

It is often necessary to move some node (including it's sub-nodes) or a group of nodes around the Event Tree or copy the sub-tree from another EP document (which in this case serves as a template). The system provides the following means to accomplish this task:

- **Copy, Cut and Paste.**

The usage of these operations is similar to the Windows ones (Windows Explorer for example). The system will place the copied node(s) including all sub-nodes as child(s) of the node that is a destination for the Paste operation. There is one important extension: if the user selects the group of nodes as a destination and chooses the Paste operation the node(s) that were previously placed on the Clipboard using Copy or Cut operation will be copied as sub-nodes to ALL the nodes user have selected.

Note: If the destination sub-tree already has some nodes with the same name(s), the system will not replace them with copied once. This restriction applies only to the particular sub-tree into which the dragging operation is performed.

- **Drag and Drop**

The functionality of these operations is similar to the Windows functionality with the same restriction as in the note from previous paragraph. The only difference is that by default the system copies dragging node(s) as opposed to moving them in Windows. To move dragging node(s) from one place to another press the SHIFT button during the dragging operation.

- **Paste Special**

It is often necessary to place the copied sub-tree between two nodes of the destination tree and not as a child of one of them. In this case invoke the "Paste Special/Before" or "Paste Special/After" command from the right-click menu on the selected node. The system will insert the sub-tree before or after the selected node correspondingly.

Working with Sub-Modules

User works with sub-modules exactly in the same way as with other tree nodes except that in order to add or copy the Sub-Module he uses the ***Event*** tree column header as a target for operation. For example, he invokes the right-click menu or drags data into the Event column header instead of any Main Tree node. The default Sub-Module names are Module_1, Module_2 etc. The Sub-Module also can be added through the Insert **Event/After** or **Insert Event/Before** or **Paste Special/Before** or **Paste Special/After** invoked on any top-most nodes (including the Main node) of the Event Tree.

Changing the event status

- **Enabling/Disabling the events**

Sometimes it is necessary to temporarily disable the execution of an event or group of events without actually deleting them. In order to accomplish this, select the event(s) and invoke the **Disable** command from the right-click menu. Later the previously disabled events can be enabled using the "Enable" command. It is possible also to enable/disable the single event by double-clicking on the icon of the appropriate node in the Event Tree.

Note, that this feature is especially useful while working with multi-site scans to exclude some locations which are no longer active (containing no cells, for example) from the processing.

- **Toggling the Physical Action marker**

The meaning of the Physical Action marker was discussed in **How It Works/In Depth** chapter above. In order to set/remove the marker move the cursor over the cell in the column left from the Event Tree. While moving the cursor over the cell the cursor will change an appearance and the underlying cell will transform into the button. Pressing this button will toggle the marker on and off and if it is on the Physical Action icon will appear in the cell. In the process of debugging it is sometimes useful to temporarily disable all Physical Action markers. It can be achieved this by depressing the "Enable Physical Action" button on the Active Spreadsheet toolbar.

- **Toggling the Breakpoint.**

The importance and meaning of the Breakpoint was discussed in **How It Works/In Depth** chapter as well. Breakpoint markers are displayed in the left-most column of the Active Spreadsheet and the user works with them in a same way he works with the **Physical Action** marker. To enable/disable the breakpoint use "Enable Breakpoint" button from the Active Spreadsheet toolbar. Additionally, it is possible to toggle the Breakpoint or remove all Breakpoints using the "Toggle Breakpoint" and "Remove all breakpoints" buttons correspondingly.

Note, that the system remembers the event status along with all other Experiment Log document components. It means that when saved and closed Event Log document is opened again all the markers will have the same status and appearance as at the end of the previous editing session.

Tree transformation

Repetitive task disassembly

Let's suppose we would like to create an X-Y montage of adjacent fields. Using the notion of a hierarchical tree and the LOOP command introduced in **How It Works/In Depth** chapter above, we can create the Event Log accomplishing this task containing two enclosed loops that would look like one represented on Fig.13a (the loop example will be discussed in details in the **Programming Technique** chapter below). This example uses the fact that montage fields are positioned in the nodes of the regular grid in order to reduce the number of events in the Event Log. Now let's suppose we have the multi-well plate where wells are also positioned within the rectangular grid. It seems that our example log would also work in this case but in practice the situation is different. The field of view can be much less in size than the whole well so the user always needs to offset the fields of interest from the grid nodes. These offsets will be different for each well so instead of using the compact two loops representation we need to create a list (see Fig.13b) in which every field of interest has it's own X-Y coordinates initially belonging to the regular grid. After the list has been created user can modify each individual XY coordinates using the Feedback mode (see below). There are multiple types of multi-well plates available that differ in well size, shape and the number of rows and columns so the task of creating multiple long lists for each plate type would be very laborious.

The Experiment Planner RAD environment provides an elegant way of transforming the compact loop-based representation into a list of individual events using the "Disassembly Loop" command from the right-click menu. The steps to accomplish this are following:

- Create (or copy from the template document) a sub-tree implementing the regular grid montage using the LOOP command.
- Adjust the number of repetitions and step size for each coordinate
- Select the upper level node containing the LOOP directive and invoke "Disassembly Loop" from the right-click menu.

The system will start the execution in the simulation mode (it means that virtual devices will be modified according to the flow of operation but no physical device actions will be performed) creating the list nodes each time it repeats the operation within the Loop. At the end the loop selected for disassembly (and all underlying loops) will be transformed in to the list containing real data for each loop step. In our case it would be the individual X-Y coordinates that later can be modified using the Feedback mode (see Fig. 13c).

As an example of the efficiency of this approach consider the case when the 1536 well plate (48x32) needs to be implemented. In order to do this using loop disassembly the following operations need to be performed:

- One Drag and Drop operation (from X-Y montage template)
- Four editing operations (2 loop parameters and X-Y steps) and
- 1 "Disassembly Loop" command from right-click menu

i.e. 6 operations as opposed to at least 1536*2 editing operations in case of direct node creation and editing.

Another example use for this technique is for multi-site time-lapse where target fields are sampled approximately on a grid over a large area.

- First create a rectangular grid over the search area.
- Then convert the grid to a list
- Finally modify each location to view an interesting target cell or cluster nearby.

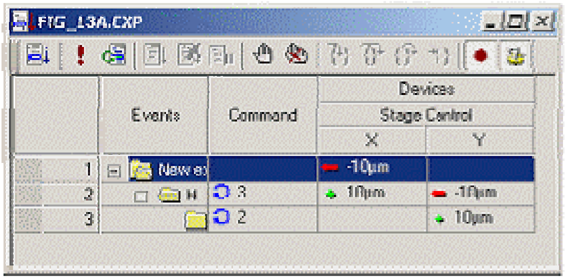


Figure 13a. Two-dimensional montage

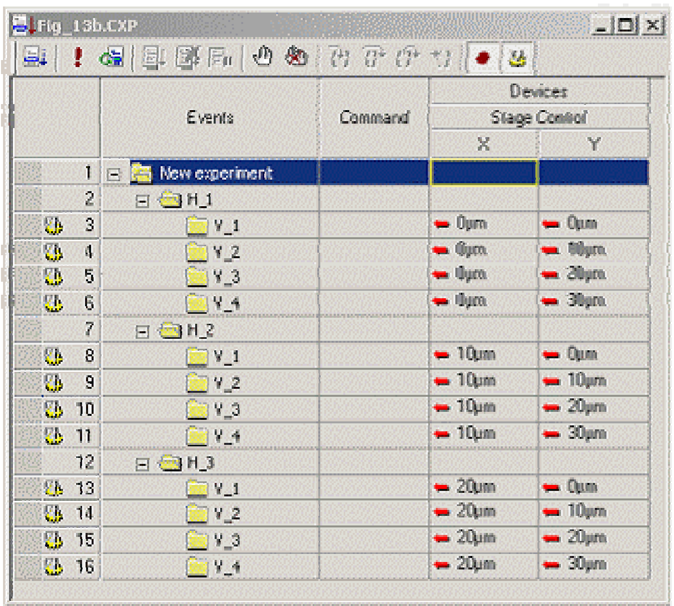


Figure 13b. Hierarchical list

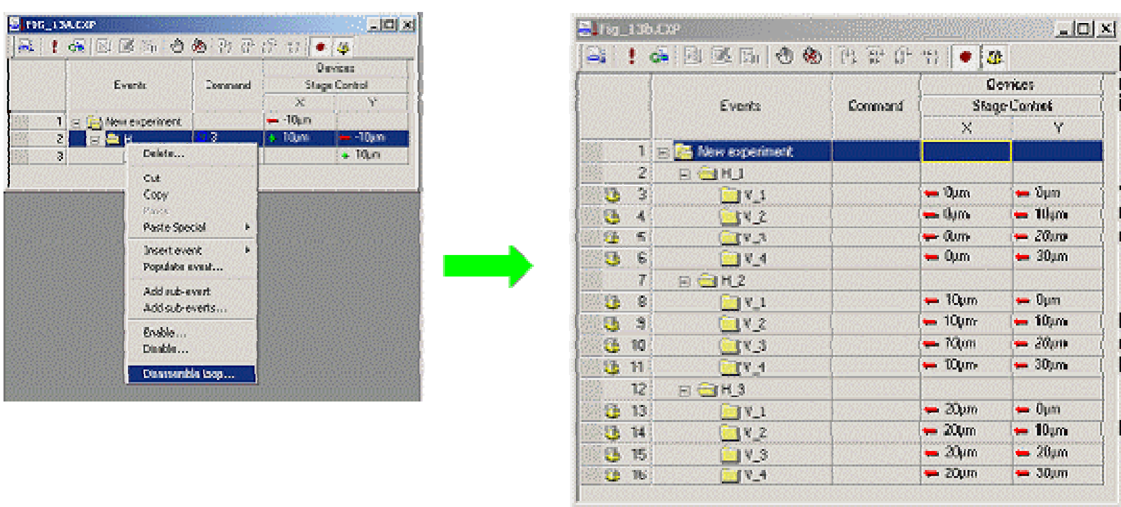


Figure 13c. Using the Disassembly loop command

Working with Device Resources

Overview

The hierarchical tree of devices is displayed as the header of the Active Spreadsheet and defines what devices are to be controlled during the execution of the Event Log. The hierarchical structure of the Device Resources can include a low-level group of devices that compose a higher-level device. For example, the Stage device consists of X,Y and Z low-level devices, the Acquisition device consists of Setup and Capture devices etc.

Note, that all devices in the Active Spreadsheet environment are virtual ones (see How It Works chapter above) so the real physical device implementation is hidden from user. Typically the user works with the device resources in the same way he works with the Event Tree (with some exceptions listed below) except that there is a single source for all devices available in the system- the Device Resource Tree (see Fig.14). The Device Resources Tree can be opened in the separate window using the command View/Device resources from the main application menu.

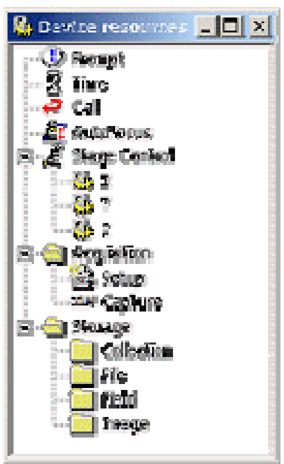


Figure 14. Device Resources window

Adding the devices

When the new Experiment Log document is created the Device Resource Header is initially empty. Use ***Copy/Paste*** or ***Drag/Drop*** operations to get needed devices from the Device Resource Tree window (the Cut operation is not available) or Copy/Cut/Paste (Drag/Drop) from another or the same Experiment Log document. Each lowest-level device occupies the entire column of the Active Spreadsheet and the devices may not be renamed. When using ***Paste*** or ***Drop*** on the device that is already present the system will create the new copy of the device and assigns it the default name using the format <***OriginalName_N>*** where N is a device copy number. For example it is possible to have the devices X, X_1, X_2 etc., though all of them will control the single virtual device X. This duplication provides the opportunity to place several commands for the same device into the single line of the spreadsheet.

The recent version of the experiment planner has the following exceptions:

- Only the lowest level devices can be duplicated. For example, the devices X, Y, Z, Time etc. can be duplicated but the devices Stage, Acquisition etc. may not.
- The lowest level device may not be copied unless the whole sub-tree (including higher-level device) is copied or unless the higher-level device is already present in the Active Spreadsheet header.

Removing the devices

Remove the device selecting it and invoking the ***Delete*** command from the right-click menu.

When the device is deleted the corresponding column in the spreadsheet disappears but all non-empty cells are kept intact by the system. If later the same device is added, these cells will appear again in the device column. This behavior prevents users from accidentally removing important information from the spreadsheet and also allows user to change the device order using the Cut/Paste Special operation described in the next paragraph.

Changing the device order

As it was mentioned before (see ***How it Works*** chapter above) the system executes the device commands in order they are listed in the header (from left to right). This means that sometimes the device order is important and needs to be altered or the device needs to be placed into the particular position from the very beginning.

It can be achieved by using the ***Paste Special/Before*** or ***Paste Special/After*** command from the right-click menu on particular device that serves as a reference point for the insertion. If the device needs to be moved from one place to another use ***Cut*** command before applying ***Paste Special***.

Note, that low-level devices will always be placed within the group of columns belonging to the higher-level device.

Enabling/disabling the device(s)

Each device or group of devices can be enabled or disabled using ***Enable/Disable*** command from the right-click menu. When the device is disabled the whole corresponding column in the spreadsheet changes the appearance (becomes grayed) and the system excludes it and all corresponding cells from the execution. When the upper level device is disabled all lower level devices belonging to the same group will be disabled also.

