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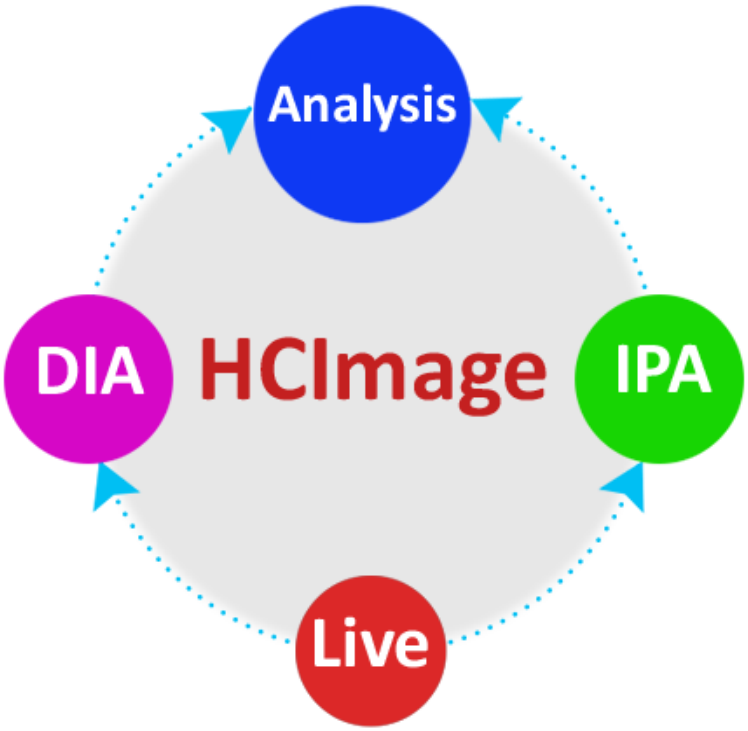
HCImage

Getting Started Guide



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INSTALLATION

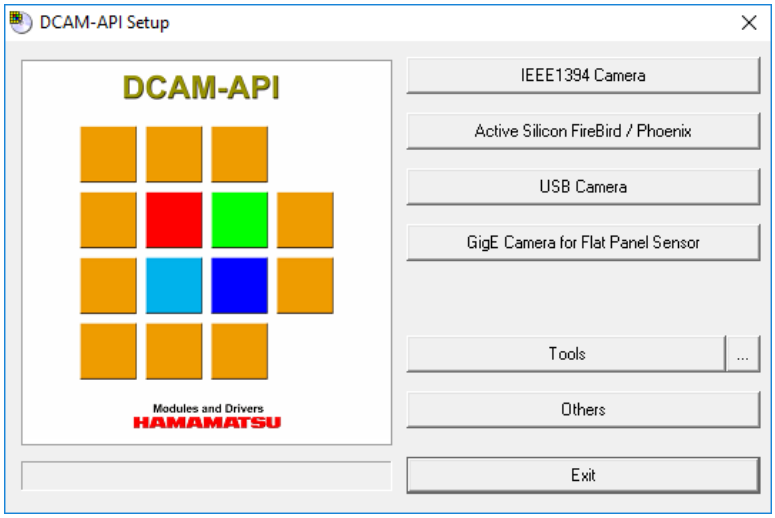
Install HCImage

1. Insert the HCImage installation DVD into the DVD-ROM drive. If autoplay is enabled, the HCImage setup will run automatically. If autoplay fails to start, locate your DVD-ROM drive and double-click on **setup.exe**.
2. Click **Yes**, if prompted by the User Account Controls.
3. To begin the installation wizard, click **Next**.
4. Review the Software License information and click **Yes**.
5. Review the README section for up-to-date information on software compatibility and support. When you are ready, click **Yes**.
6. On the Personalize screen, enter your registration information and click **Next**.
7. Choose the Destination Folder and click **Next**. It is recommended to install the software in the default path.
8. If you are ready to proceed with the installation, click **Install**.
9. Follow the instructions on each installation page.
10. Securely connect the dongle () to a USB port after the software installation has finished.
11. Install the appropriate DCAM-API drivers, see the instructions below, then turn your camera on prior to launching HCImage.
12. Click the **HCImage** icon on your Desktop to launch HCImage.
13. Register the software to receive technical support, please go to www.hcimage.com and click **Register**.

Install DCAM-API Drivers

Before installing the camera driver, make sure that the camera is turned off.

1. Open Windows Explorer, go to HCImage installation DVD, expand the **Drivers folder**, open the **Cameras folder** and open the **DCAM folder**. If you downloaded HCImage, please go to http://www.dcam-api.com/ and download the DCAM-API drivers for Windows.
2. Double-click **Setup.exe** to launch the DCAM-API Setup dialog.
3. Click **Yes**, if prompted by the User Account Controls.
4. Select the appropriate driver for your Hamamatsu camera from the DCAM-API Setup dialog. If you are unsure of which driver to install, please consult the DCAM-API Compatibility Note or contact your local Hamamatsu representative. To view DCAM-API Compatibility Note, select **Others** and then click **Compatibility Note**.



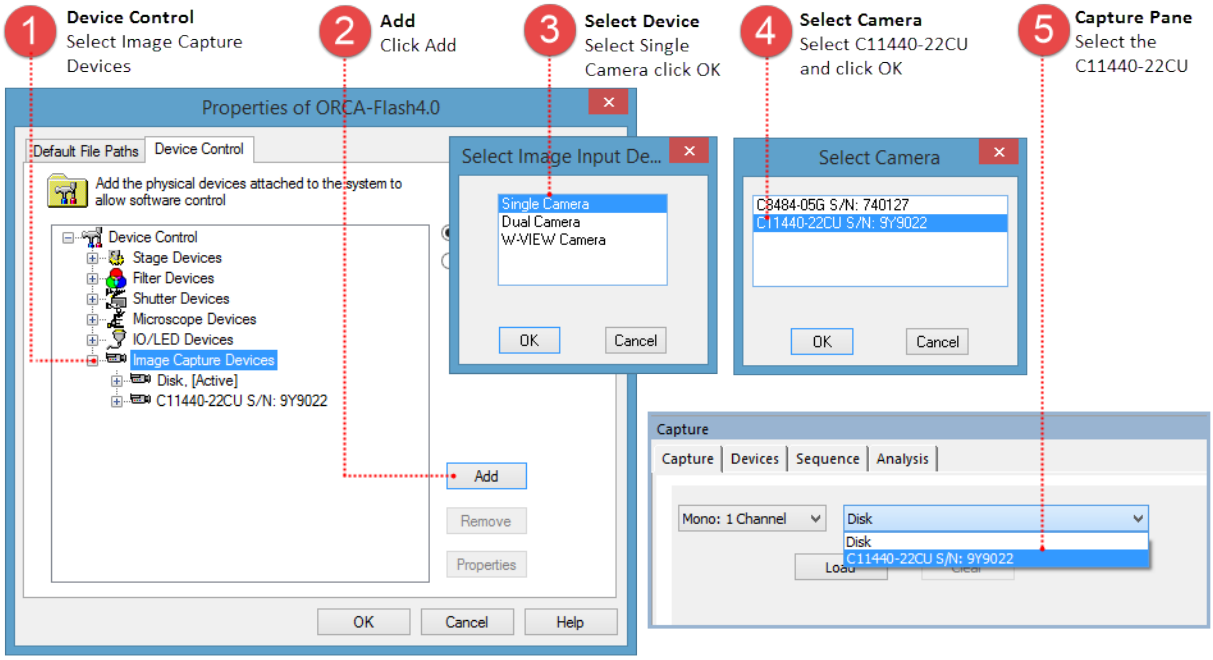
5. Click **Next** to begin the installation.
6. Follow the instructions on each installation page and click **Finish** when the installation is complete.

ADD DEVICES TO A PROFILE

Add and setup peripheral hardware devices to the profile. Supported hardware includes: cameras, stages, filters, shutters, I/O and microscope devices. For a list of supported devices, please visit our website at http://hcimage.com/support/hardware.htm.

Add a Camera

Launch HCImage, go to File, select Current Profile and then follow the steps below to add a camera to the profile.



Add an Olympus IX-83 Automated Microscope

Olympus 64-bit Drivers from the HCImage DVD

Be advised that this application installs the Olympus Camera and Microscope drivers, as well as copying all of the necessary dlls into the HCImage directory.

1. Open the DVD contents in Windows Explorer and navigate to Drivers\Microscopes\Olympus\Olympus 3 Series\x64.
2. Double-click on **Olympus_x64 Install.exe** and follow the installation instructions.
3. Click **Yes**, if prompted by the User Account Controls

Note: If using a Hamamatsu 1394 camera, this driver installation may supersede the Hamamatsu driver causing communication problems. To recover from this issue, please see "**Unable to communicate with Hamamatsu 1394 camera**" on page 1.

Configure with the Touch Panel Controller

The microscope drivers have been installed, time to configure it using the touch panel controller (TPC) and then add it as a device in HCImage. The first step is to turn on the IX3-CBH (microscope control box) and then the touch panel controller.

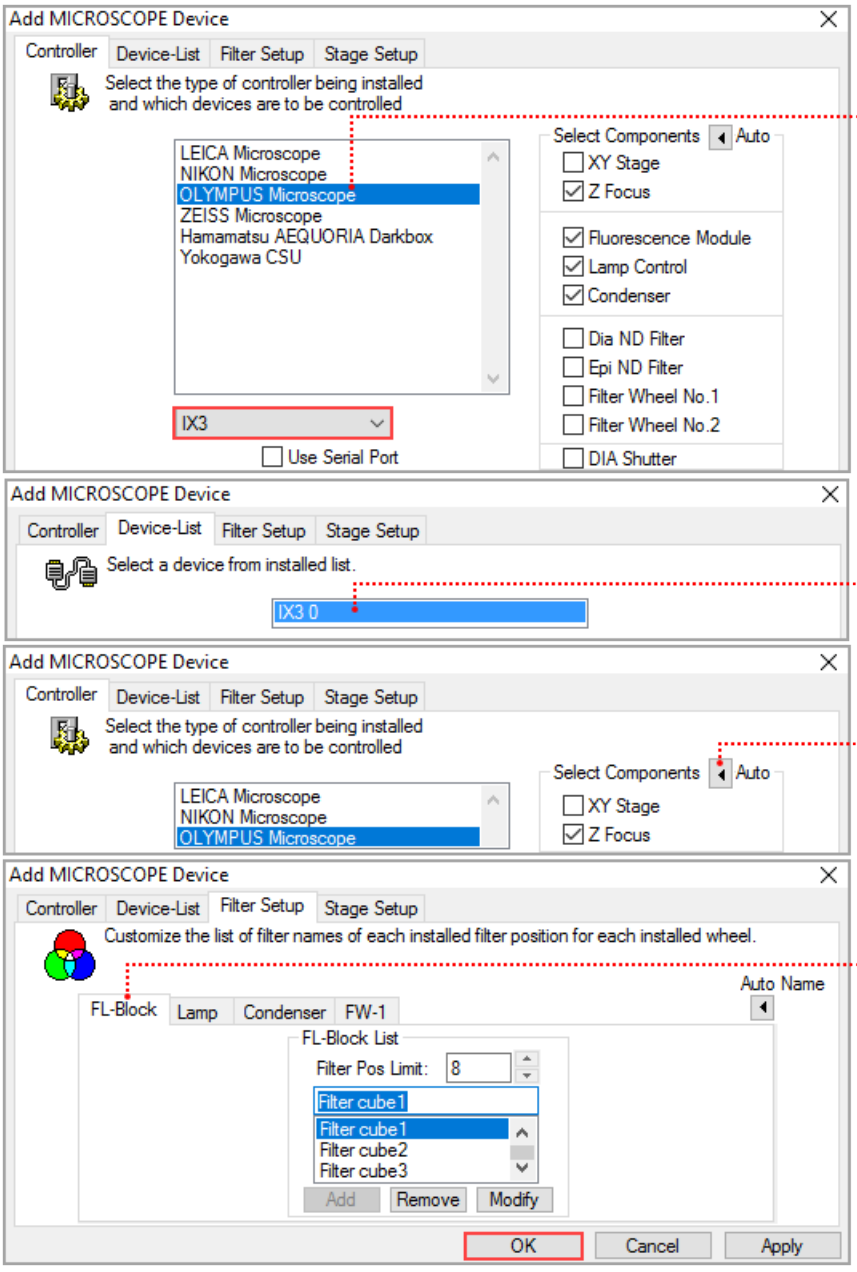
Note: The "Power On" sequence for turning the equipment on before use should be: Light Source > PC > Camera > IX3-CBH > Touch Panel Controller > Launch HCImage.