Note, that the disabled spreadsheet cells remain accessible for editing.

This functionality can be used in the process of debugging the Event Log to temporarily disable the device or group of devices that are not important at the given debugging stage.

Working with Active Spreadsheet Cells

Cell Status

Each non-empty cell contains the data (optional) and the operation that will be performed when the system executes the particular line of the spreadsheet. All cells in the same column belong to the same lowest-level device in the header of the column and the set of operations available for the given cell depends on the device the cell belongs to.

Note, that the cells in the **Command** column have the same properties and functionality as any device cell in a spreadsheet.

Each cell can be in one of three states:

- Inactive cell (empty)
- Activated cell (contains operation and data)
- Cell in the process of editing

In order to perform some operation on the cell it has to be previously selected. The selected cell has a yellow border around it. The next click on the selected cell changes its state (from inactive to activated, from activated to editing etc). The right click on the cell selects it and invokes the right-click menu simultaneously.

Inactive (empty) cell

An Inactive cell does not contain any operation or data and does not initiate any device operation while the system executes the given line of the spreadsheet. An empty cell in the command column is interpreted by the system as "do not alter the default flow of operation" command.

Activated cell

The activated cell contains the operation and data (optional) and causes the system to perform the particular device operation or the command in case the cell belongs to the ***Command*** column. An empty cell can be activated by selecting it (the yellow border around the cell appears) and then clicking on it again. The activated cell initially contains the default operation and data (if any) specific for the given device.

Cell in the process of editing

Each Active Spreadsheet cell contains up to three components:

- Operation icon (always)
- Cell data (optional)

- Cell dialog button (optional)

Cell in the process of editing

Once the cell is active and selected its properties can be changed as described below.

- **Operation selection**

Move the cursor over the operation icon. The icon will change the appearance and transform into a button. Pressing the button opens the popup menu containing the list of all operations available for the particular device (icon plus operation name). After choosing a new operation the menu is closed and icon in the selected cell is updated.. Each operation has its own type of data (numbers, names, file path etc. or no data at all) and the system will automatically match it with the chosen operation type. The system also saves the data corresponding to the previous operation so they will be restored if user will choose that operation later. Some operations allow multiple types of data. In this case user can choose the appropriate data type through the commands Type/Number, Type/File Path etc. from the cell right-click menu.

- **Data editing**

There are two types of data editing: in-place editing and editing through the dialog.

Typically, if the data are simple and single-component (like numbers, names, time, length etc.) they can be edited in place. If the cell data are multi-component (for example, the Acquisition/Setup virtual device contains dozens of parameters including lamp and filter parameters, image size, depth etc.) or editing is not a simple editing (for example, file selection) the special modal dialog is provided.

- **In-Place editing**

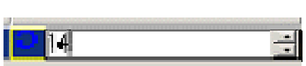
Select the cell and click on the cell data. The system switches to the editing mode and replaces the cell data with the appropriate edit control depending on the currently selected operation. The user can finish and commit the editing by pressing Enter or clicking on another cell in the spreadsheet. Pressing ***Esc*** finishes the editing and disregards all changes made.

Cell control types

The examples of the controls are:

Text editing control

Provides simple text editing functionality without context control



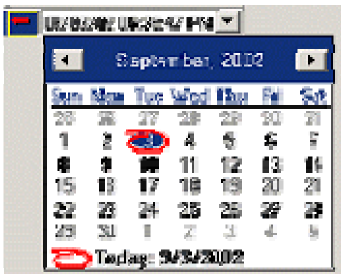
Number edit control with spin buttons

Allows user to type numbers only and spin up and down using spin buttons (in case of integers).

Automatically controls the number range if appropriate.

Choice control with drop-down list

Provides a choice between alternatives through the drop-down list.



Date and time control

This control allows defining date and time through the calendar control.

Physical units edit control with parser

This allows user to enter length, time etc. in physical units with the range control if appropriate. An example: 12cm 13mm (length) or 1h 14ms (time).

Provides the syntactical control of data entered (does not allow to type the wrong numbers or units). For example, it will not allow user to type 1h 78m or 2s 34h. Or, if he enters 135s the system will transform it into 2m 15s after the editing is finished etc.

Note: to enter the micron units use ***Alt+M*** combination on the keyboard.

Editing using the dialog

If the given cell is suitable for this type of editing it has the right aligned button

Pressing it invokes the appropriate dialog that stays active until user selects "OK" or "Cancel". The type of the dialog is device and operation specific (see ***Commands, Devices and Operations*** chapter below).

Note, that dialog-activated button appears only in the currently selected (highlighted) line of the spreadsheet.

Cell Manipulation

Removing the cell

Delete the cell using the "Remove" command from the cell right-click menu. The removed cell becomes inactive (empty) cell.

Copying the cell content

The cell content (including the operation selected) can be copied or moved to another cell using ***Copy/Cut/Paste*** commands from the cell right-click menu. There is one important restriction: the target cell has to belong to the same device column or to its duplicate.

For example, the cell belonging to the column "X" column can be copied/moved to any cell of the column "X", "X_1", "X_2"…, but not to the columns named "Capture" "AutoFocus", "Y" etc.

If the cell from the wrong column is chosen for ***Paste*** operation, the system automatically will automatically disables the ***Paste*** item of the right-click menu.

Expanding the cell content.

In some cases (the "Call" device, "Ext. Call" operation, see ***Commands, Devices and Operations*** chapter below) the cell contains the name of the another Event Log document. This Event Log document can be opened using the "Expand" command from the right-click menu.

Executing the Event Log

Introduction

The execution of the Event Log is controlled either via the Active Spreadsheet toolbar or ***Run*** sub-menu of the Main application menu (see Fig.15 a, b).

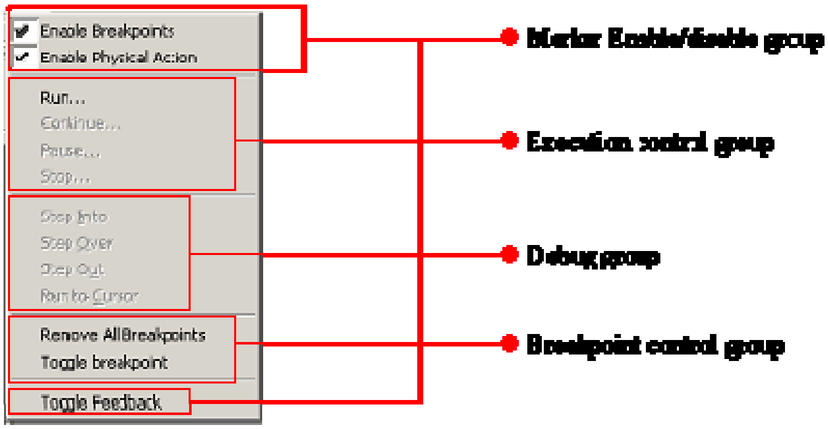


Figure 15a. Run sub-menu

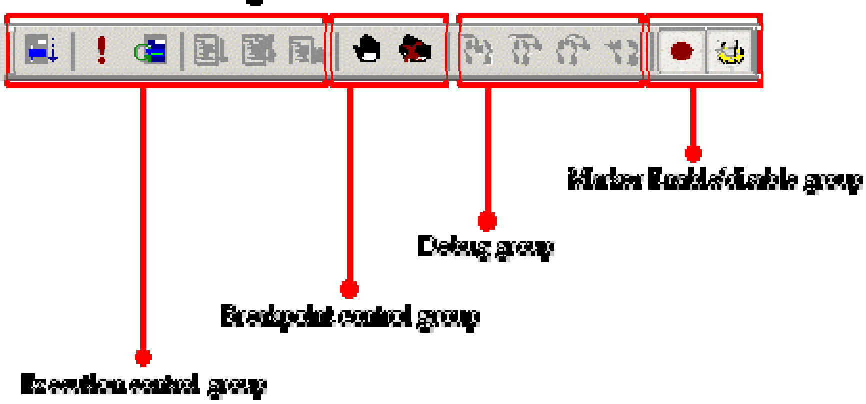


Figure 15b. Active Spreadsheet toolbar

There are three modes of execution available:

- **The real-time mode** when Event Log actually runs the experiment.
- **The debug mode** command allowing user to execute the Event Log step by step."Pause", when the system interrupts the real-time execution at the breakpoint or on
- **The feedback mode** when the system repeatedly executes the selecting line of the spreadsheet updating the cell data while user performs some manual adjustments on the devices. For example, when user moves the stage using a joystick, the system automatically updates data in the corresponding X-Y cells.

Here is a list of commands and corresponding toolbar buttons available to control the execution of the Event Log

Execution control group

Running line on/off

If on, causes the system to select (highlight) the line of the Event Log that is currently executed. If the particular spreadsheet cell performs the read-out operation the system will also automatically update data in that cell. If the cell contains the reference to the external Event Log document (the "Ext. Call" command, see ***How It Works*** chapter above) the system automatically opens it in a separate window and continues to display the running line in that document (if the running line mode is off, the system also opens the external document internally but does not display it). Note, that the running line mode can be turned on and off during the Event Log execution.

Run

Starts the execution of the Event Log from the top-most Event Tree node.

Toggle Feedback

Toggles the feedback mode on and off (see ***Working in the Feedback Mode*** chapter below) allowing user to adjust the cell parameters directly manipulating the physical devices.

Continue

Switches the system from debug (step) mode into the real-time mode.

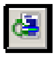
Abort

Stops the execution of the Event Log in any of three modes

Pause

Switches the system from the real-time to the debug (step) mode

Breakpoint control group



Toggle Feedback

Sets or removes breakpoint in the selected spreadsheet line

Remove All Breakpoints

Removes all breakpoints in the spreadsheet

Debug group (debug mode only)

Note, that in the debug (step) mode the system selects (highlights) the line in the spreadsheet that is about to be executed at the next step (not already executed!). In further writing such an action will be referred to as "switching to the next node".

Step In

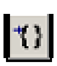
If the current Event Tree node has sub-nodes, the system executes the current node and switches to the first child node. If the given node does not have child the system switches to the next sibling node. If the current node is a last child node of some parent node the system switches to the node next to the parent.

Step over

The system executes the current node and all its sub-nodes and switches to the next sibling node. If the current node is a last child node of some parent node the system switches to the node next to the parent.

Step out

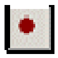
The system executes the current node and all remaining sibling nodes of the same parent. It then switches to the node next to the parent.



Run to cursor

When in debug mode the user selects some another line of the spreadsheet the system resumes the execution until the newly selected line is reached

Marker Enable/disable group



Enable Breakpoints

This command enables/disables all breakpoints in the spreadsheet. If the breakpoints are disabled the system will ignore them and run in the real-time mode but the breakpoints are not deleted and can be enabled again at any time.

Enable Physical Action

This command enables/disables all Physical Action markers in the spreadsheet. If Physical Action markers are disabled, the system updates the virtual devices but does not perform any actions on physical devices (simulation mode). This option can useful to perform a logical structure check of the newly created Event Log document.

Execution Modes

Working in Real-Time mode

In order to start the execution, press the Run button. The system runs the Event Log from the top-most node of the Event Tree and stops when the last node in a tree is achieved. Note, that Sub-Module(s) will never be executed during the normal flow of operation. The system switches to the Sub-Module(s) execution only as a result of ***Call*** or ***Ext. Call*** commands. To terminate the execution, user needs to press the ***Abort*** button. The system automatically switches into the Debug (step) mode if it meets the breakpoint or if user presses the ***Pause*** button. If during the execution the Physical Device Action markers are disabled the system runs in the simulation mode (it updates data in virtual devices but does not perform any physical actions.

Working in Debug (step) mode

After switching to the Debug mode user can execute the Event Log step by step using the Debug Control group of buttons. To resume the execution in the real-time mode, user can press the Continue button. Pressing the Abort button stops the execution of the Event Log.

Working in the Feedback Mode

To run the system in the Feedback mode select the line of the spreadsheet in which the data need to be adjusted and press the ***Toggle Feedback*** button. The system starts the execution in the Feedback mode performing the following steps:

1. First, it switches in to the simulation mode (all physical actions are disabled) and starts to execute the Event Log modifying the virtual devices content until it reaches the selected line.

2. After the selected line is reached, the system switches to the real mode and executes the line committing all changes into the physical devices. At this point, the status of all

physical devices will be exactly the same as if the system ran the Event Log in the real-time mode. For example, the system will move the stage to the position that is a result of all previous actions but without any intermediate movements.

3. The system starts to execute selected line repeatedly, reading data from physical devices and updating the spreadsheet cells data taking into account the operation selected in each cell. The special marker (icon) will start blinking constantly in the left-most column of the spreadsheet line selected for the feedback. User can modify the status of the physical devices using manual device controls (joystick for Stage device, for example) or Capture Dialog in the core application to adjust the stage position, image depth, light source etc.

If during the Feedback session user selects another spreadsheet line and presses the ***Toggle Feedback*** button again the system finishes the current Feedback session and restarts it again repeating the steps described above for the newly selected line. If user presses the ***Toggle Feedback*** button without selecting the new line the system finishes the Feedback session. It also can be finished any time by pressing the ***Abort*** button.

Commands Devices and Operations

Introduction

During this chapter we introduce the first example of the Event Log that solves simple but real problem illustrating the practical aspects of using the Experiment Planner. We will start from the very basic functionality adding new features at the end of each paragraph so finally the complete Event Log document will be created. In further writing this problem will be referred to as a "Real World Problem". Note that after initial problem will be completed we will continue to add some new aspects to it so at the end of the chapter a few slightly different problems will be solved as well.

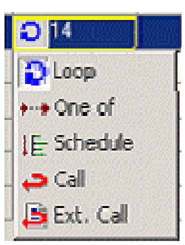
The "Real World Problem" can be formulated as follows:

Multiple position time-lapse experiment

"During the experiment the image sequence needs to be acquired in 3 different positions on a slide using monochrome acquisition for first position, two-color for second and 3-color for third. The process needs to be repeated 10 times and the period between repetitions needs to be 15 seconds. The images acquired during the experiment need to be stored in subsequent fields of the file with name "Problem1.cxd", 3 images per field, so that each field corresponds to one loop pass and contains monochrome, two- and three- color images (Image1, 2 and 3 respectively) belonging to the same pass. At the beginning of the experiment the message needs to appear reminding user to turn on the lamp ".

Note: For the description of SimplePCI file formats see the core application help.

Commands



 **Loop**

Format: ***<u>Loop <N></u>***, where ***<u>N</u>***>0 is an integer number

Function: The system executes the given node (and all it's sub-nodes) N times

Usage: Use the Loop command when some action or group of actions needs to be performed repeatedly.

Note, that any Loop node may contain another Loop (enclosed Loops).

Editing: number edit control with spin buttons

Example: Acquiring the image 10000 times (see Fig.16)

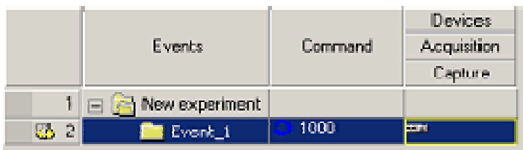


Figure 16

One of

Format: ***One of <N>*** where N>0 is an integer number

Function: If the system passes the node containing this command repeatedly it will execute it only each Nth pass (the first pass, the N+1 pass etc.)

Usage: Used to perform some specific operation within the Loop not every time but periodically.

Editing: number edit control with spin buttons

Example: Perform an auto-focus operation while acquiring images once per 100 images (see Fig.17)

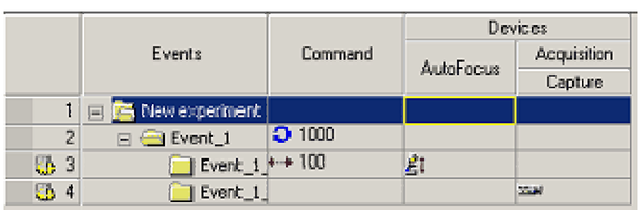


Figure 17

Schedule

Format: ***Schedule <N,M,L…>*** where N, M, L… is a list of integer numbers >=0.

Function: When used within the loop the system executes the first child of the node containing the Schedule command at the first N passes, the second child – at the next M passes, the third child at the L next pass etc. repeating the process when it reaches the end of the list.

Note, that only one child node of the Schedule node is executed at each pass and the Schedule node itself is executed each time.

Usage: This command is typically used to perform different grous of actions with different frequencies.

Editing: list of numbers edit control

Example: Let's suppose that 3 groups of wells in the micro-well plate need to be observed with different frequencies due to the different grows factor en each group and that the ration of frequencies is 8:2:1. It means that the first group needs to be observed at each pass, the second at each fourth pass and the third at each eights. The Event Log fragment accomplishing this task and the explanation of how two solve it can be found in the next chapter (***see Programming Technique/Using the Schedule operation,*** Part 2)

Call

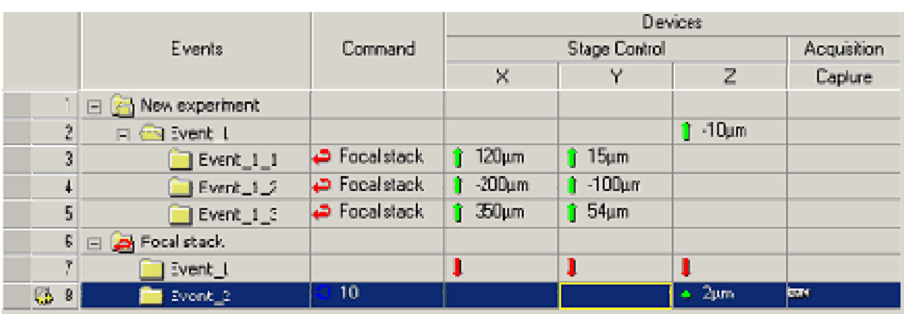
Format: ***Call <Module_Name>***, where Module_Name is a name of sub-module (see Working with the Event Tree)

Function: The system executes the Module_Name sub-module and returns to the normal flow of operation.

Note, that the system executes the line containing the ***Call*** command before it calls the sub-module. It allows the user to save the necessary sub-module parameters in the Parameters Buffer before the sub-module is called.

Editing: text edit control

Example: Get the focal stack of images for 3 XY locations (see Fig.18)



	Events	Command	Devices			
			Stage Control			Acquisition
			X	Y	Z	Capture
1	New experiment					
2	Event_1				-10µm	
3	Event_1_1	Focalstack	120µm	15µm		
4	Event_1_2	Focalstack	-200µm	-100µm		
5	Event_1_3	Focalstack	350µm	54µm		
6	Focal stack					
7	Event_1					
8	Event_2	10			2µm	

Figure 18

Ext. Call

Format: ***Ext.Call <File_Name>***, where File_Name is a name of another Event Log document containing the implementation of some standard task.

Function: The system opens the external Event Log document, executes it and returns to the normal flow of operation. It executes the line containing the ***Ext***. ***Call*** command before calling the external document.

Editing: Open File dialog invoked through cell dialog button

Example: the same as before but the focal stack processing is placed into the external Event Log document (see Fig. 19).

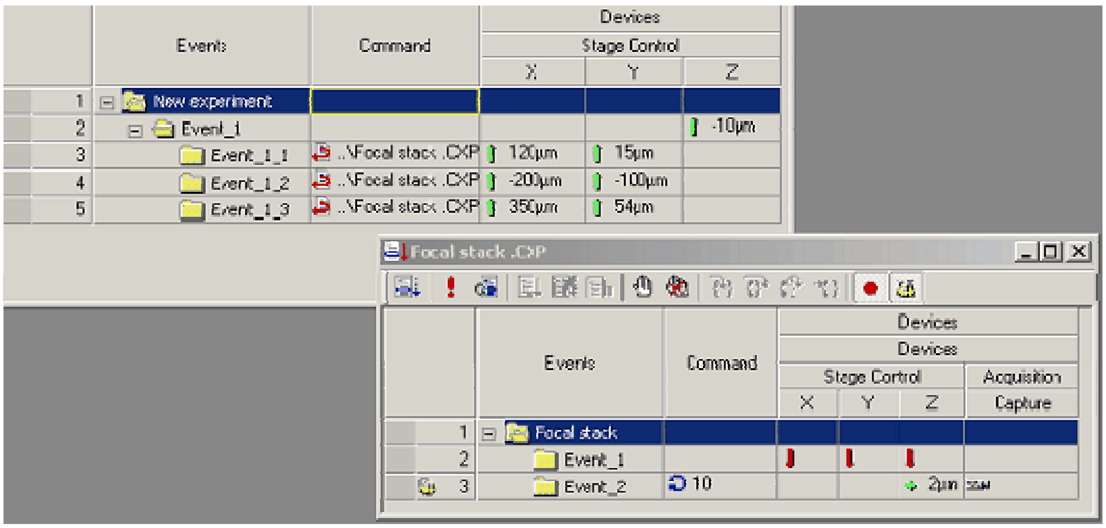


Figure 19

Real Word Problem - Step 1: building the Event Tree

Create the Event Tree using the technique discussed in the RAD environment/Working with the Event Tree chapter above as follows:

1. Create new Event Log document using File/New/Log Document command from the application main menu.
2. Change the name of the root Event Tree node from "New Experiment" to the "Real World Problem".
3. Add sub-event to the "Problem 1" node and change its name to the "Time-lapse loop"
4. Activate spreadsheet cell in the Commands column of "Time-lapse loop" event and select the ***Loop*** command. Change number of repetitions to 10.
5. Add 3 sub-events to the "Time-lapse loop" node using the ***Add Sub-Events*** command from the right-click menu.
6. Change names of sub-events to "Position 1", "Position 2", "Position 3".
7. Save the resulting Event Log document using File/Save As command from the main application menu.

The resulting Event Log document is represented on Fig. 20.

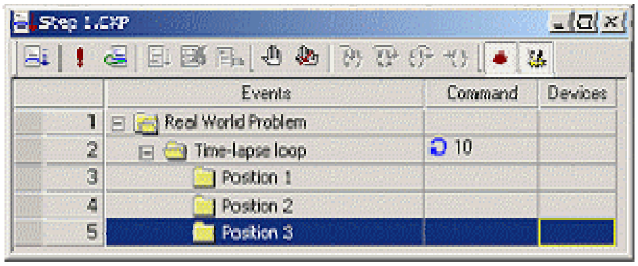


Figure 20. Real World Problem, Step 1

At this stage the system can already execute the Event Log though no real actions will be performed. Place the breakpoint at the first line of the spreadsheet and then press the ***Run*** button in the Active Spreadsheet toolbar. The system will start the execution and immediately stop at the very beginning switching into the Debug (step) mode. Using the ***Step In*** button from the Active Spreadsheet toolbar execute the Event Log step by step observing how the system highlights different lines of the spreadsheet while traversing the Event Tree.

Note: in further practicing, if executing the Event Log in real-time mode, it is a good idea to turn the running line on (by pressing the Running Line button in the toolbar) to observe the selected line change. If the maximum speed needed the Running Line mode is not recommended as updating the display slows down the execution process.

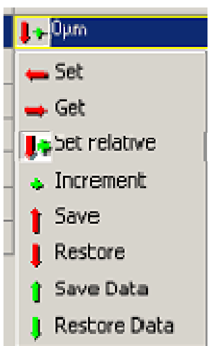
Stage Device Group

Overview

The Stage Device Group contains X,Y and Z devices that have the identical set of operations. Allows user to move the stage to the specified position or read the stage position for display.

The operation descriptions below contains the ***Formal Description*** which uses data exchange diagram notation introduced in the ***How it Works/ The generic classification of the Experiment Planner device operations chapter*** above.

Operations



 **Set**

Format: ***Set <F>***, where F is an axis coordinate in physical units from centimeters to microns

Function: Moves the corresponding axis to the absolute position F.

Editing: edit control with physical units parser

Formal description: CD->VD

 **Get**

Format: ***Get <F>*** where F is an axis coordinate in physical units ranging from centimeters to microns

Function: Reads the absolute axis position and places it into the data cell.

Note, that the system will display the new data only if the Running Line mode (see ***Executing the Event Log chapter above)***

Editing: edit control with physical units parser

Formal description: VD->CD

Set Relative

Format: ***Set Relative <F>*** where F is coordinate offset in physical units ranging from centimeters to microns

Function: Takes the value from corresponding Parameters Buffer and combines it with the cell data to calculate the absolute coordinate. The Parameters Buffer in this case can be thought of as an origin for relative coordinates containing in the cell data.

Editing: edit control with physical units parser

Formal description: (PB+CD)->VD

Increment

Format: ***Increment <F>,*** where F is coordinate increment in physical units ranging from centimeters to microns (can be negative)

Function: Takes the current coordinates from the device and increments it by F. Often used to perform the scanning over X, Y and Z.

Editing: edit control with physical units parser

Formal Description: (VD+CD)->VD

Save

Format: Does not have any parameters

Function: Reads data from the device and places it into the Parameters buffer. Used to preserve the initial stage position in order to use it later in Set Relative operation as an origin, for example.

Editing: no editing available

Formal Description: VD->PB

 **Restore**

Format: Does not have any parameters

Function: Reads the coordinate F from the Parameters Buffer and use it to move the axis to the position F. Often used in Loop sequences to initially reset the coordinate on which the increment is performed.

Editing: no editing available

Formal Description: BP->VD

 **Save Data**

Format*:* **<u>Save Data <F>,</u>** *where F is coordinate in physical units ranging from centimeters to* microns

Function: Loads coordinate from the data cell into the Parameters Buffer. Used to set up the origin that will be used later in Set Relative operation

Editing: edit control with physical units parser

Formal Description: CD->PB

 **Restore Data**

Format*:* ***<u>Restore Data <F>,</u>*** where F is coordinate in physical units ranging from centimeters to microns

Function*:* Reads the coordinate F from the Parameters Buffer and puts it into the cell. Used to dynamically display the content of the Parameters Buffer.

Editing: edit control with physical units parser

Formal Description*:* PB->CD

Real Word Problem - Step 2: Moving stage over the 3 positions

Activate the stage device and its sub-devices, add the stage movement operations and adjust the stage position as follows:

1. Open the Device Resources window using View/Device Resources command from the application main menu.

2. Select the Stage device in the Device Resources window and drag it onto the Device column header of the spreadsheet. The Stage device and X, Y and Z sub-devices will appear in the spreadsheet header. Remove the Z device from the header as we do not need it at this stage

3. Clicking on X and Y spreadsheet cells that belong to the events "Position 1", 2 and 3 activate the cells and then select **Set** operation for all of them. User can also practice in modifying the cell data using the editing mode at this stage.

4. Add the Physical Action marker (see chapter above) to the events "Position 1", 2 and 3. The resulting Event Log document is represented on Fig.21.