An initial system setup is required when using the microscope for the first time or after replacing one of the components. The microscope is setup and configured using the TPC.

1. Go to **System Setting** in the **Menu** screen.
2. Select **Unit**, enter the components connected to the IX83 for each module and tap **OK** to save the settings.
3. Select **Optical**, enter and configure the objectives, mirror units and condenser.
4. Select **Customized**, enter the focus limits and parfocality correction.
5. When the setup is complete, tap **X** to exit to the **Menu** screen.

Add Microscope to a Profile

Once the microscope has been setup from the touch panel controller, the next step is to add the microscope to a profile and configure it in HCImage. Launch HCImage, go to File and select Current Profile. In the Device Control tab, select Microscope Devices and click Add.



1 Device Control
Select the microscope manufacturer and the model

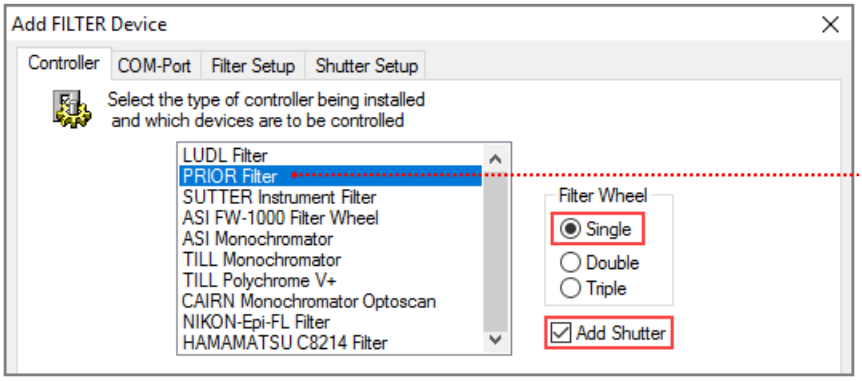
2 Device List
Select the device that is listed

3 Device Control
Select Auto and HCImage will update the components list

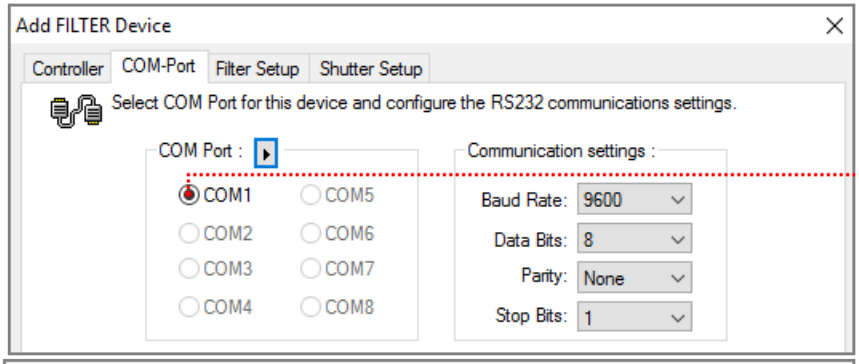
4 Filter Setup
Verify the settings for the individual components, modify if necessary and click OK

Add a Filter Wheel and a Shutter

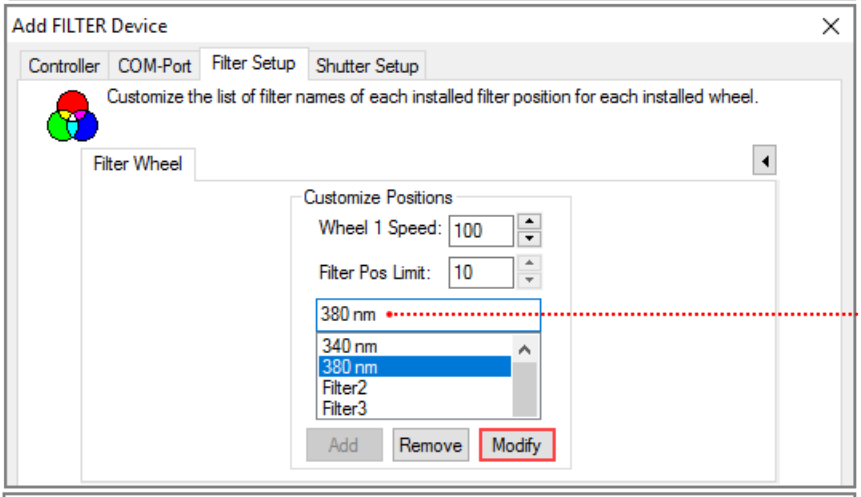
Launch HCImage, go to File and select Current Profile. In the Device Control tab, select Filter Devices, click Add and follow the instructions below.



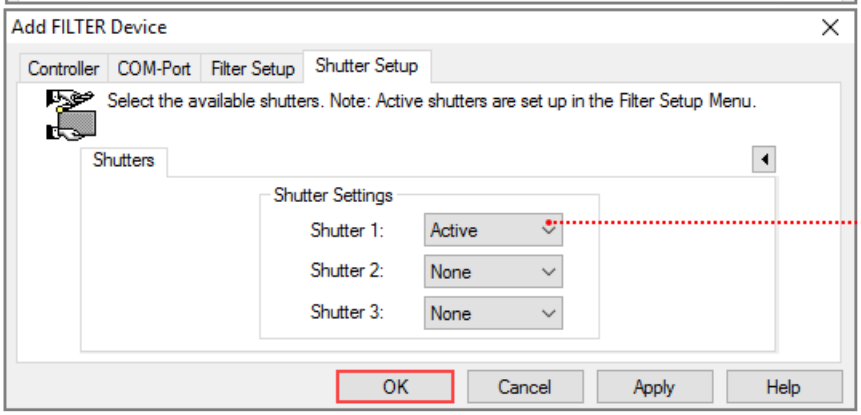
1 **Device Control**
Select the controller, the type of filter wheel and enable Add Shutter



2 **Enable COM Port**
Select the COM Port for the device



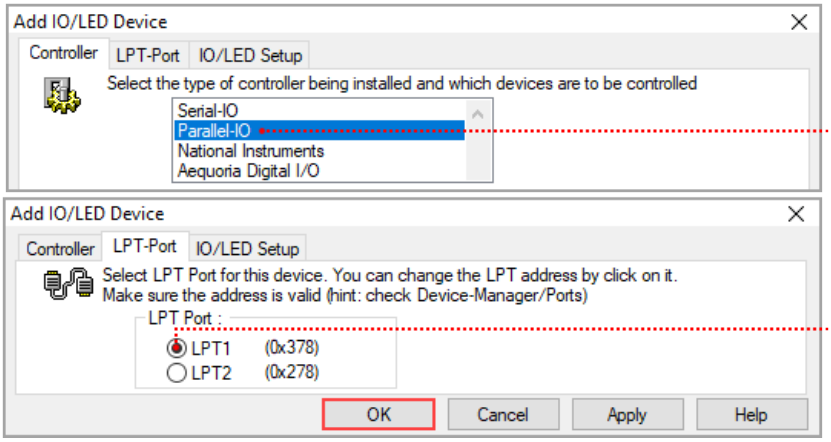
3 **Filter Setup**
Select the filter position, enter the name and click Modify. Repeat for each filter position



4 **Shutter Setup**
Set the status to Active and click OK

Add a Parallel Port as an IO/LED Device

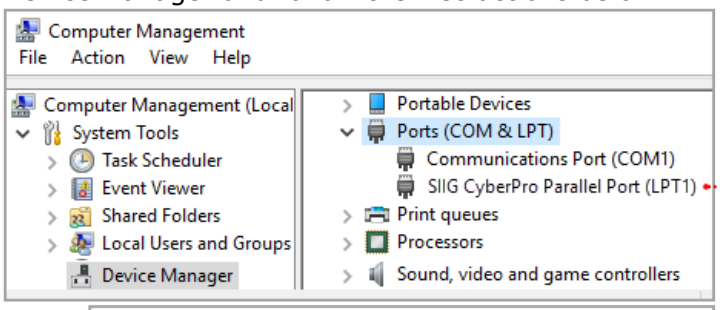
In the Device Control tab, select IO/LED Devices and follow the instructions below.



1 **Device Control**
Select Parallel-IO

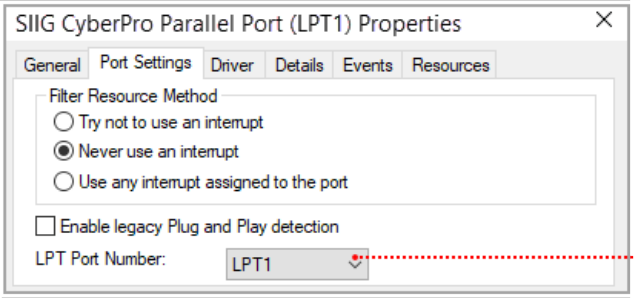
2 **Enable LPT Port**
Select LPT1 and click OK

If the computer doesn't have a parallel port, we recommend the SIIG CyberParallel PCIe port card (http://www.siig.com/it-products/serial-parallel/parallel/pcie/dp-cyberparallel-pcie.html). Install the parallel port card and driver as per the instructions provided with the card and then launch the Device Manager and follow the instructions below.

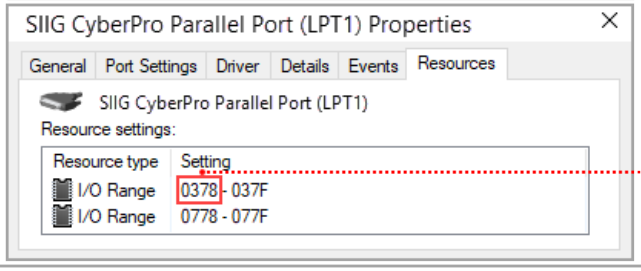


1 **Find the SIIG Parallel Port**
Select Device Manager, expand Ports (COM & LPT) and the SIIG card should be listed below.

2 **SIIG Parallel Port Properties**
Right-click on SIIG CyberPro Parallel Port and select Properties.

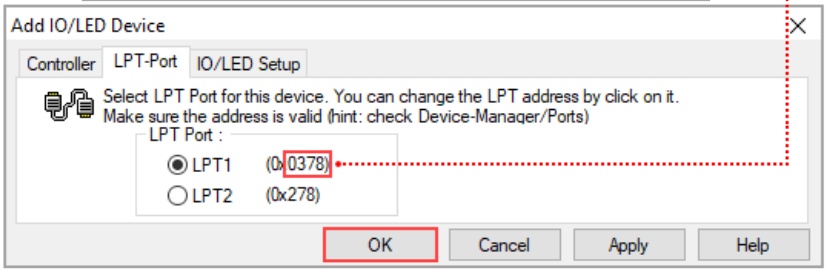


3 **Verify Port Settings**
Select the Port Settings tab, the LPT Port Number should be LPT1 if there are no other LPT ports on the system or LPT2 if there is an on-board parallel port. If there are no other LPT ports on the system and the and the card is not listed a LPT1 or LPT2, select LPT1 from the list.



4 **Copy Resource Settings**
Select the Resources tab, highlight and copy the first four digits under Settings.

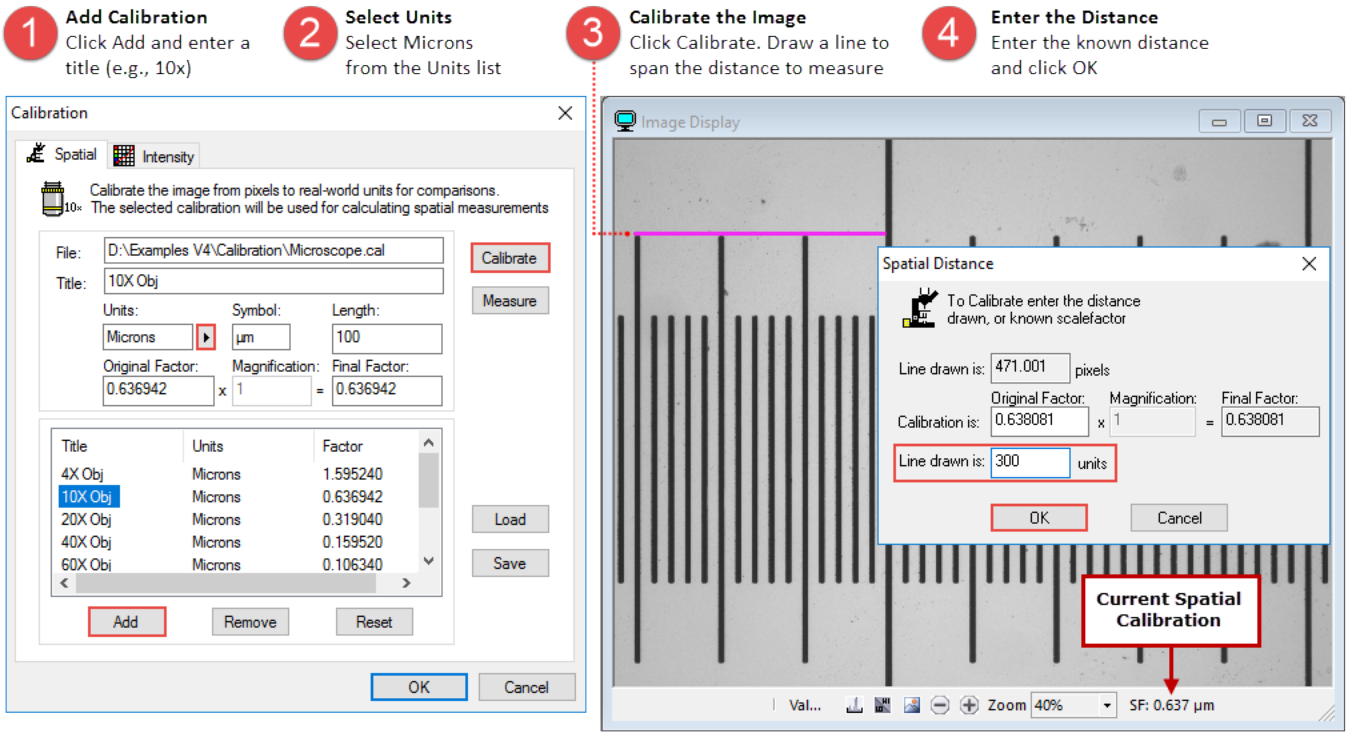
5 **Add LPT Port to a Profile**
Launch HCImage, go to File, select Current Profile, click Device Control, select IO/LED Devices and click Add. Select Parallel I/O, go to the LPT Port tab and select the LPT Port for the SIIG card. Highlight the last three digits after 0x and paste the content. Click OK.



CALIBRATION

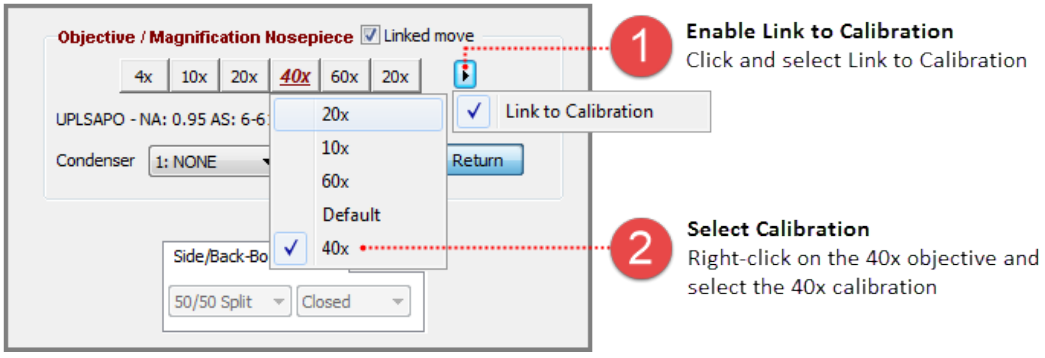
Calibrate an Image from Pixels to Microns

Open or capture an image with some known distance, for example a micrometer. Click on the Calibration Properties icon (Calibration) on the Analysis toolbar and follow the instructions below.



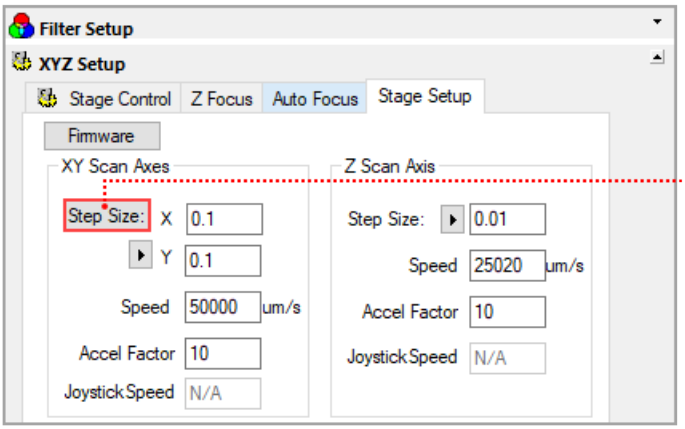
Link Calibration to Objective

To link the calibration to an objective, go to the Microscope Setup panel in the Devices pane and follow the steps below.

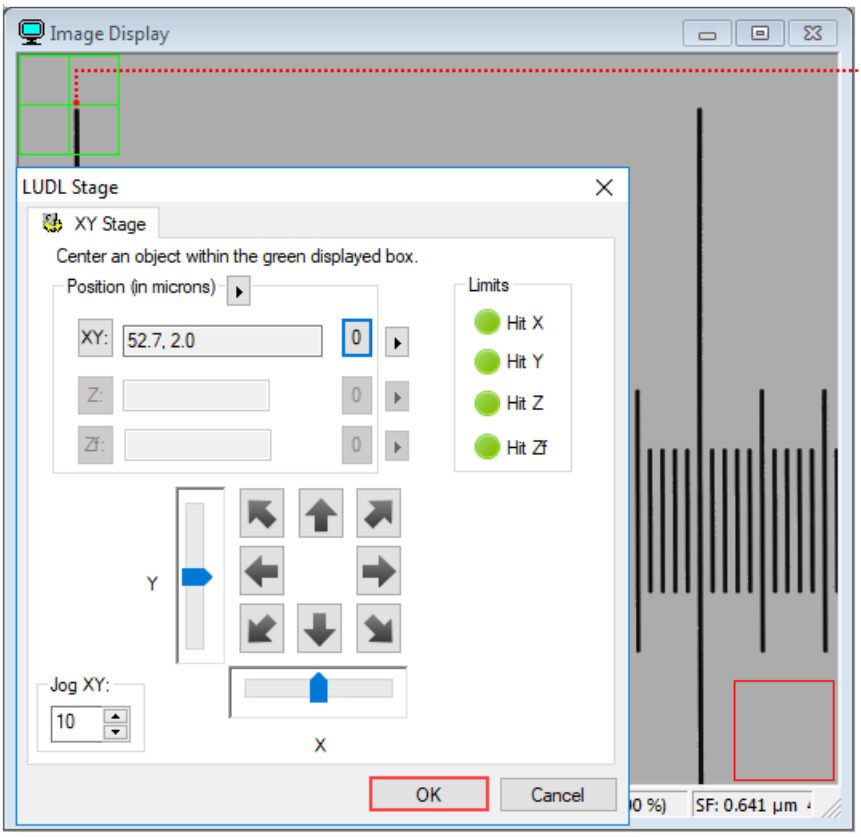


Calibrate a Stage

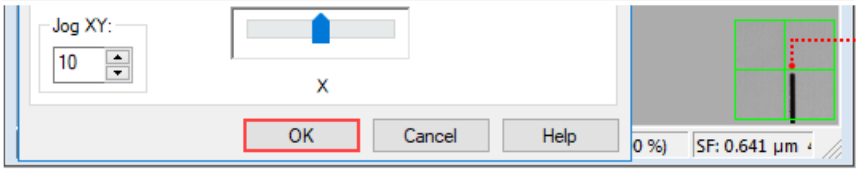
Before calibrating the stage, make sure to load the correct scale factor for the selected objective and then follow the instructions below.