5. Using the Feedback mode modify the stage coordinates for each position. In order to do that, select the spreadsheet line corresponding to the "Position 1" event and then press "Toggle Feedback" button. The stage will move into the position according to the data in X and Y cells and switch into the Feedback mode displaying the blinking icon in the selected line. Using joystick move stage to the preferred position observing how system updates data in the spreadsheet cells. Repeat the process for positions 2 and 3.

Note: there is no need to select the position corresponding to some meaningful filed of the slide at this stage.

6. Press the Abort button in the spreadsheet toolbar to finish the Feedback mode.

7. Save the resulting Event Log document.

8. Run the Event Log on Real or Debug (step) mode as was described above observing the stage movement.

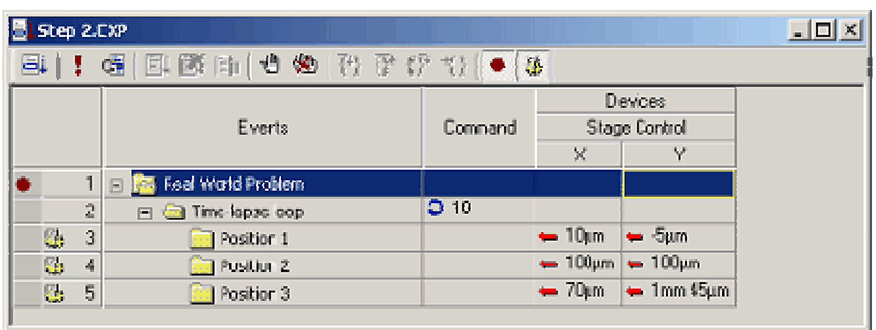


Figure 21. Real World Problem, Step 2

Acquisition Device Group

Overview

Contains 2 sub-devices: Setup and Capture. The first one is responsible for control the acquisition settings and the second one performs the acquisition itself.

Operations

 **Setup**

Format: contains multi-component data (not displayed in the cell data field. Parameters are modified through the specific dialog.

Function: Sets all the parameters necessary to perform the capture (image depth, camera exposure, type of filter etc.)

Editing: There are dozens of parameters that are controlled during this operation, so user sets them invoking the modal dialog (Capture Dialog from the SimplePCI core application) by pressing the spreadsheet cell modal dialog button (see above.) This dialog can be invoked in the process of editing as well as while running the Event log in the Feedback mode.

Note, that if there is no Setup operation performed, the Capture device is using the application capture settings which can be modified through the Capture Dialog of core application.

Formal Description: CD->VD

Operations

 **Capture**

Format: does not have any parameters

Function: cases the system to perform image capture using the setting provided by the Setup operation. The acquired image is displayed in the Mil Display.

Note, that if in the Feedback mode the system monitors the spreadsheet line where the Capture cell is active, then system uses the Focus mode to constantly update the Mil Display.

Editing: no editing available

Formal Description: CD->VD

The Real Word Problem - Step 3: acquiring the images

Add the Acquisition device and its sub-devices. Activate corresponding spreadsheet cells adding the Setup and Capture operations and adjust the acquisition settings as follows:

1. Add the Acquisition device and its sub-devices from the Device Resources window in the same way the Stage devices were added

2. For events "Position 1", 2 and 3 activate the cells corresponding to the Setup and Capture sub-devices. There is only one operation available for each of them (Setup and Capture operations correspondingly) so no operation selection required.

3. Adjust parameters for Setup operation invoking the Capture dialog through the Setup cell dialog button. Change the image depth for events "Position 1" 2 and 3 to monochrome, two-color and three color respectively adjusting the acquisition depth and exposure if necessary using the Focus mode of the Capture dialog. At this point the focus adjustment may be needed if it was not done before through the core application capture dialog. The resulting Event Log document is represented on Fig.22

4. Choose the meaningful X-Y positions for all 3 positioning events using the Feedback mode as described above.

Note, that now it can be done easily, as the system will display the live image in the Mil display during the Feedback mode because the Capture device is present and corresponding Capture spreadsheet cells are activated.

5. Save the resulting Event Log document.

6. Execute the Event Log observing images changing in the Mil Display.

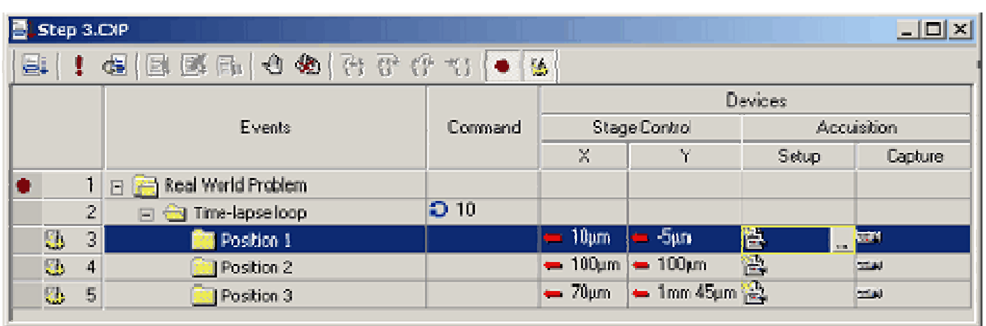


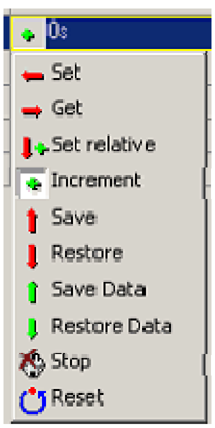
Figure 22. Real World Problem, Step 3

Time Device

Overview

Contains the timer which measures the current world time with 1ms precision. Also contains the internal register where some pre-defined time can be set. Typically, when physical action on timer is performed, it compares the current world time with pre-defined time makes the decision if it needs to interrupt the Event Long execution until the predefined time is reached or abort the execution etc. depending on the operation performed.

Operations



 **Set**

Format*:* **<u>Set <T></u>**, *where T is a word time in the format "mm/dd/yy hh:mm:ss AM/PM"*

Function: causes timer to interrupt the Event Log execution until T time is reached

Editing: calendar control

Formal Description: CD->VD

 **Get**

Format: ***<u>Get <T></u>***, where T is a word date/time in the format "mm/dd/yy hh:mm:ss AM/PM"

Function: reads the current time in to the cell

Editing: calendar control

Formal Description: VD->CD

Set Relative

Format: ***<u>Set Relative <T></u>***, where T is a time span in the format "N1d N2h N3m N4s N5ms"

Here N1d is number of days, N2h is number of hours etc. down to milliseconds and any field can be omitted.

Function: takes the world time from the Parameters Buffer and combines it with cell data to produce the resulting world time Twait. Causes the timer to interrupt the Event Log execution until Twait is reached

Editing: Edit control with physical units parser

Formal Description: (PB+CD)->VD

Increment

Format: ***<u>Increment <T></u>***, where T is a time span in the format "N1d N2h N3m N4s N5ms"

Here N1d is number of days, N2h is number of hours etc. up to milliseconds and any field(s) can be omitted.

Function: increments the content of internal time register. Causes timer to interrupt the Event Log execution until new pre-defined time is reached. Used to perform operation or set of operations at the regular time intervals.

Editing: Edit control with physical units parser

Formal Description: (VD+CD)->VD

Save

Format: does not have any parameters

Function: Saves the current world time in the Parameters Buffer. Used to preserve some time origin.

Editing: no editing available

Formal Description: VD->PB

Restore

Format: does not have any parameters

Function: Loads the world time from the Parameters Buffer into the timer.

Editing: no editing available

Formal Description: PB->VD

Save Data

Format: ***<u>Save Data <T>,</u>*** where T is either date/time or time span

Function: Loads time T from the data cell into the Parameters Buffer. Used to set up the time origin that will be used later in Set Relative operation

Editing: edit control with physical units parser

Formal Description: CD->PB

Restore Data

Format: ***<u>Restore Data</u>*** ***<u>T,</u>*** where T is either date/time or time span

Function: Reads time T from the Parameters Buffer and puts it into the cell. Used to dynamically display the content of the Parameters Buffer.

Editing: edit control with physical units parser

Formal Description: PB->CD

Stop

Format: ***<u>Stop <T></u>***, where T is a date/time

Function: Finishes the execution of the Event Log when the current data/time exceeds T

Editing: calendar control

Formal Description: if (VD>CD) then Abort

Reset

Format*:* does not have any parameters

Function*:* forces the content of internal register to become equal to the current world time. There are two important applications for this operation*:*

- Timer re-initialization before starting the time-lapse loop.
- Implementing a delay. The delay can be implemented by using the Reset followed by Increment operation. If the Time device is duplicated (the spreadsheet has two adjacent columns Time and Time_1) it be done in the single lime of spreadsheet*.*

Editing*:* no editing available

Formal Description*: (*current time)->(internal register)

Real Word Problem - Step 4: performing a time-lapse

Add time device, timer initialization event and set loop time period as follows:

1. Add Time device to the spreadsheet

2. Insert the event before the "Time-lapse loop" event using ***Insert Event/Before*** command from the right-click menu on "Time-lapse loop" tree node. Change the name of the new event to "Reset timer" and put the Physical Action marker into the line.

3. Activate Time cell in the new event line and select the ***Reset*** operation from the operation list.

4. Add sub-event to the "Time-lapse loop" node using ***Add Sub-Event*** command and change its name to the "time-lapse". Put the Physical Action marker into the line.

5. Activate the Time cell in the "time-lapse" event line, select ***Increment*** operation and change cell data to "15s". The resulting Event Log is represented on Fig.23

6. Save the resulting Event Log document.

Disable all breakpoints by un-pressing Enable Breakpoints button in the spreadsheet toolbar and run the Event Log. Observe, the system now will wait 15 seconds, counting from the beginning of the previous pass before performing the next pass within the loop.

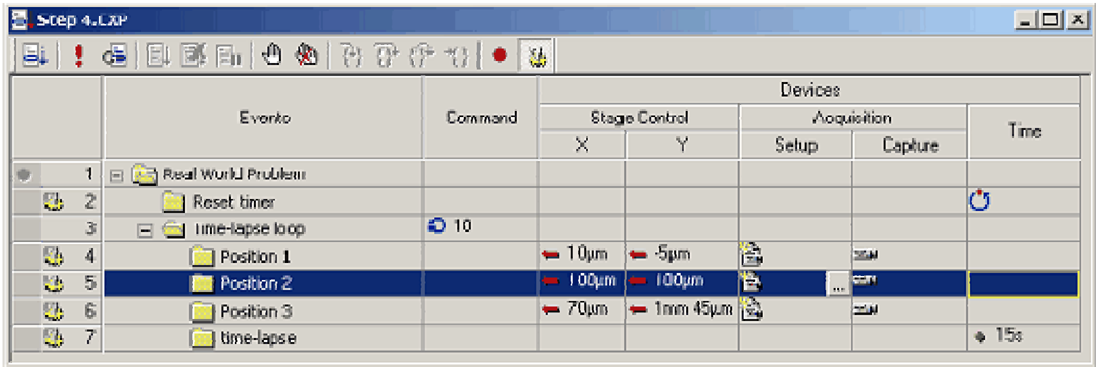


Figure 23. Real World Problem, Step 4

Storage Device Group

Overview

Contains the following devices:

Collection device Allows creation and manipulation of set of Summary Documents (.cxs files) serving as a repository for data documents

File device Allows creation and manipulation of set of Data Documents (.cxd files) serving as a repository for data fields collection

Field device Allows creation and manipulation of Data Document .fields serving as a repository for data and set of images

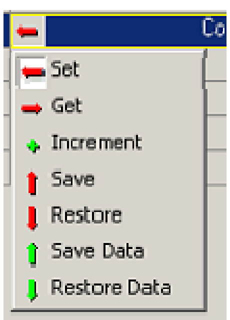
Image device Allows creation and manipulation of set of images acquired during the execution of the event log

Collection and File Device

Overview

Both devices control the process of creation and modification of files and have the similar set of operations providing the possibility to work with individual files as well as with groups of files. It means that they support the creation of enumerated group of files with names composed of the "root name" followed by enumerator. The example of such a group would be "Test_1.cxd", "Test_2.cxd" etc. The higher-level storage device automatically incorporates such a group into the Summary (.cxd) file if the Collection device is present and activated. In order to provide such a dual functionality the Collection and File devices have two internal registers: one for the root name and the other for file number and the system combines the content of this registers to generate the resulting file name. If the file number is not initialized (equal to 0) the system considers the file as an individual and uses root name as a final name of the file. As a result, some device operations support two types of data: file name (for root name) and numerical (for the file number) and user chooses one of them using the Type/… command from the cell right-click menu. If the system detects that some existing file is about to be overwritten as a result of device operation it provides the warning allowing user to change the name, overwrite the existing file, overwrite all existing files or abort the execution.

Operations



Set (file name)

Format: ***Set <FileName>***, where FileName is a name of the file (including the path) to be created

Function: Sets the file name and simultaneously resets file number to zero.

Editing: File Open Dialog usin the cell dialog button

Formal Description: CD->VD (file name)

Set (number)

Format: ***<u>Set <N></u>***, where N>=0 is a file number

Function: creates the file "FileName_N" if N>0 or file "FileName" if N=0

Editing: number edit control

Formal description: CD->VD (file number)

Get (file name)

Format: ***<u>Get <FileName></u>***, where FileName is a name of the file (including path) to be created

Function: reads the file root name into the cell

Editing: File Open Dialog invoked through the cell dialog button

Formal Description: VD->CD (name)

Get (file number)

Format: ***<u>Get <N></u>***, where N>=0 is a file number

Function: reads file number into the cell

Editing: number edit control

Formal description: VD->CD (file number)

Increment

Format: ***<u>Increment <N></u>***, where N is an integer (can be can be negative)

Function: Increment the file number and creates the file combining the root name with file number

Editing: Number edit control

Formal description: (VD+CD)->VD (file number)

Save

Format: does not have any parameters

Function: Saves file name and number from the device in the Parameters Buffer.