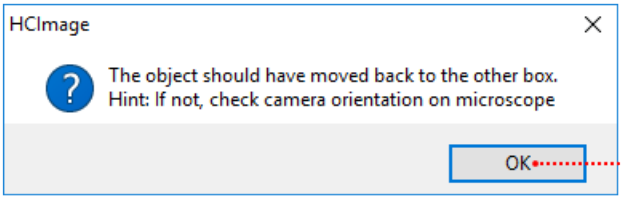
1 Define Step Size
Go to the Stage Setup tab, located in the XYZ Setup panel and click Step Size



2 Center Top Left
Center an object to the green crosshair and click OK



3 Center Bottom Right
Center the same object to the green crosshair and click OK



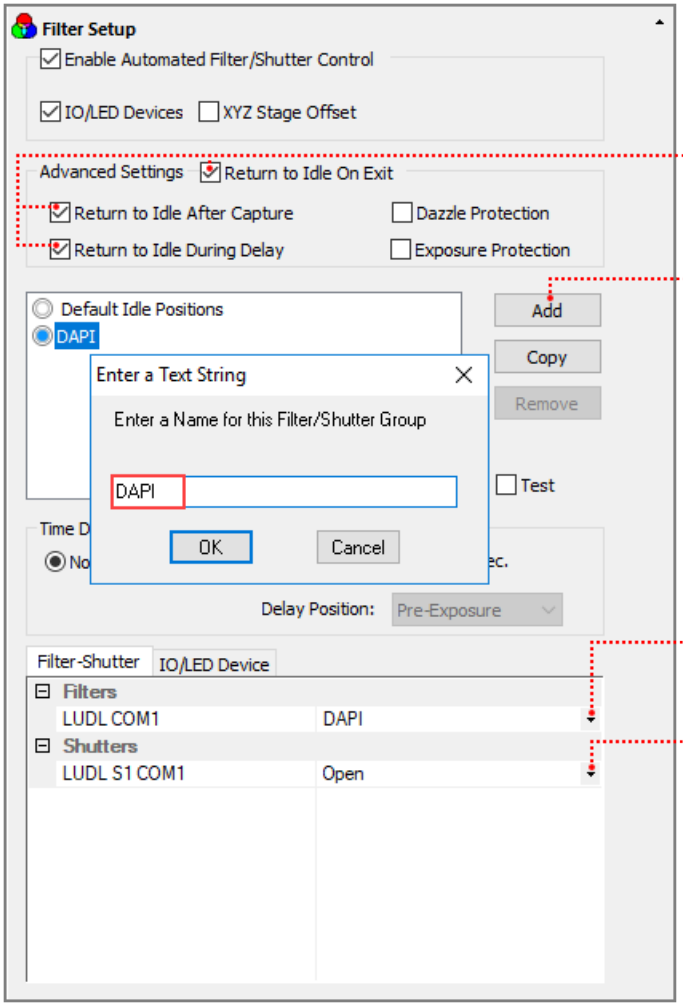
4 Object Centered
If the object moved back to the center of the top left crosshair, the calibration was successful, click OK. If the object did not move to the same location, check the camera orientation to the microscope and repeat Steps 1-3.

FILTER SETUP

Once the filter device has been added to the profile it will need to be configured in the Filter Setup. The examples below outline the basic steps for configuring two commonly used filter devices, a filter wheel with a shutter and a Lambda DG-4.

Filter Wheel and Shutter Setup

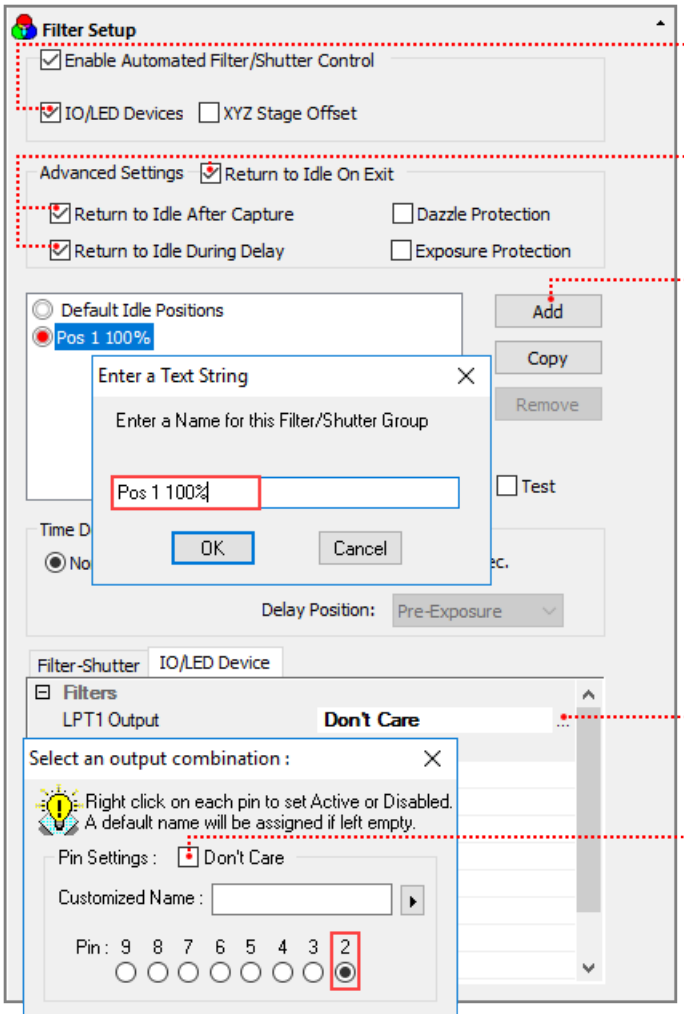
After the filter wheel and shutter have been added to the profile, go to Filter Setup in the Device pane and follow the instructions below.



1. **Enable Return to Idle Conditions**
   Select Return to Idle on Exit, After Capture and During Delay
2. **Add Filter Group**
   Click Add, enter name and click OK
3. **Enable Filter Settings**
   Right-click on the filter group that was just created and select a filter tint
4. **Select Filter Position**
   Select the filter from the list
5. **Define Shutter Setting**
   Select Open
6. **Add Remaining Filters**
   Repeat the steps to add the remaining filters
7. **Define Default Idle Settings**
   Select Default Idle Positions, under Filters select Don't Care and for Shutters select Closed

Lambda DG-4 Filter Setup as an I/O Device

TTL can be used to control many types of devices. This example explains how to configure a Lambda DG-4 as an I/O Device controlled through the parallel port. In the Device pane go to Filter Setup and follow the instruction below.



1 Enable IO/LED Devices
Select IO/LED Devices

2 Enable Return to Idle Conditions
Select Return to Idle on Exit, After Capture and During Delay

3 Add Filter Group
Click Add, enter name and click OK

4 Enable Filter Settings
Right-click the filter group that was just created and select filter tint

5 Enable IO Pin Settings
Click ellipsis button

6 Define Pin Settings
Select Don't Care (un-check), enable Pin 2 and click OK

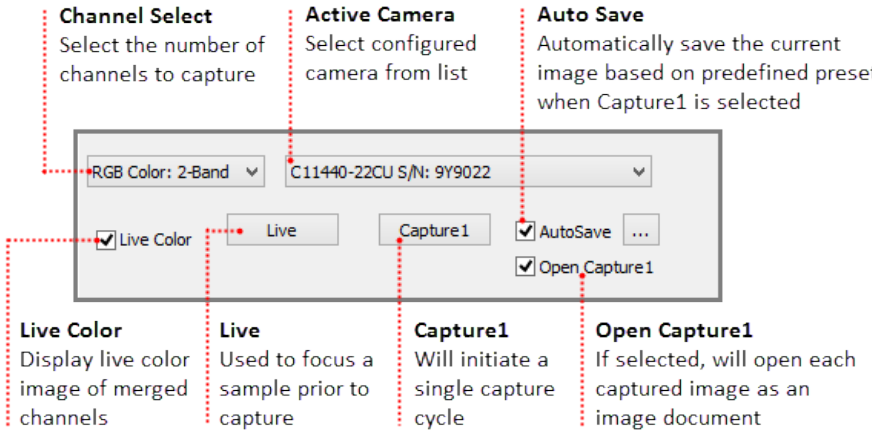
7 Add Remaining Filters
Repeat the steps to add the remaining filters and attenuations using the pin settings in the table below

8 Define Default Idle Pin Settings
Select Default Idle Positions, then for the pin settings select Don't Care (un-check) and click OK

Filter Position	Attenuation		
	100%	50%	33%
1	Pin 2	Pins 2 & 4	Pins 2 & 5
2	Pin 3	Pins 3 & 4	Pins 3 & 5
3	Pins 2 & 3	Pins 2, 3 & 4	Pins 2, 3 & 5
4	Pin 4	Pin 5	Pins 5 & 4

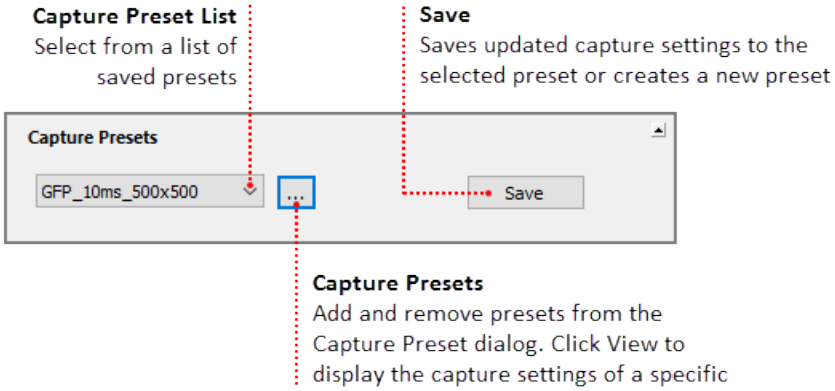
CAPTURE

The Capture Pane provides a flexible and comprehensive method to access camera features and functionality. The Capture Pane is organized by functionality into panels that can be expanded when in use or collapsed when space is needed. The capture controls at the top of the pane (shown below) are always visible and used for controlling how images are acquired and displayed.



Capture Presets

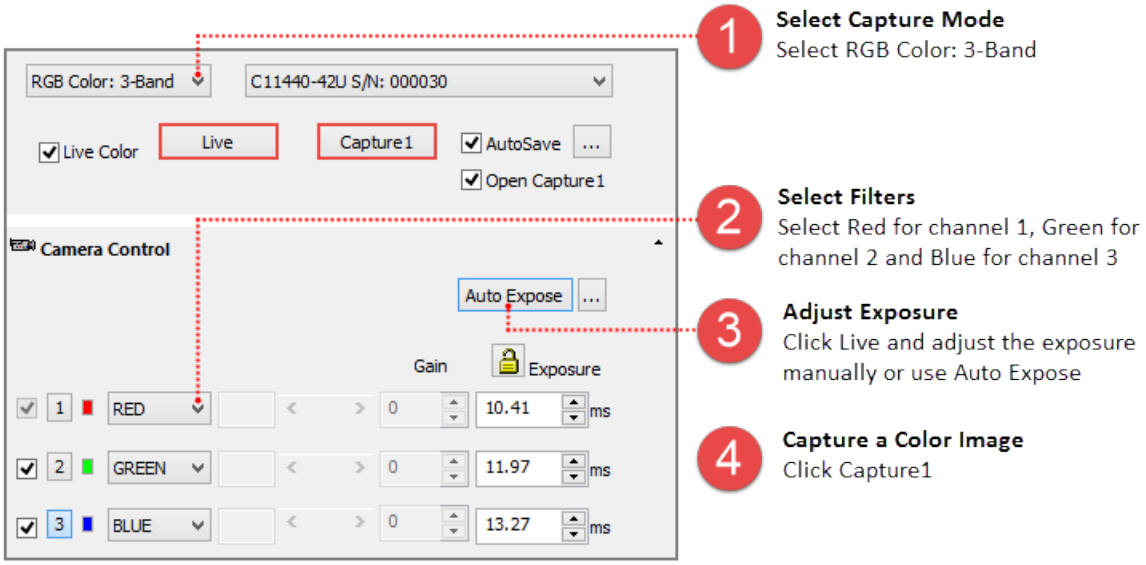
Capture presets save basic settings such as the capture mode, channels, filters, exposure times, as well as output trigger settings and advanced camera properties. For a list of the camera settings that are saved, select a capture preset from the Capture Presets dialog and click View. HCImage will load the capture settings from the previous session when launched.



Note: Capture presets are not automatically saved before changing presets or exiting the software. To make changes to a saved capture preset, select the capture preset from the list, adjust the capture settings and click Save.

Capture a Color Image

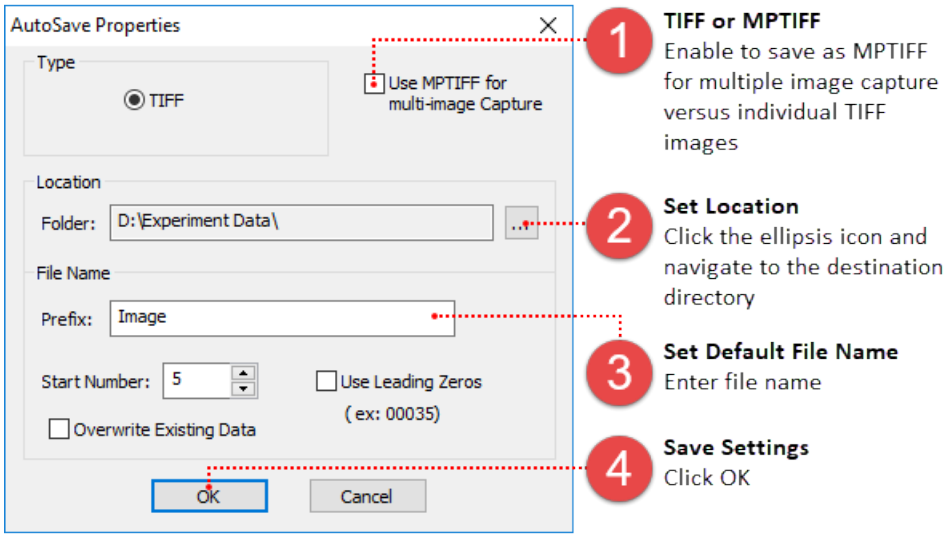
Capturing a color image requires filter setup, for instructions on configuring filters, please see "**Filter Setup**" on page 10.



Hint: In order to achieve the best possible speed when acquiring color images, set the same exposure for each channel. Once each of the exposures have been entered, click the Exposure Lock icon () to lock the exposure settings. Now any exposure adjustments will be made to all of the channels.

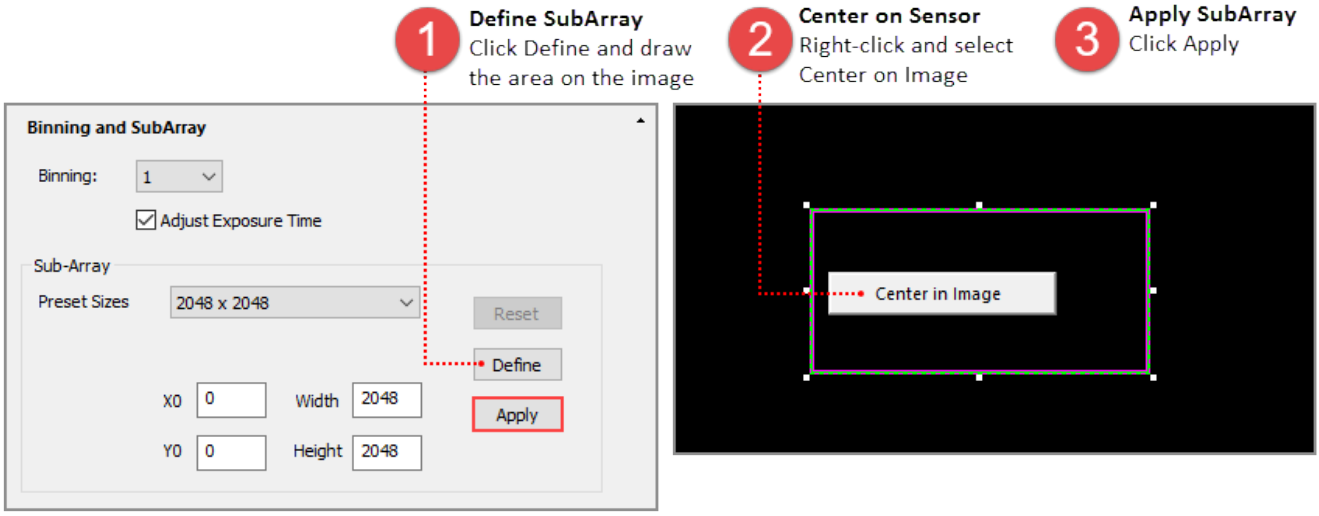
How to use AutoSave

Enabling AutoSave will automatically save the current image every time Capture1 is selected. The captured image is saved as a TIFF based on the file name and destination directory defined in the AutoSave Properties dialog. Enable AutoSave and then click on the ellipses to open the AutoSave Properties dialog.



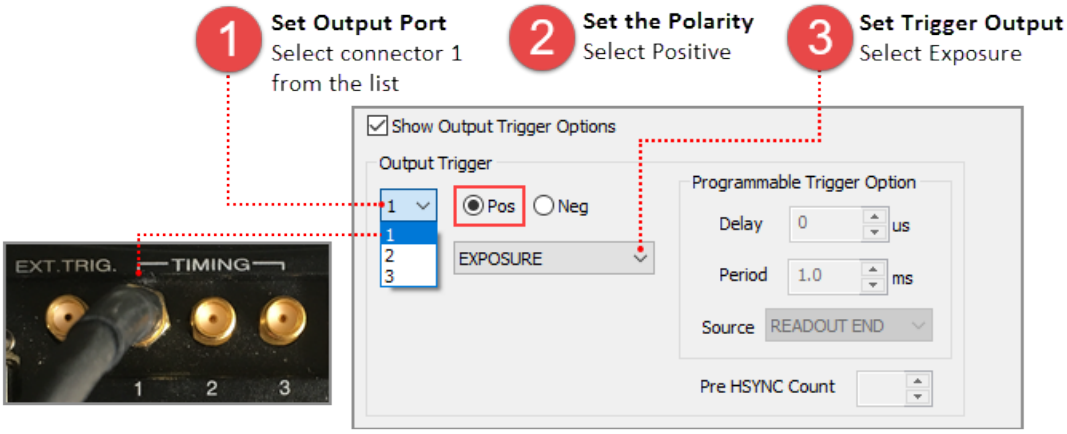
Define a Custom SubArray for Maximum Speed

Click Live, focus on the sample and move the area of interest into the center of the image. Follow the steps below to define a custom subarray.



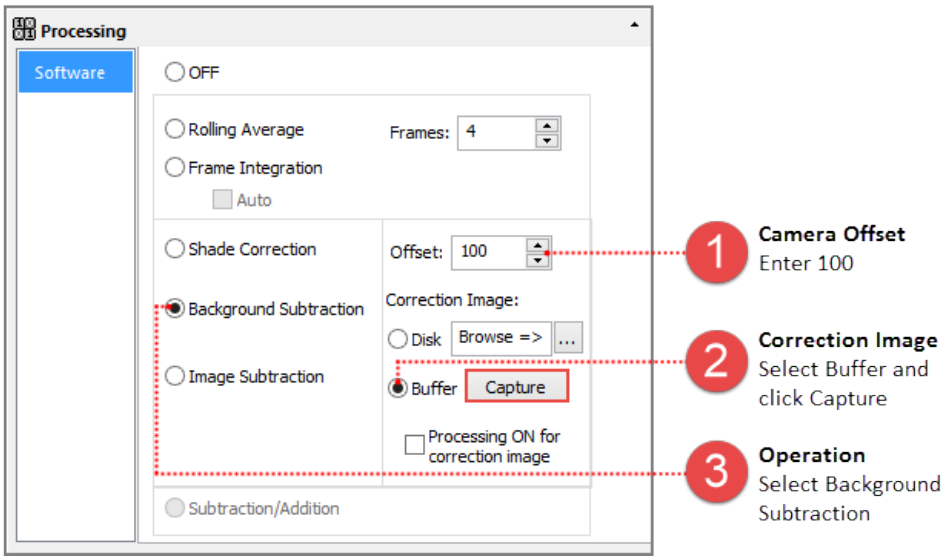
Control an LED using Output Trigger from the Camera

Some cameras provide a range of output trigger signals to synchronize with an external instrument where the camera becomes the master and the external instrument becomes the slave.

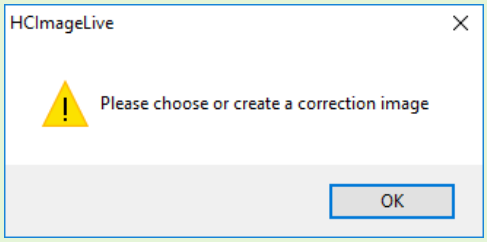


How to Setup a Background Subtraction

Typically used in fluorescence microscopy, a background subtraction can be used when the image presents a dark non-uniform background. To perform a background subtraction click Live, bring the sample into focus and then move the stage off of the sample so that only the background is visible. Next, follow the steps below, when finished move the stage to bring the sample into view and the background subtraction is applied.

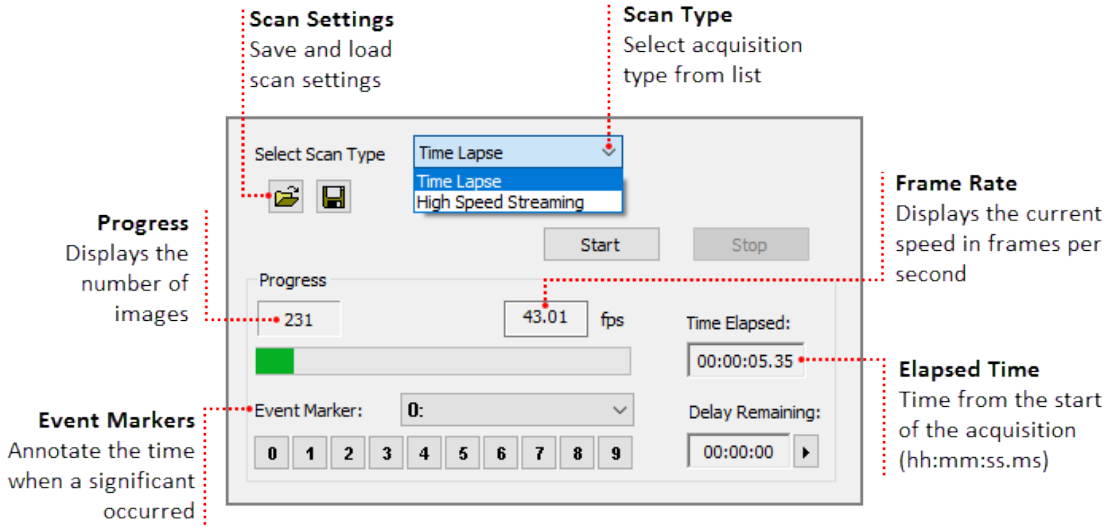


Hint: HCImage remembers the capture settings from the previous session, if background subtraction was left enabled, the following message will appear the next time HCImage is launched.