Editing: no editing available

Formal Description: VD->PB

Restore

Format: does not have any parameters

Function: loads the file name and number from the Parameters Buffer into the device.

Editing: no editing available

Formal Description: VD->PB

Save Data (file name)

Format: ***Save Data <FileName>,*** where FileName is a root file name

Function: Saves file name from the data cell in the Parameters Buffer.

Editing: File Open Dialog invoked through the cell dialog button

Formal Description: CD->PB (file name)

Save Data (number ***)***

Format: ***Save Data <N>*** where N>=0 is file number

Function: Saves file number from the data cell in the Parameters Buffer.

Editing: number edit control

Formal Description: CD->PB (file number)

Restore Data (file name)

Format: ***<u>Restore Data <FileName>,</u>*** where FileName is a root file name

Function: Reads file name from the Parameters Buffer into the data cell.

Editing: File Open Dialog invoked through the cell dialog button

Formal Description: PB->CD (file name)

Restore Data <u>(number)</u>

Format: ***<u>Save Data <N></u>*** where N>=0 is file number

Function: Reads file number from the Parameters Buffer into the data cell.

Editing: number edit control

Formal Description: PB->CD (file number)

Fields and Image Devices

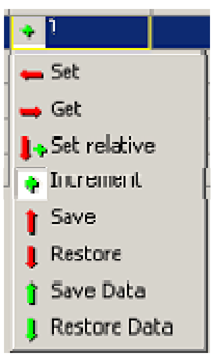
Overview

Fields and images distinguished only by their numbers and have an identical set of operations similar in functionality to the one provided for Stage sub-devices. The only difference is that Field and Images operations manipulate with integers rather than physical units as in case of stage operations.

Note, that fields and images can be assigned any number in the process of creation providing the opportunity to break a tie between the order fields and images are collected and the order they have in the Data Document.

This means for example that one can optimize the stage trajectory during the field montage minimizing the traveling time and still have the Data Document with fields ordered by rows

Operations



 **Set**

Format: ***Set <N>***, where N is field/image number N>0

Function: Sets the Field/Image number to N

Editing: number edit control

Formal description: CD->VD

 **Get**

Format: ***Get <N>,*** where N is field/image number N>0

Function: Reads the Image/Field number and places it into the data cell.

Note, that the system will display the new data only if the Running Line mode (see ***<u>Executing the Event Log chapter above)</u>***

Editing: number edit control

Formal description: VD->CD

Set Relative

Format: ***<u>Set Relative <N></u>*** where N is Field/Image number offset

Function: Takes the value from corresponding Parameters Buffer and combines it with the cell data to calculate resulting the Field/Image number.

Editing: number edit control

Formal description: (PB+CD)->VD

Increment

Format: ***<u>Increment <N></u>*** where N is Field/Image number (can be negative)

Function: Takes the current Field/Image number from the device and increments it by N.

Editing: number edit control

Formal Description: (VD+CD)->VD

Save

Format: Does not have any parameters

Function: Reads data from the Field/Image device and places it into the Parameters buffer.

Editing: no editing available

Formal Description: VD->PB

Restore

Format: Does not have any parameters

Function: Reads the Field/Image number from the Parameters Buffer and use it as a device File/Image number

Editing: no editing available

Formal Description: BP->VD

Save Data

Format: ***Save Data <N>*** where N is Field/Image number (can be negative)

Function: Loads Field/Image number from the data cell into the Parameters Buffer. Can be used later in Set Relative operation

Editing: number edit control

Formal Description: CD->PB

Restore Data

Format: ***Restore Data <N>,*** where N is Field/Image number (can be negative)

Function: Reads the Field/Image number from the Parameters Buffer and puts it into the cell. Used to dynamically display the content of the Parameters Buffer.

Editing: number edit control

Formal Description: PB->CD

Real Word Problem - Step 5: storing the images

Add the Storage device and File, Filed and Image sub-devices add storage control operations and adjust data as follows:

1. Add **Storage** device and its sub-devices. Remove Collection device as it is not used in this problem
2. Insert the new event before the "Reset timer" event and give it the name "Init". In the spreadsheet line corresponding to this event activate the **File** cell, choose Set operation and provide the file name (Problem1.cxd) using the cell File Open dialog. The recommended file path is "…\SimplePCI\Experiment Planner\Practice" and it needs to be created if necessary (of course, any other file path can be used)

3. Activate **Field** cell in the "Time-lapse loop" line, choose Increment operation and set data to 1, so at the beginning of each loop the system will increment the field number.

Note, that executing this line the system modifies the field number but does not perform any storing operations (physical Action marker is not present), as the actual storing will be performed during the image acquisition ("Position 1", 2 and 3 events)

4. Activate Image cells for the events "Position 1", 2 and 3 and choose Increment operation with data set to 1 instructing the system to increment the image number before each storing action.

5. Initialize the **Field** loop increment. This is extremely important step that is present in any Event Log dealing with loops. The problem is that system increments the field number using the starting number initially stored in the **Field** device. If this number is not initialized properly, the wrong field sequence will be generated. As the **Increment** operation is always performed before data are committed into the physical device, the starting number in this case needs to be zero. To accomplish this, activate the Field cell in the "Init" event line and put **Set 0** into it.

6. Initialize the Image loop increment. There is the same problem and the same solution as with **Field** increment, but in this case the Image number needs to be initialized at the beginning of each loop pass, i.e. in the "Time-lapse loop" node. The resulting Event Log file is represented on Fig 24.

Note, that it is a common situation when the Event Log manipulates the data (the File Name and Field and Image numbers) without performing the actual physical actions until all data are being prepared.

Run the Event Log. After the execution is finished, open the Data Document created during the experiment. It has two have 10 fields with 3 images in each field, ranging from monochrome to three-color.

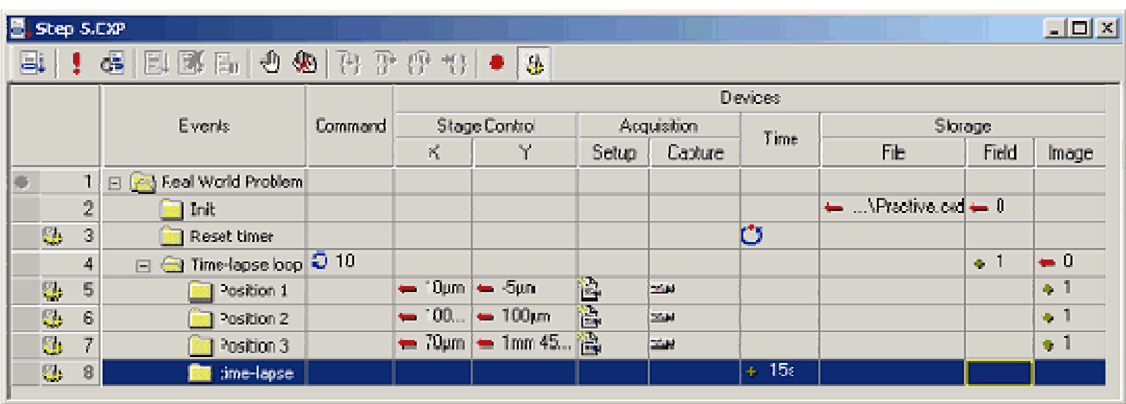


Figure 24. Real World Problem, Step 6

Prompt Device

Overview

This device provides the interaction between the system and user while running the Event Log. If the corresponding device cell in the current spreadsheet line is active the system interrupts the execution and pops-up the dialog displaying the message containing in the cell. This message may prompt the user to adjust the stage position, turn-on the lamp, add some reagent into the well of the multi-well plate etc. After necessary actions are performed user can resume the Event Log execution by pressing the Continue button or stop the experiment by choosing the "Abort".

Note: the Prompt device is an only device that always performs the physical action even if the corresponding event does not contain the Physical Action marker. This allows user to check the logic of interaction in the simulation mode

Operations

There is a single operation available:

 **Prompt**

Format: **<u>Prompt <Message></u>**, where Message is any text user enters into the cell.

Editing: edit control

Formal Description: CD->VD

Real Word Problem - Step 6: prompting user to turn on the lamp

1. Add the **<u>Prompt</u>** device into the spreadsheet.
2. Activate the **<u>Prompt</u>** cell in the root node of the Event Tree and type-in the following text: "Turn on the lamp!". The resulting Event Log is represented on Fig.25.
3. Save the resulting Event Log document.

Run the Event Log. At the beginning of the execution the message box containing the Prompt cell text will popup and the system will stop the execution. To resume the execution, press the "Continue" button. At this point the execution can be stopped any time as the remaining portion of the Event Log is exactly the same as before.

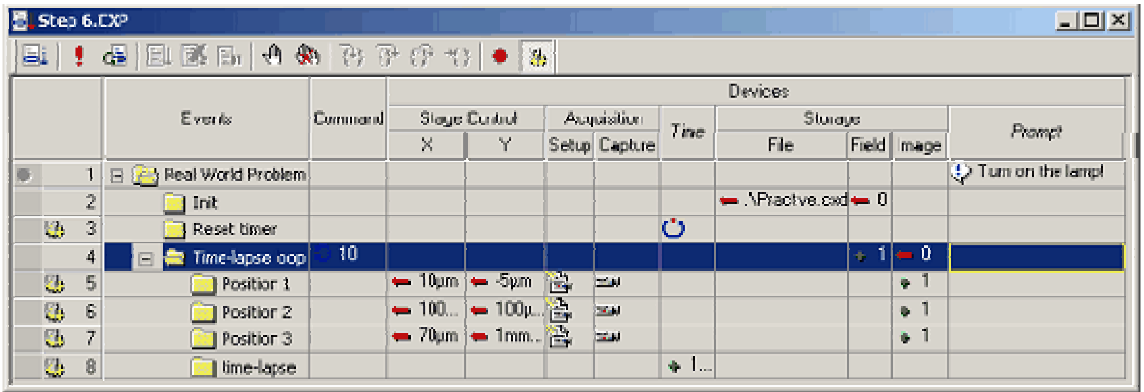


Figure 25. Real World Problem, Step 6

At this point, the initial Real World Problem is completely solved. To explore the functionally of remaining devices the additional task needs to be included into the problem:

"At the beginning, obtain Z coordinate for given 3 X-Y positions using the Auto-Focus capability and then reuse them while acquiring the images within the time-laps loop. Also use the Sub-Module functionality to perform the auto-focus procedure."

Call Device

Overview

Provides the same functionality as ***Call*** and ***Ext. Call*** command (see Commands chapter above) but allows placing few calls into the same line of spreadsheet by the device duplication.

Operations

Call

The same as Call commands

Ext. Call

The same as Ext. Call command

Real Word Problem - Step 7: creation of the Auto-Focus Sub-Module.

1. *Add Sub-Module into the Event Tree using* ***Add Sub-Module*** *command from the right-click menu on the* ***Events*** *column header.*
2. *Change the name of the Sub-Module to "Auto-Focus"*
3. *Add 3 events to the sub-module and rename them as "Focus 1", 2 and 3. In the X and Y columns, enter the same operation and coordinates as for the events "Position 1", 2 and 3. Activate the Physical Action marker for all 3 events.*
4. *Add the Call device to the spreadsheet.*
5. *Activate the Call cell in the "Init" event line; choose the Call operation and type in the name of the sub-module. The resulting Event Log is represented on Fig.26.*
6. *Save the resulting Event Log.*

Enable the breakpoints if necessary and run the Event Log in Debug (step) mode. After reaching the "Init" event line the system will jump to the "Auto-Focus" sub-module, run through it and then return to the main event tree. At this point the execution can be stopped any time.

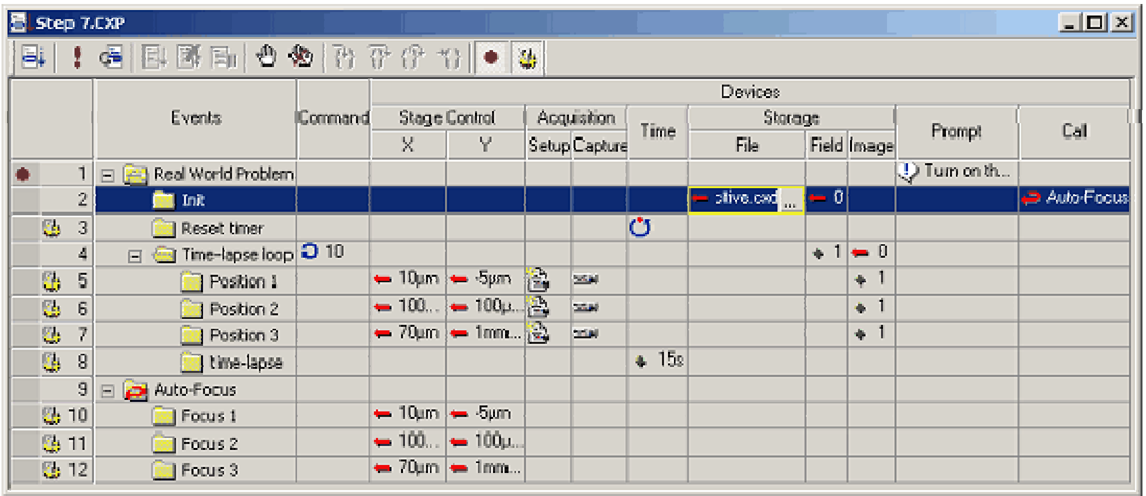


Figure 26. Real World Problem, Step 7

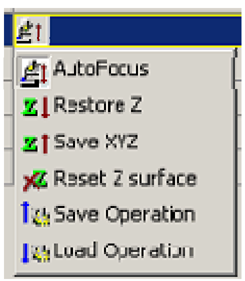
Auto-Focus Device

Overview

The Auto-Focus device is used to automatically find the right focus Z at a given X-Y position and allow the reuse of this information later on in the experiment. For example we may want to update the Z position of a field in a time lapse experiment to allow for Z stage drift.