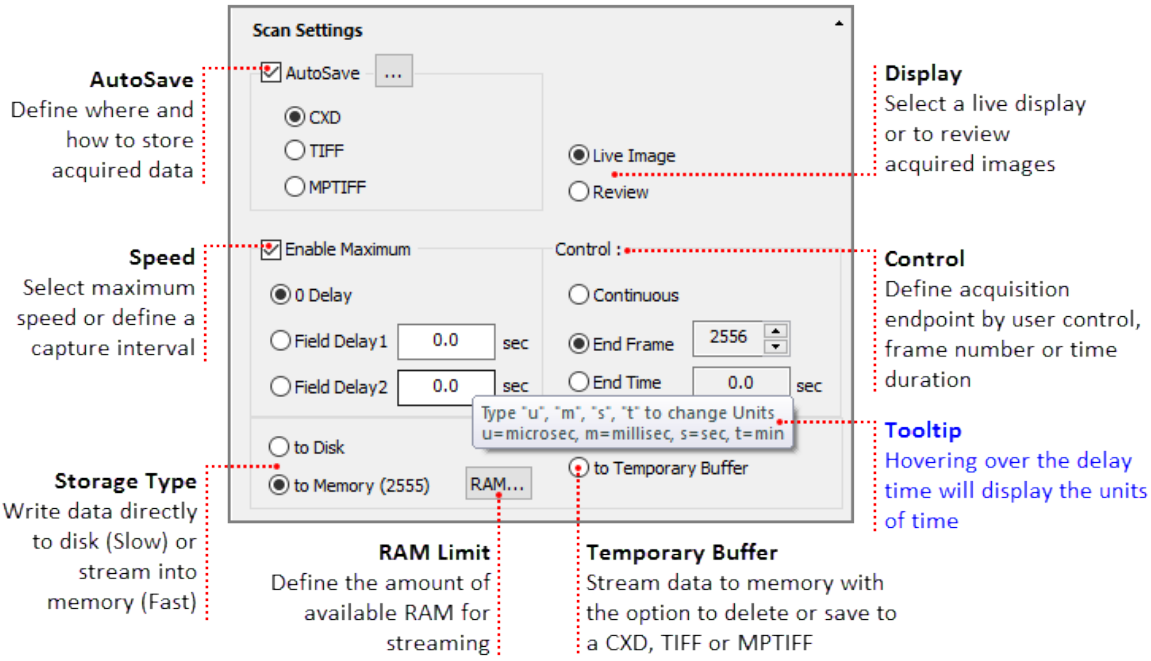
SEQUENCE

The Sequence pane provides a variety of options for defining a time lapse or high speed streaming. The sequence controls at the top of the pane (shown below) are always visible and used for selecting the scan type and reporting in real time, information about an ongoing sequence. This sections covers the basic steps for setting up a typical time lapse and high speed streaming.



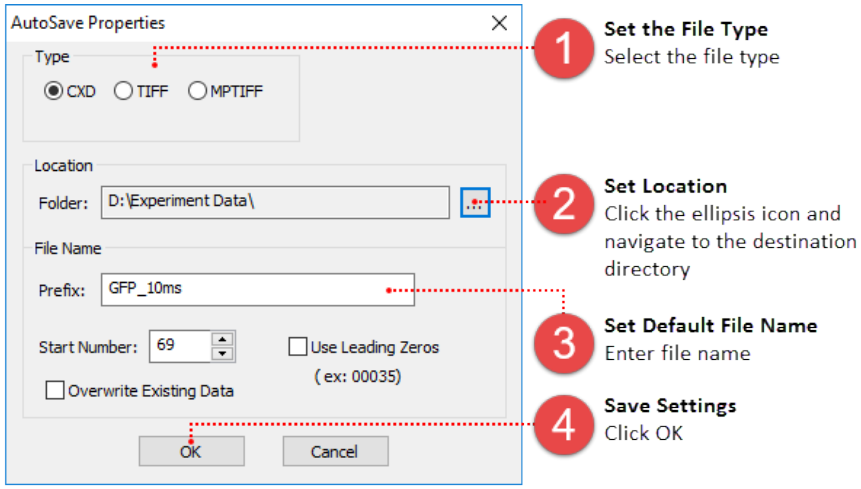
Setting up a Time Lapse

The Scan Settings panel provides a variety of options for defining a time lapse to fit the needs of your application. This section provides three examples of typical time lapse settings, using each of the storage options.



How to Use AutoSave

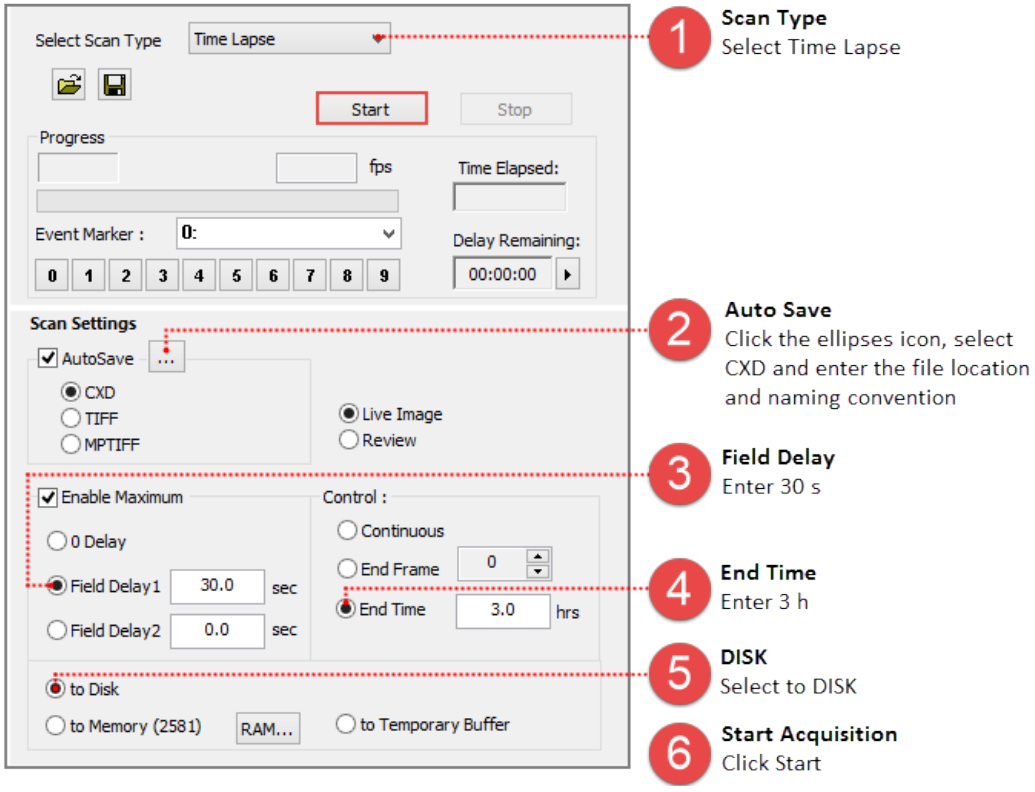
In the AutoSave Properties dialog, the user can determine how and where to store the acquired data. Image data can be saved as a CXD, TIFF or MPTIFF. The example below provides a description of the Auto Save Properties dialog.



Note: MPTIFF files have a 65,000 image limit or 4 GB size limit. For image sequences exceeding these limits, multiple MPTIFF files will be saved and numbered sequentially.

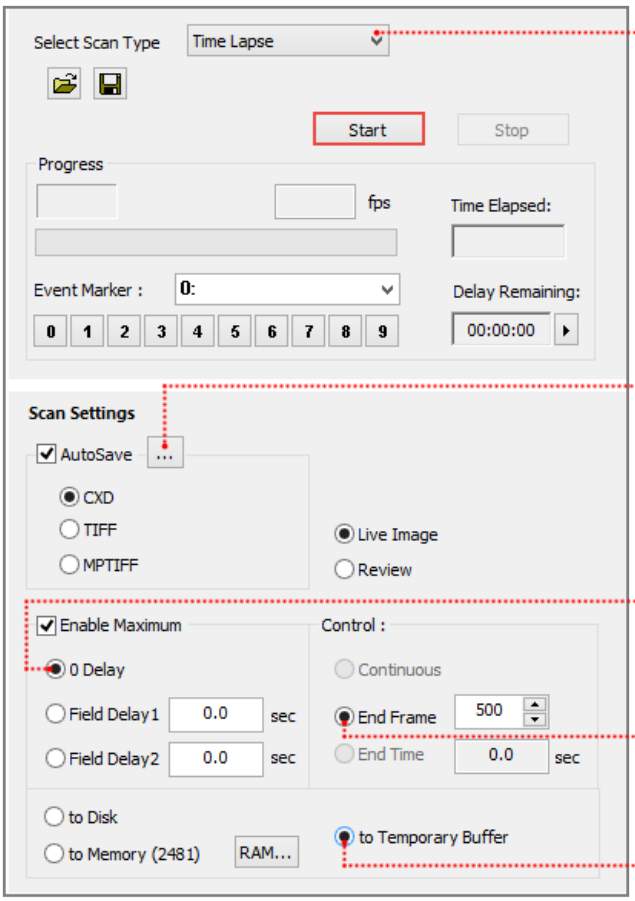
Setup a Time Lapse - Save to Disk

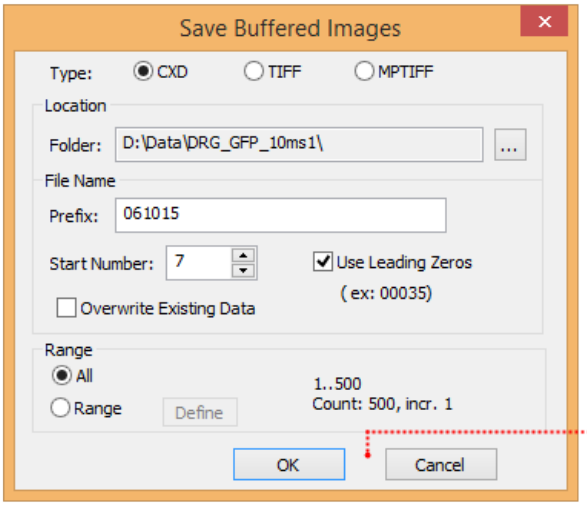
The time lapse in this example will acquire an image every 30 seconds for 3 hours and the data will be saved as a cxd. Once your are satisfied with capture settings and the sample is in focus, go to the Sequence pane and follow the steps below.



Setup a Time Lapse - Save to the Temporary Buffer

Acquired data is stored in memory with the option to review the image sequence before saving or deleting it. When Temporary Buffer is selected, End Frame is automatically enabled and display the maximum number of frames that can be streamed to memory. Once your are satisfied with capture setting and the sample is in focus, go to the Sequence pane and follow the steps below.



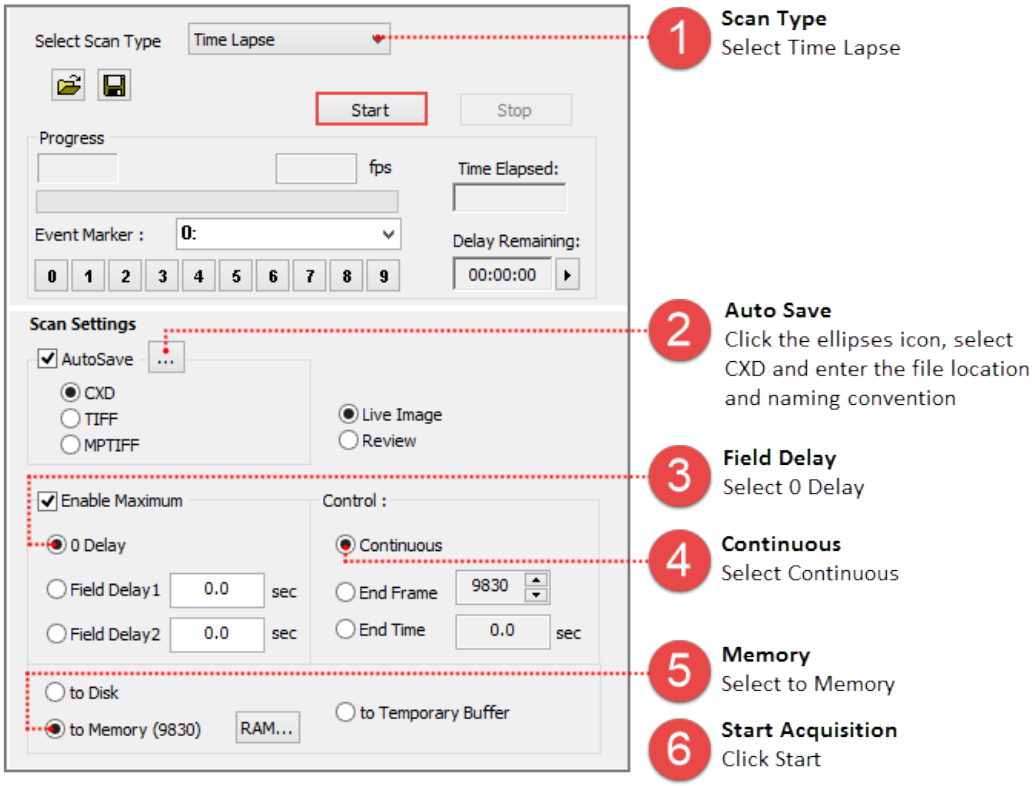


1. **Scan Type**
   Select Time Lapse
2. **Auto Save**
   Click the ellipses icon, select CXD and enter the file location and naming convention
3. **Field Delay**
   Select 0 Delay
4. **End Frame**
   Enter 500
5. **Temporary Buffer**
   Select to Temporary Buffer
6. **Start Acquisition**
   Click Start
7. **Acquisition Complete**
   Review acquired data using the playback controls in the Image Display
8. **Save or Delete**
   Save - click OK
   Delete - click Cancel

Note: Streaming to the Temporary Buffer is very useful because it provides the option to review the image sequence when trying to capture specific event and for demonstrating camera speeds.

Setup a Time Lapse - Save to Memory

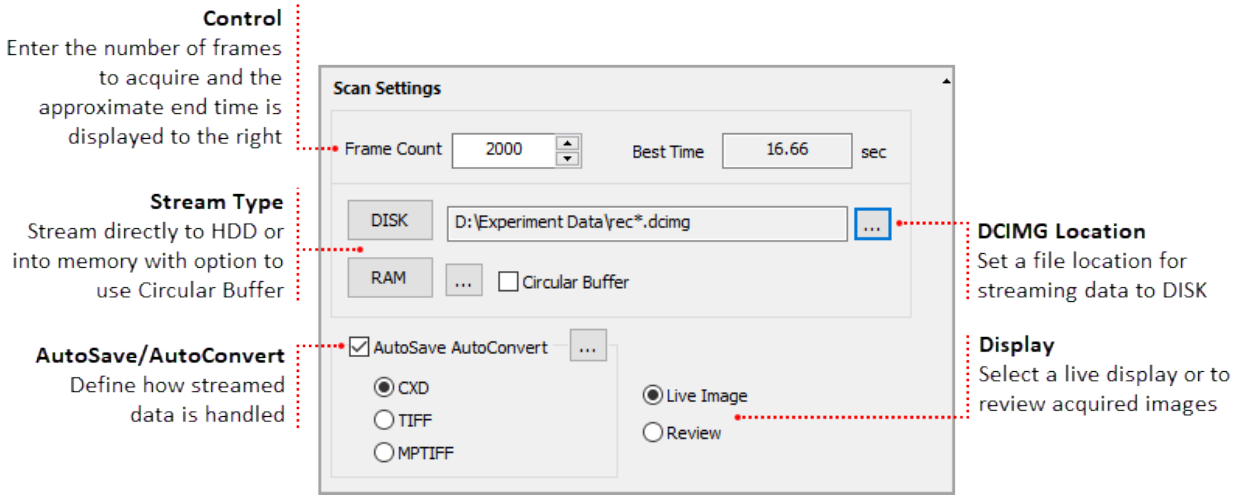
The time lapse in this example will store images in memory until the acquisition is stopped or runs out of memory at which point the acquired images are saved to disk for the remainder of the time lapse. Once your are satisfied with capture settings and the sample is in focus, go to the Sequence pane and follow the steps below.



High Speed Streaming

High Speed Streaming is used to obtain the fastest acquisition speed from the camera. This scan is optimized for single channel streaming to RAM or directly to the computer's solid state drives (SSD) configured in a RAID 0.

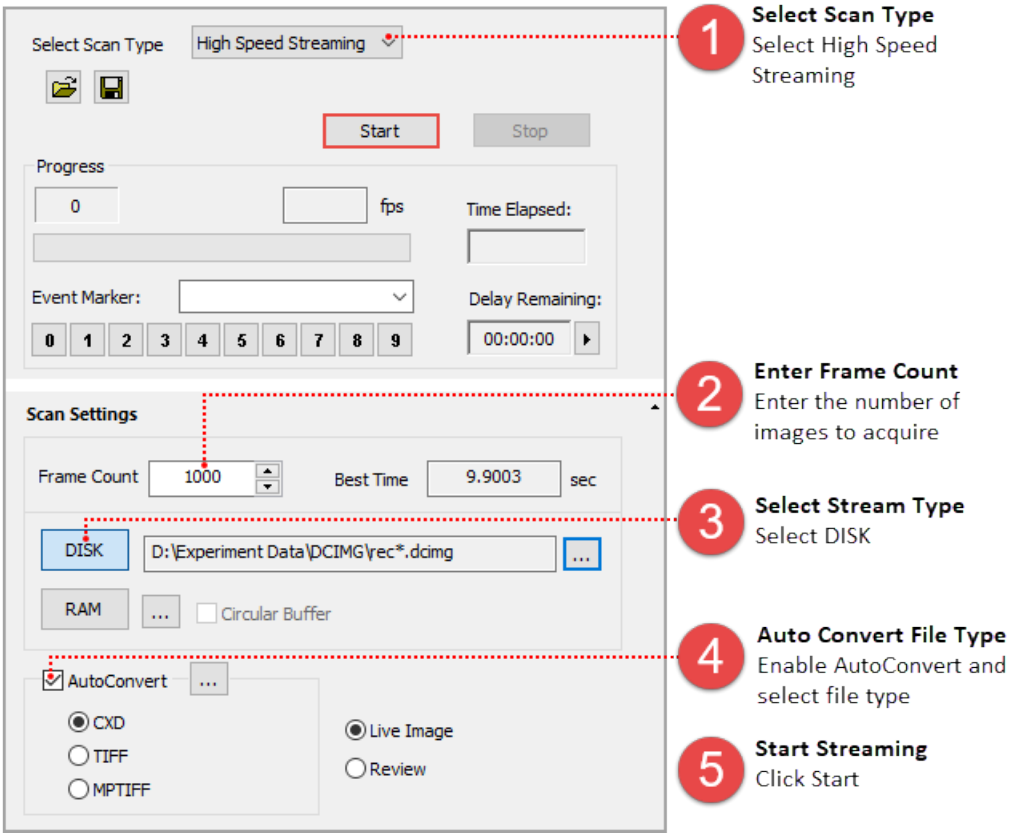
Note: Acquisition rates will vary based on the PC configuration, for information about the computer requirements, please see the PC Recommendations for ORCA-Flash4.0 V3 / LT+.



Note: High Speed Streaming does not support multi-channel acquisition, camera registration features (i.e., flip, rotation and pixel shift) or software processing operations (e.g., shade correction and rolling average).

Steps for Streaming to Disk

When streaming to disk, a temporary file (.dcimg) is created to store the data while it is being acquired, the temporary file location needs to be located on the RAID array, SSD drive, or the fastest drive available. Configure the capture settings, go to the Sequence pane and follow the steps below.



1 **Select Scan Type**
Select High Speed Streaming

2 **Enter Frame Count**
Enter the number of images to acquire

3 **Select Stream Type**
Select DISK

4 **Auto Convert File Type**
Enable AutoConvert and select file type

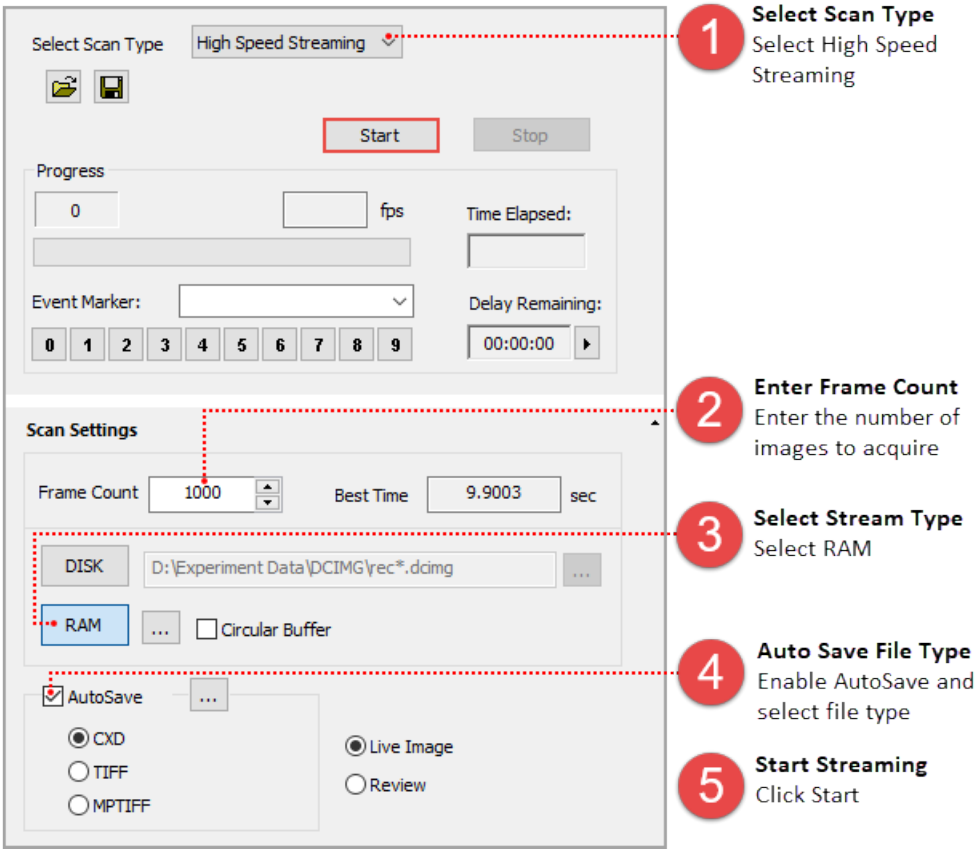
5 **Start Streaming**
Click Start

Note: To leave the streamed data as a DCIMG file disable AutoConvert.

Steps for Streaming to RAM

Acquired data is stored in memory with the option to review the image sequence before saving or deleting it. In the AutoSave Properties dialog, the user can determine how and where to store the acquired data. Once your are satisfied with capture settings and the sample is in focus, go to the Sequence pane and follow the steps below.

Note: The Circular Buffer stores streamed data in memory, once the frame count has been reached, the previous acquired data is replaced sequentially. The cyclic process repeats until the acquisition is stopped, leaving the most recent images stored in RAM.