In order to accomplish this task an internal list of X-Y-Z positions (the surface profile) is supported. This list is updated any time auto-focusing is performed and can be later reused to pick the right Z for a given X-Y position without additional refocusing (see Fig.30). Using the information about X, Y and Z, means that the Auto-Focus device is actively interacting with the Stage devices during the operation execution.

Operations



 **AutoFocus**

Format: does not have any parameters.

Function: Performs auto-focus and saves it in the surface profile for the current X-Y.

Note, that if X or Y devices are not present the auto-focus will be performed anyway, but the surface profile will not be updated.

Editing: no editing available

Formal Description: VD->(auto-focus)->VD

 **Restore Z**

Format: does not have any parameters.

Function: Restores Z for a given X-Y using the previously accumulated surface profile.

If X or Y devices are not present or the current X-Y can not be found in the surface profile, no operation will be performed.

Note: in the current version, the system finds a match in the profile if the distance between the given X-Y position and closest point in the profile does not exceed 10 microns.

Editing: no editing available

Formal Description: (XY)->VD(surface profile)->Z

Save XYZ

Format: does not have any parameters

Function: Adds the current X-Y-Z position to the surface profile, updating the surface profile directly, without using the auto-focusing.

One of the possible scenarios of using this operation is following: user creates the Event Log containing X,Y,Z and Auto-Focus devices, adding the instructions for visiting the multiple XYZ positions on the slide using X, Y and Z ***Set*** operations and Auto-Focus ***Save XYZ*** operation in each line of the Event Log. After adjusting the focus (Z) using the Feedback Mode for each of X-Y position the file can be saved and reused later as an instant surface profile generator.

Editing: no editing available

Formal Description: (XYZ)->VD(surface profile)

Reset Z

Format: does not have any parameters

Function: removes all data from surface profile. Used to cleanup the surface profile starting the creation of the new one.

Editing: no editing available

Formal Description: (remove surface profile list)->VD

Save/Load Operation group

It is often the case that during a long experiment the slide or micro-well plate goes out of focus because of vibration, heat deformations etc. It means that the system needs to periodically switch back and forth between auto-focus and surface profile reusing modes. The set of Auto-Focus device operations includes 2 operations allowing the elegant solution of this problem.

One of them (***Save Operation***) allows saving the device operation in Parameters Buffer and the second one (***Load Operation***) loads it into the cell and actually executes it. Using these operations the user can, for example, create a Sub-Module that performs the task of acquiring images in multiple positions using ***Load Operation*** instruction to obtain the right Z. Depending on what operation was saved in the Main module, the sub-module will obtain Z either in the auto-focus or surface profile reusing mode. The alternation between operations in the main module can be achieved using the ***Schedule*** command (see above) The example of altering between auto-focus and profile reusing can be found in ***Programming Technique*** chapter below.

Save Operation

Format: ***Save Operation <Operation_Name>***, where Operation Name is either ***AutoFocus*** or ***Restore Z***

Function: stores operation in the Parameters Buffer

Editing: drop-down list control

Formal Description: CD->PB

Load Operation

Format: ***Load Operation <Operation_Name>***, where Operation Name is either ***AutoFocus*** or ***Restore Z***

Function: loads the operation from the Parameters Buffer and executes it

Editing: drop-down list control

Formal Description: PB->CD

Real Word Problem - Step 8: performing the auto-focus operation

1. Add the Auto-Focus device to the spreadsheet between **Stage** and **Acquisition** devices using **Copy** and then **Paste Special/Before** command on Acquisition device.

2. Activate the Auto-Focus cells in the event lines "Focus 1", 2 and 3, choose the **AutoFocus** operation and set the Physical Action markers for all of them.

3. Activate the Auto-Focus cells in the event lines "Position 1", 2 and 3 and choose **Restore Z** operation for them. The resulting Event Log is represented on Fig.27

4. Save the resulting Event Log.

Move the stage slightly out of focus and then run the Event Log. While executing the "Init" event the system will switch to the sub-module and after auto-focusing is finished return to the time-lapse image acquisition. At this point even though initially the microscope was out of focus the system will restore the focus and reuse it for subsequent acquisitions.

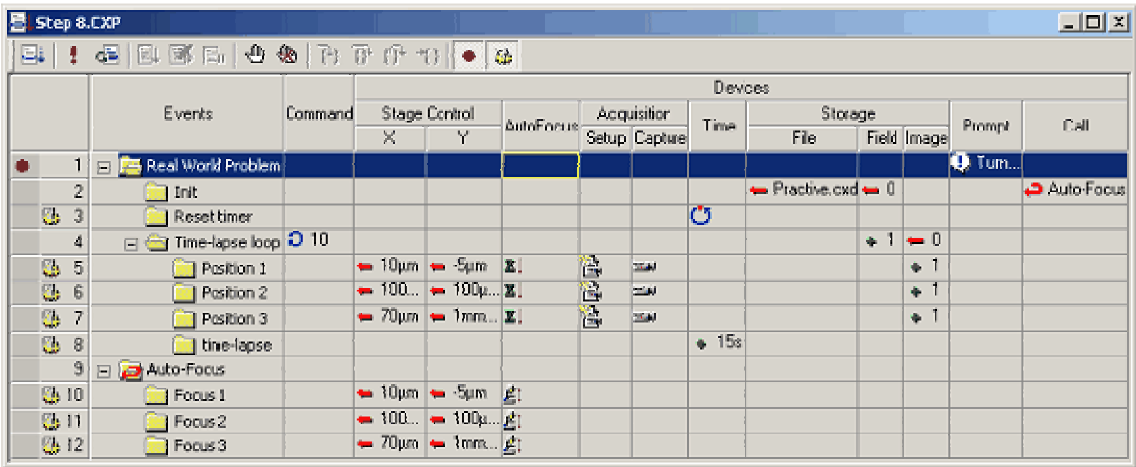


Figure 27. Real World Problem, Step 8

Programming – Loops

Introduction

The enclosed loops are widely used in the situations where multi-dimensional scanning is performed, for example, in case of 2D or 3D montage. There are two aspects to be considered while developing the enclosed loops in the Experiment planner: the loop order and the loop initialization. In order to illustrate these aspects consider the creation of <XY> montage with 3 rows and 4 columns where stage travels by rows, the X and Y increments are 100 and 50 microns respectively and the starting upper-level coordinate of the montage is <0,0> see Fig.28

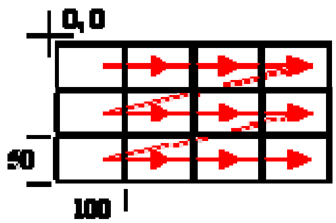


Figure 28. 4x3 montage

The stage movement accomplishing this task can be described as follows: for each Y the X coordinate needs to be set to four incremental values X1, …X4, where Xi is a column coordinate (horizontal scan). The Y itself needs to be set to 3 values Y1,…Y3 where Yi is a row coordinate (vertical scan).

Note, that stage needs to be moved to the next Y position after all X positions are visited.

Loop enclosure order

The horizontal and vertical scans can be implemented using the Loop command and the Increment operation on X and Y devices correspondingly. The phrase "for each Y…" above means that for each Y loop pass the complete X loop needs to be implemented. In terms of the Event Tree it means that X loop has to be a child of the Y loop event and the Event Tree fragment accomplishing this task is represented on Fig.29

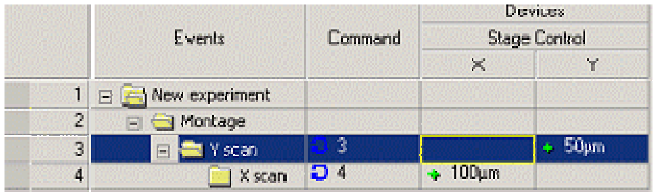


Figure 29. 4x3 montage enclosed loops

This approach may look very unfamiliar because in the normal conversation this process would be described as "perform the XY montage with X scan followed by Y scan" but it is exactly the same approach used in any programming language when implementing the enclosed loops.

The following rule can be suggested: to eliminate the possible confusion the user always needs to describe the multi-dimensional scan as following: "for each Y perform the scan within which: {for each X perform the scan within which: {…for each Z perform the scan…}}". Here the left-most statement will be implemented in the upper level loop, the next will be enclosed into the first and so on and the right-most will be the lowest-level loop in the hierarchy.

Loop initialization

As was discussed above (see the "Real Word Problem Step5: storing the images"), the data that will be incremented within a loop always need to be initialized. In our case the first montage position is <0,0> and X and Y increments are 100 and 50 microns respectively. It means that the X has to be set to –100 microns and Y to –50 microns before the first increment will occur.

The following rule can be suggested: if the starting loop value is ***V*** and the loop increment is ***dV***, the data need to be set to the value ***V-dV*** before the first increment takes place.

The next problem that needs to be solved is where exactly in the Event Log the initializations need to be placed. Note, that each time the stage moves to the next Y position the X position needs to set to the beginning of the row. It means that Y position needs to be initialized before the Y loop ("Scan Y" event) starts and the X position needs to be initialized before the X loop ("Scan X" event) but within the Y loop. The reason is that if, for example, the initialization action will occur before the Y loop starts, the X position will be initialized only once and stage will not return to the first column while moving to the next row.

Taking into account that the "Scan Y" event line is executed at each Y loop pass, the X initialization can be put into this line and the Y initialization can be put into newly inserted event "Init Y". The resulting Event Log is represented on Fig.30.

The following rule can be suggested: put the lower-level loop initialization into the upper loop line and put the highest-level initialization before the first loop.

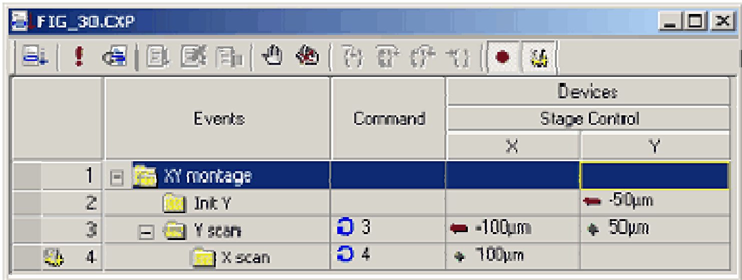


Figure 30. Loop initialization and the resulting XY montage Event Log

Using Sub-Modules or External Event Log Documents and the Parameters Independence

Consider the situation when the same 4x3 montage needs to be performed for multiple positions on slide. In this case, it is reasonable to move the montage procedure into the sub-module or into the external Even Log document and modify it in such a way that it will work correctly no matter for the montage origin is so it can be reused for all montage positions through the Call or Ext. Call command. In order to accomplish this task, the loop initialization has to be modified in such a way that it will use the Buffer Parameters values for initialization instead of absolute coordinates. Using the ***Set Relative*** operation and taking into account the <-100,-50> initial offset, the position-independent external Montage document would look like as represented on Fig.35

The following rule can be suggested: to make the montage position independent, use Set Relative -F operation for initialization, where F is a value increment.

The Event Log using the position-independent montage procedure as a Sub-Module to create the Montage for 3 positions is represented on Fig.31.

Note, that capture operations are also included into this event log for completeness.

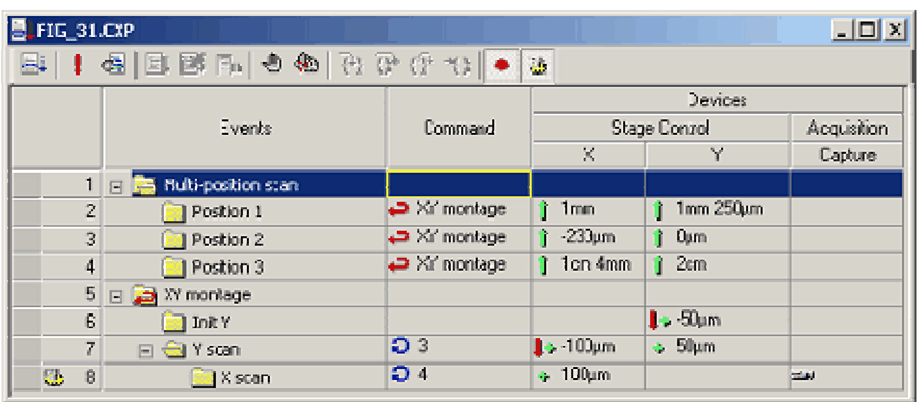


Figure 31. Using sub-modules and position independence

The "XY montage" sub-module shown on Fig. 31 still uses numbers to perform X-Y increment and the initial offset. To implement the completely universal XY montage sub-module, the X and Y increments need to be supplied to the sub-module as parameters and the increment needs to be implemented using ***Increment By Parameter*** operation. As there are two parameters (origin and increment) need to be supplied for ***X*** and ***Y*** devices each device needs to be duplicated as ***X_1*** and ***Y_1*** devices correspondingly. The Event Log performing the same task as above and using the increment-independent XY montage sub-module is represented on Fig. 32.

Note, that in this example the montage origin coordinates are modified in such a way that now they incorporate the initial offset (-100 microns for X and –50 microns for Y).

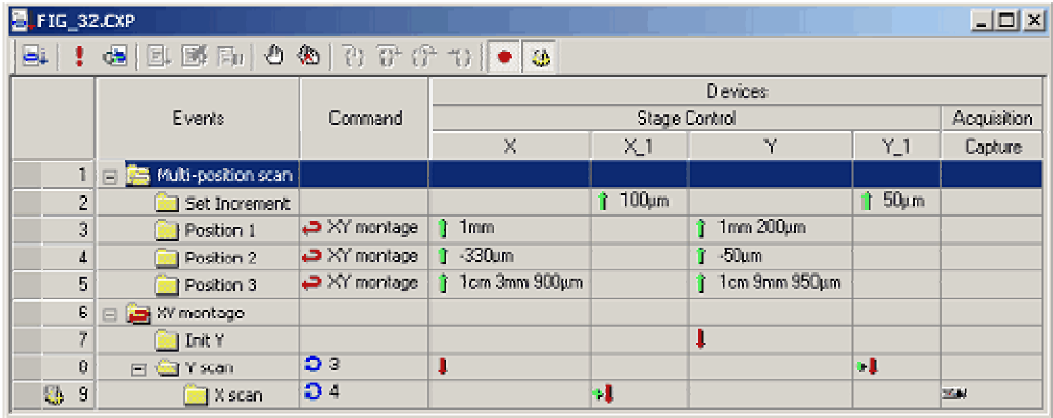


Figure 32. Sub-module position and increment independence

Incorporating the offset into the origin is not a very convenient approach, as it requires user to perform some calculations and hides the real origin increasing the possibility to make a mistake. There are two different methods to have deal with this problem. The first method uses an additional device duplicate to transfer the initial offset to the sub-module (see Fig. 33).

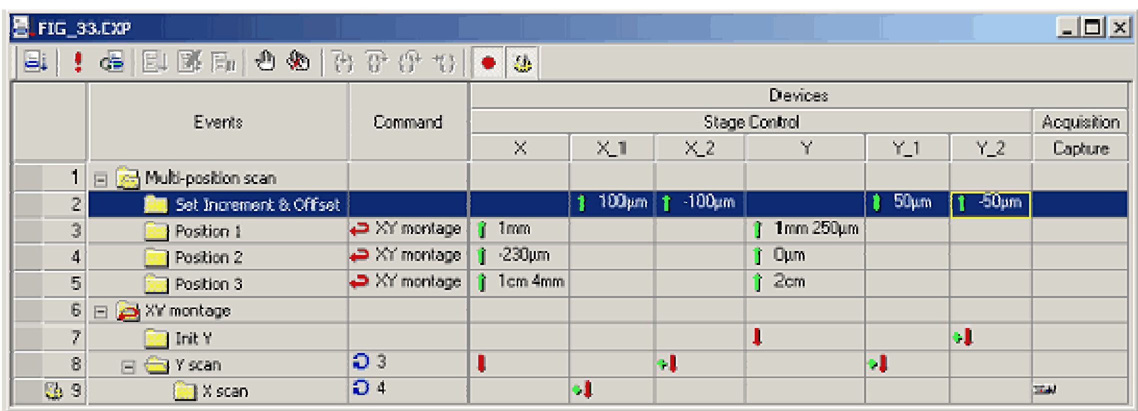


Figure 33. XY-montage completed sub-module, Variant 1

The second, more elegant method modifies the sub-module in such a way that the initial offset is completely eliminated (see Fig. 34). Here the order of operations is altered so the acquisition is performed before the first increment takes place, which means that the first acquisition will be applied at the origin position. The acquisition operation is placed into the separate line preceding any coordinate increments, which is the only line in the sub-module marked with Physical Action tag. This means that any coordinate modifications made at the given loop pass will be applied only at the next pass and the increment at the last loop path will not cause any unnecessary stage movements.

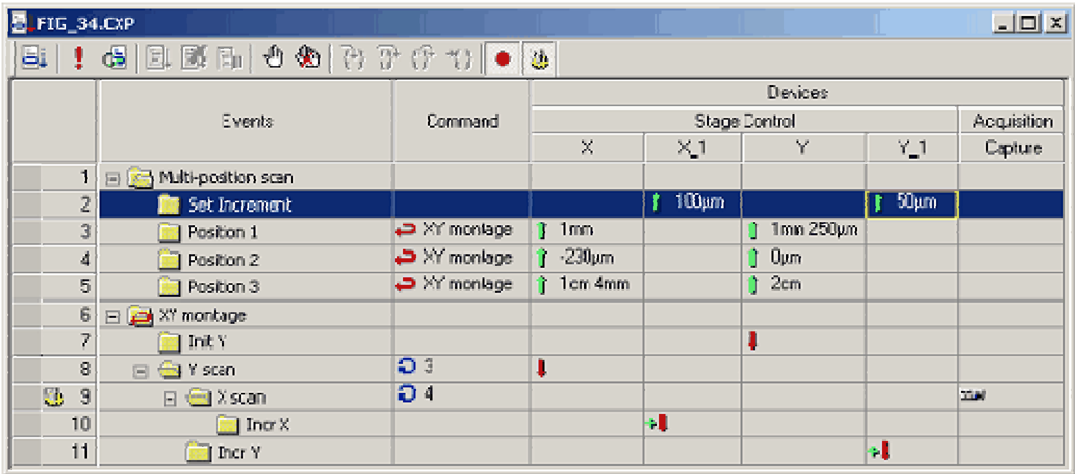


FIG_34.CXP

	Events	Command	Devices: Stage Control: X	X_1	Y	Y_1	Acquisition: Capture
1	Multi-position scan						
2	Set Increment			100µm		50µm	
3	Position 1	XY montage	1mm		1mm 250µm		
4	Position 2	XY montage	-230µm		0µm		
5	Position 3	XY montage	1cm 4mm		2cm		
6	XY montage						
7	Init Y						
8	Y scan	3					
9	X scan	4					
10	Incr X						
11	Incr Y						

Figure 34. XY montage completed sub-module, Variant 2

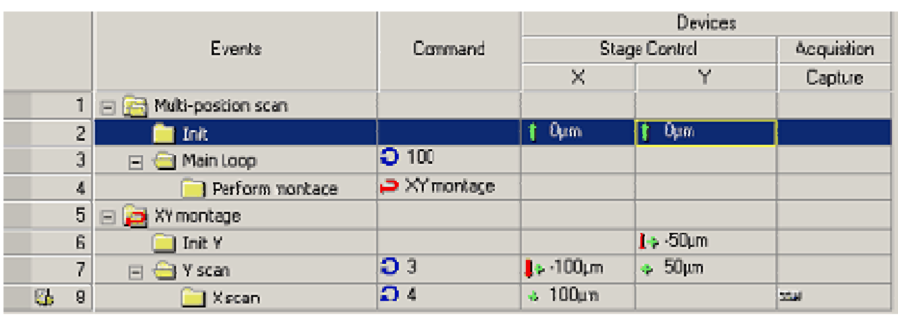
Storing Images in Order Different they are Acquired

It can be the case that images acquired sequentially need to be distributed over different files depending on the logic of the experiment. For example, consider the situation when the same 4x3 XY-scan is performed on the multi-well plate so each image belongs to the different well and that the scanning process repeats 100 times during the experiment. In order to analyze the experiment on per-well basis, it is reasonable to store all the images belonging to the same well in the single Data Document file, one image per field. It means that as a result of the experiment 6 files containing 100 fields each will be created and the images number 1,7,13 etc. will be stored in the first file (one image per field), the images number 2,8,14 etc. will be stored the second and so on. Here the image number denotes the order in which the images where acquired in the process of the XY-scan. To provide the easy access to the files they need to be included into the Summary Document file so the resulting set of files created will consist of (file names are arbitrary): one Summary Document file named "Multi-well Experiment.cxs" and 6 Data Document files with names "Well_1.cxd", "Well_2.cxd", … , "Well_6.cxd" respectively.

The solution of this problem is based on the fact that the order the Storage devices listed in the Active Spreadsheet header does not necessary define the order the operations and that the File device internally supports the file number (see the File device description in the previous chapter) so the field and file numbers can be manipulated independently.

In order solve this problem do the following:

1. Create the skeleton Event Log as shown on the Fig.35, using the Montage sub-module from the previous example. Here the "Init" event in the Main Tree is used to perform all necessary initialization (X-Y position at this step), the "Main Loop" event performs the loop and the "Perform montage" sub-event of the "Main Loop" event invokes the "Montage" sub-module.



	Events	Command	Devices: Stage Control X	Devices: Stage Control Y	Devices: Acquisition Capture
1	Multi-position scan				
2	Init		0µm	0µm	
3	Main Loop	100			
4	Perform montage	XY montage			
5	XY montage				
6	Init Y			-50µm	
7	Y scan	3	-100µm	50µm	
8	X scan	4	100µm		

Figure35. Storing Images - skeleton Event Log

2. Add Storage devices into the spreadsheet header and remove the Image device, as only one image per field will be used. In the "Init" event line, initialize the Collection cell as Summary Document file name ("…\Multi-well Experiment.cxs") and the Field number as zero.

3. As the File number needs to be incremented for each subsequent image, increment the File number in the "X scan" line of the sub-module. The first image acquired in the sub-module is always stored in the file number one. It means that the file number needs to be

reset to zero before the montage is starting, for example in the "Perform montage" event of the Main tree. Initializing the File cell as a file name ("…\Well.cxd") using Set operation will set the proper file name and reset the Field number to zero simultaneously.

4. Each image of the current montage has the same field number. It means that field number has to be incremented before the current montage starts, for example in the same "Perform montage" event of the Main tree.

The final Event Log document is represented on Fig.36

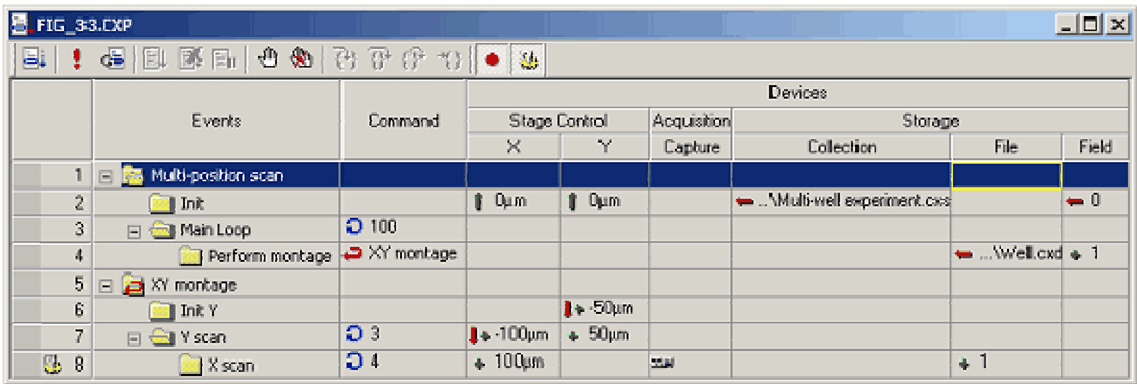


Figure 36. Storing images - final Event Log

Altering between the auto-focus and reusing the surface profile

As was mentioned in the discussion of the Auto-Focus device, it is necessary to perform refocusing from time to time if the surface profile of the Auto-Focus device is used. To explore the method of using the Schedule operation for this purpose, consider the following addition to the last variant of the Montage problem.

Let suppose that the Auto-Focus device functionality is used to retrieve the Z position of the montage fields. Let's suppose also that re-focusing is needed at each 10th pass of the Main Loop while in other passes the surface profile can be reused. The file storing portion of the problem can be omitted for clarity at this stage.

The idea of using the Schedule command in this case is following: if during the stage movement within the "XY montage" sub-module the Z retrieving operation will be performed and the ***Load Operation*** (see Auto-Focus device in the chapter above) is used for this purpose, the system will execute the operation it retrieves from the Parameters Buffer. If, in the Main tree, we will alter the content of the Auto-Focus Parameters Buffer (using the ***Save Operation*** instruction) in such a way that it will contain ***AutoFocus*** operation at each 10th pass (including the first one) and ***Restore Z*** otherwise, the task will be accomplished.

The resulting Event Log is represented on Fig.37 Here the Auto-Focus device is inserted before the acquisition device so the Z retrieval precedes the acquisition. In the Main tree the "Schedule" event is inserted which contains two sub-events: "Auto-Focus" and "Surface Profile" that use the Parameters Buffer to store the ***Auto-Focus*** and ***Restore Z*** operations respectively. Both-events are calling the same "Montage" module that is modified in such a way that that it loads and executes the Z retrieving operation right before the image acquisition. The "Schedule" event itself contains the ***Shedule 1,9*** command that instructs the system to pick for the execution the first sub-event ("Auto-Focus") at the first pass and the second sub-event ("Surface Profile") at passes 2 to 9, repeating the process until the end of the experiment.

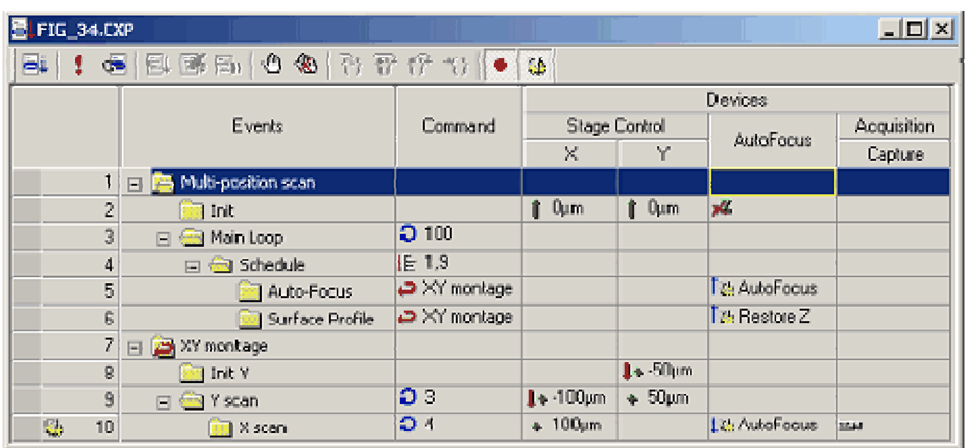


Figure 37. Altering between the auto-focus and reusing the surface profile

Observing different groups of wells with different frequencies

An example, illustrating the method of solving this problem was initially introduced in the previous chapter (see ***<u>Commands, Devices and Operations,</u>*** the ***<u>Schedule</u>*** command). The text of the example is duplicated below:

Let’s suppose that 3 groups of wells in the micro-well plate need to be observed with different frequencies due to the different grows factor en each group and that the ration of frequencies is 8:2:1. It means that the group 1 needs to be observed on each pass, group 2 on each fourth pass and group 3 on each eighth pass."

The following table shows what needs to be done at each pass:

Pass #	Observe groups
1	G1+G2+G3
2	G1
3	G1
4	G1
5	G1+G2
6	G1
7	G1
8	G1
9	The same as 1
10	...

The Event Log fragment accomplishing this task is represented on Fig.38

Here the G1, G2 and G3 are sub-modules representing the well groups 1, 2 and 3.

The Schedule event contains 4 sub-events, the first representing the pass 1, the second-passes 2 to 4, the third-pass 5 and the last the passes 6 to 8.

Note, that the well groups combinations (G1+G2+G3 and G1+G2) are implemented using the Call device duplication.

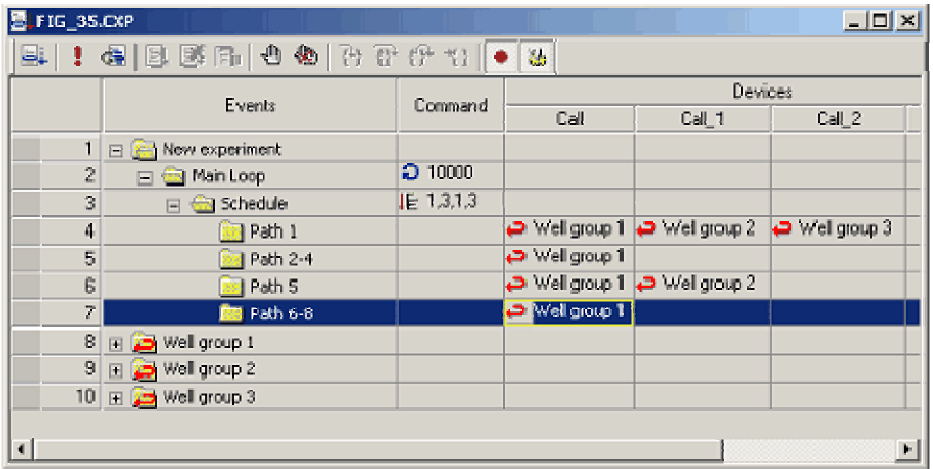


Figure 38. Observing groups of wells with different frequencies

Implementing Timing Diagrams

Sometimes it is necessary to perform the action or a set of actions at the predefined (possibly irregular) moments of time and to support the time relation between different previous and subsequent events. The graphical representation of such a relation has a special name – the Timing Diagram and is a common way to plan and analyze the timing sequences. The horizontal axis on this diagram is a time and the vertical represents different actions performed during the experiment (each action has its own horizontal axis). The non-zero value means that action was performed (or the event has happened) and the arrows denote the logical connections between the different actions.

Note, that there are four types of the relationship that can be identified:

1. Absolute time, when the event starts at some predefined data and time. An example: start the experiment on October 1, 4:30 PM
2. The delay, when the time of starting the next event is defined relatively to the end of previous event. An example: start the image acquisition 15 minutes after some solution was added to the well containing the live cells.
3. The time interval, when the time of starting the next event is measured relatively to the beginning of the previous event. An example: image acquisition at the regular 20s intervals
4. The same as the second or the third case but the time interval is measured not relatively to the previous but to some another preceding event. An example: Start the next stage of an experiment 1 hour after the solution was added to the well with live cells.

The timing diagram, reflecting all four cases is represented on the Fig. 39.

Note, that time between the beginning of the experiment and the solution adding is unspecified.

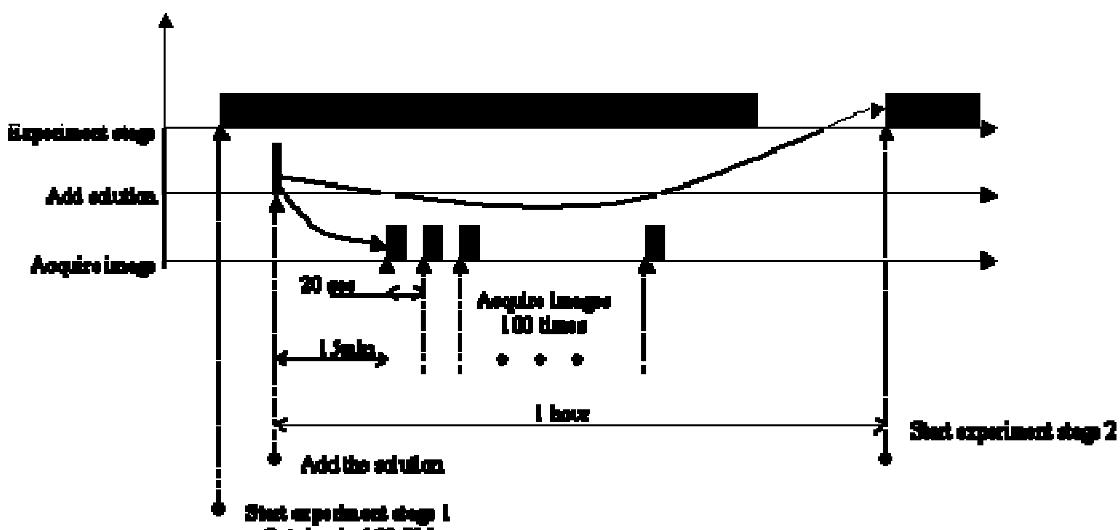


Figure 39. Example timing diagram

Here the absolute time is used to start the Experiment Stage1 event, the delay is used to start the image acquisition sequence, the regular acquisition period is implemented using the time interval and the Experiment Stage 2 is initiated relative to the Add Solution event

The Event Log implementing the timing diagram above is represented on Fig. 40. The idea behind this implementation is to consider the timing diagram above as a "timing diagram within the timing diagram", where the image acquisition sequence is an inner diagram and the experiment stage start and adding the solution is an outer one. In this case, the Parameters Buffer is used to save the time corresponding to the "Add Solution" event and the ***Set Relative*** operation is used to set the starting time of the "Experiment Stage 2". The delay is implemented using the ***Reset*** operation followed by ***Increment*** in the same line of the spreadsheet, and the regular time interval is supported using the ***Increment*** operation within each acquisition loop pass.

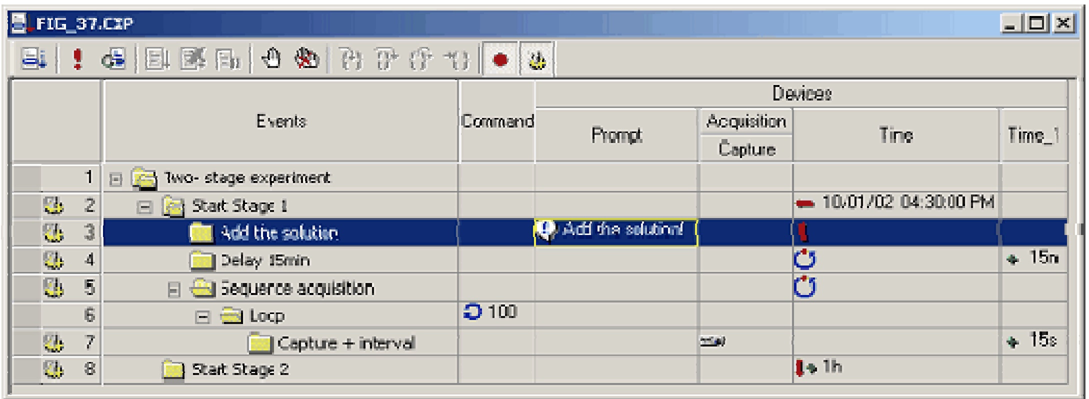


Figure 40. Timing diagram implementation

Real World Problem Examples

The Real World Problem Event Log files can be found in the following directory: " **..\Log Examples\Real World Problem\…"** and have the name format **"Step N.cxp"** where **N** is a Real World Problem step number from the manual.

Programming Technique Examples

The Programming Technique chapter examples can be found in the following directory: "..\Log Examples\Programming Techniques\…" and have the name format "Figure N. <name>.cxp" are drawing number and drawing name correspondingly. **<name>** and **N** where ,

Other Drawings

Event Log files corresponding to other drawings in this manual can be found in the directory: "..\Log Examples\Drawings\.." is a corresponding drawing number from the manual. **N** , where**"Figure N.cxp"** and have the name format

Practical Examples (Templates)

The following is a list of Event Log files that can be used as templates in various practical situations and can be found in the directory: ***"..\Lig Examples\Templates\"***

- **X List.cxp** Capture and store images at 4 different X positions
- **X Pattern.cxp** Capture and store images at 10 X positions with regular X increment
- **Y List.cxp** Capture and store images at 4 different X positions
- **Y Pattern.cxp** Capture and store images at 10 Y positions with regular Y increment
- **Z List.cxp** Capture and store images at 4 different Z positions
- **Z Pattern.cxp** Capture and store images at 10 Z positions with regular Z increment
- **XY List.cxp** Capture and store images at 4 different XY positions
- **XY Pattern.cxp** Capture and store images at 5x4 XY montage with regular X and Y increments
- **Time List.cxp** Capture and store images using 4 irregular time intervals
- **Time Pattern.cxp** Capture and store 10 images using regular 1 minute time interval
- **Settings And Capture.cxp** Capture and store images at 4 different XY locations and for different capture settings (image depth and gain varies)
- **Storage One File.cxp** Repeat process 15 times. Capture and Store 5x4 XY montage at successive fields.
- **Storage One File Per Pass.cxp** Capture and Store 5x4 XY montage repeating process 15 times, storing each XYmontage in separate file (15 files with 20 fields).
- **Storage One File Per Position.cxp** Capture and Store 5x4 XY montage repeating process 15 times, storing each XY position in separate file (20 files with 15 fields)
- **Multi Color Capture One Image Per Settings.cxp** Repeat capturing 10 times, storing all images of the same pass in a single field, 3 images per field. Capture and store images using 3 different color settings: monochrome, two and three color.

- **Autofocus Every Field.cxp** Perform auto-focus to retrieve Z coordinate for each acquisition. Capture and store images at 3 XYZ positions repeating process 10 times.
- **Autofocus Once.cxp** Perform auto-focus for all 3 positions before starting the acquisition and use obtained XYZ profile to retrieve Z during the acquisition. Capture and store images at 3 XYZ positions repeating process 10 times.
- **Autofocus Every Nth Field.cxp** operation: One Of N - Perform auto-focus every 10th pass using Capture and store images at 3 XYZ positions repeating process 100 times.
- **Time Lapse+Multicapture+Storage_One_Image_Per_Settings.cxp** Repeat process 10 times at successive fields of a single file. Perform each pass at regular time intervals (1 minute) Capture and store images at 4 XY positions and different acquisition settings, storing all images of the same pass in a single field,4 images per field.
- **Time Lapse+Multicapture+Storage_One_File_Per_Position.cxp** Repeat process 10 times at regular time intervals (1 minute) Capture and store images at 4 XY positions and different acquisition settings, storing all images of the same position in a separate file.
- **XY Pattern+TimeLapse+Capture+Storage_One_File_Per_Position.cxp** Repeat process 20 times with 10 seconds delay between passes. Capture and store images at 5x4 XY montage with regular X and Y increments, one file per position.
- **Z Pattern+TimeLapse+Capture+Storage.cxp** Repeat process 100 times with 1 minute delay between passes. Capture and store images at 10 Z positions with regular Z increment in successive fields of a single file.
- **XY List+TimeLapse+Autofocus_Schedule+Storage_One_File_Per_Position.cxp** operation to alter between auto-focus and XYZ profile reuse modes. ***Schedule*** Perform refocusing each 10th pass using Repeat process 100 times with 10 seconds delay between passes. Capture and store images at 4 XY positions storing all images of the same XY position in a separate file.
- **XY Pattern+Timelapse+Autofocus_Schedule+Storage_One_Field_Per_Position.cxp** operation to alter between auto-focus and XYZ profile reuse modes. ***Schedule*** Perform refocusing each 10th pass using Repeat process 100 times with 10 seconds delay between passes. Capture and 4x3 XY montage storing all images of the same XY position in a separate file.