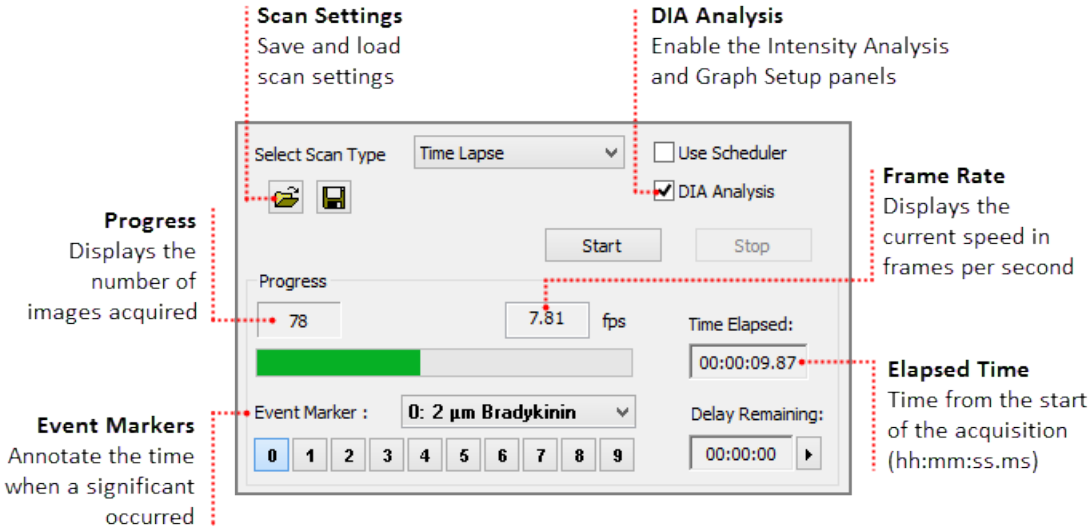


DIA OVERVIEW

Dynamic Intensity Analysis (DIA) is optimized for high speed processing and intensity analysis over time, including Live viewing of images and data simultaneously. Measuring and plotting of data is available on-line or off-line, and may be access by clicking DIA Analysis in the Sequence Pane. This functionality is only available in HCImage DIA and HCImage Analysis.

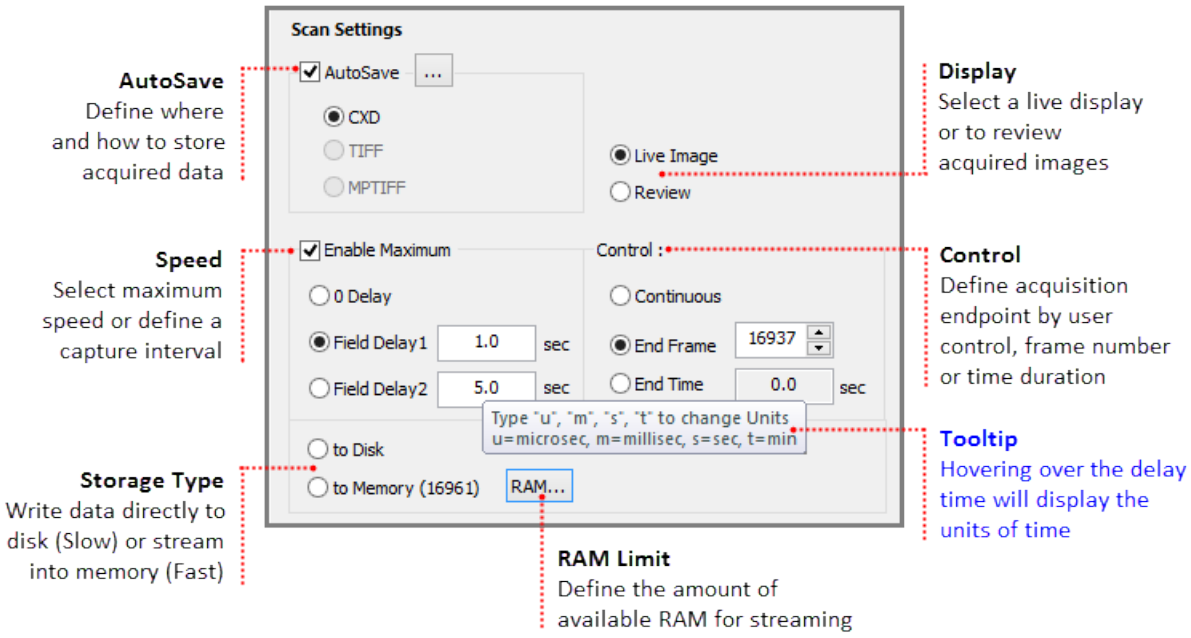
Understanding the Workspace

The Side Panel includes the Dynamic Intensity Analysis functionality, that is accessed through the Sequence pane by selecting DIA Analysis. Once enabled, the Intensity Analysis and Graph Setup panels are available, providing the tools to setup an experiment without having to switch panes.



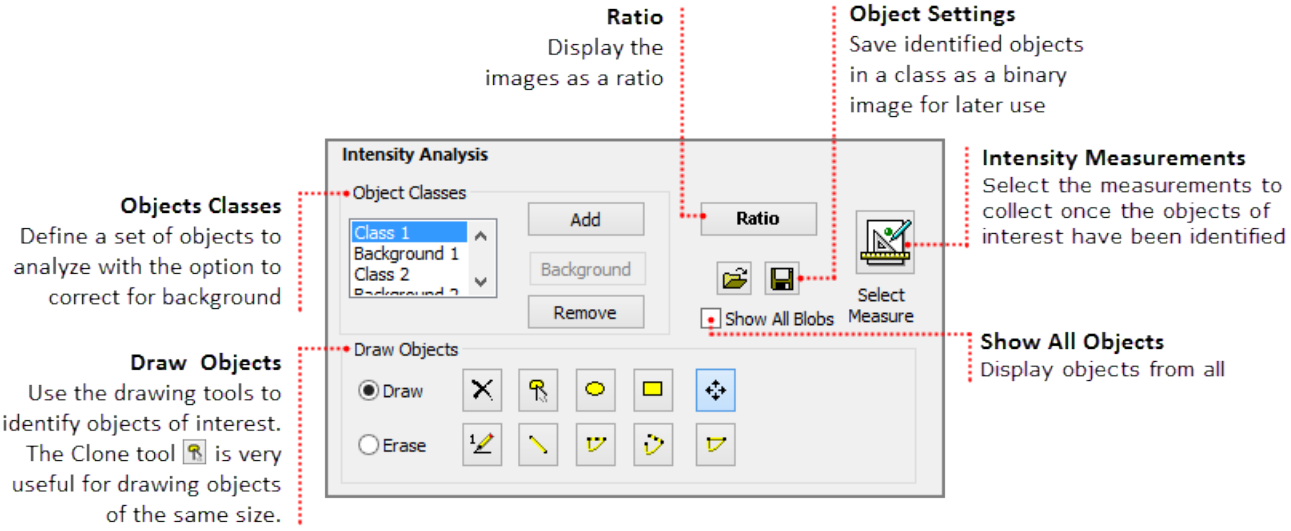
Scan Settings

The Scan Settings panel is easy-to-use, simply set the speed, define the capture interval, enter the number of images to capture and where to save the data.

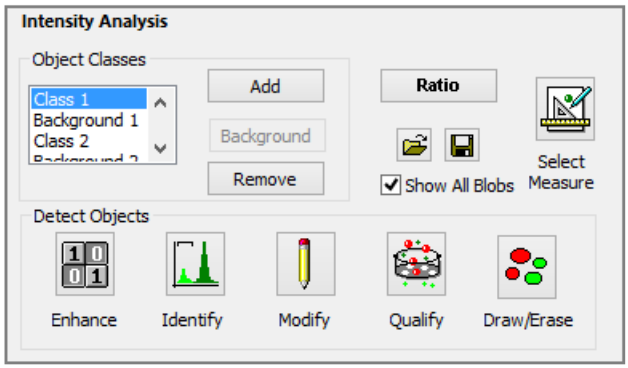


Intensity Analysis

The new Intensity Analysis panel is configured based on the selected Analysis mode: Simple or Advanced. The Advanced mode provides a comprehensive set of tools to help identify large numbers of objects and objects that are not easily differentiated. The Simple mode provides a variety of drawing tools that can be used to manually identify objects of interest.



To switch between the two analysis modes go to **View** on the menu bar, then highlight **Analysis Mode** and select **Advanced**.

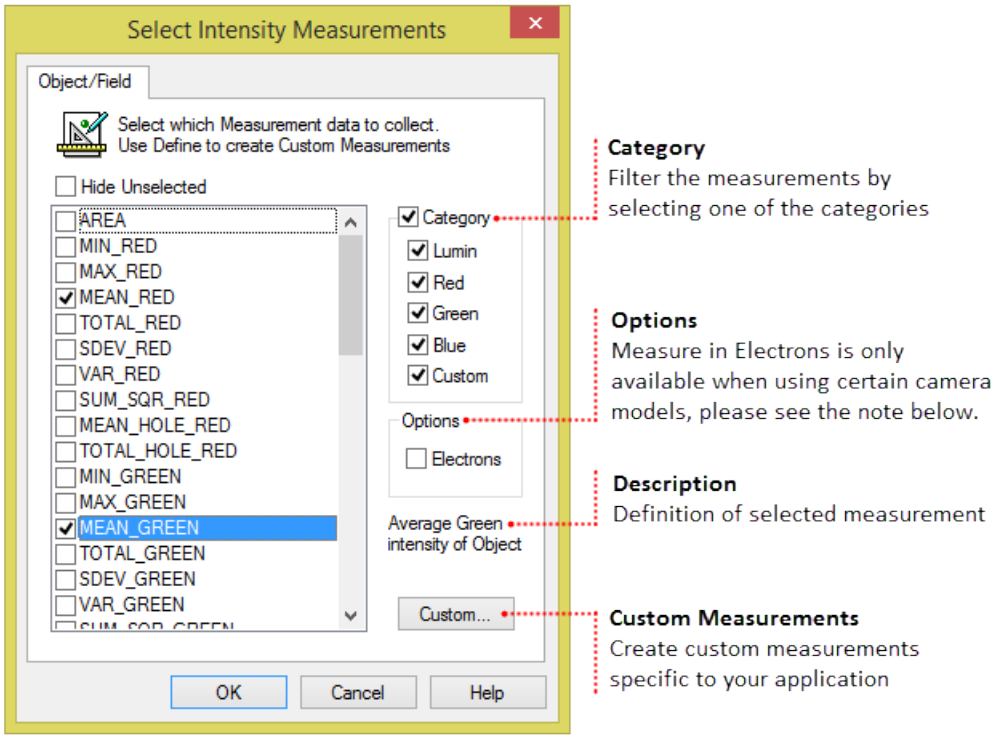


In the Advanced Mode, the user has the ability to identify a large number of objects, as well as, identify hard to detect objects because of defects in the image. The tools are grouped by function as described below:

- **Enhance** the image for detection by correcting for defects such as noise, dust, intensity variation, etc.
- **Identify** objects using an intensity threshold and create a binary image layer over the image.
- **Modify** the binary image layer, filling holes and separating connected objects.
- **Qualify** objects based on one or more measurement ranges and conditionally remove edge objects.
- **Draw/Erase** objects using a set of drawing tools.

Measure Objects

Intensity measurements are available in the Select Intensity Measurements dialog, click the Select Measurements icon to open the dialog. Select measurements by clicking the measurement check box to the left. Filter the view of the measurements by selecting one of the categories in the right. When correcting for background fluorescence, the corrected and uncorrected data for each of the selected measurements will be collected.

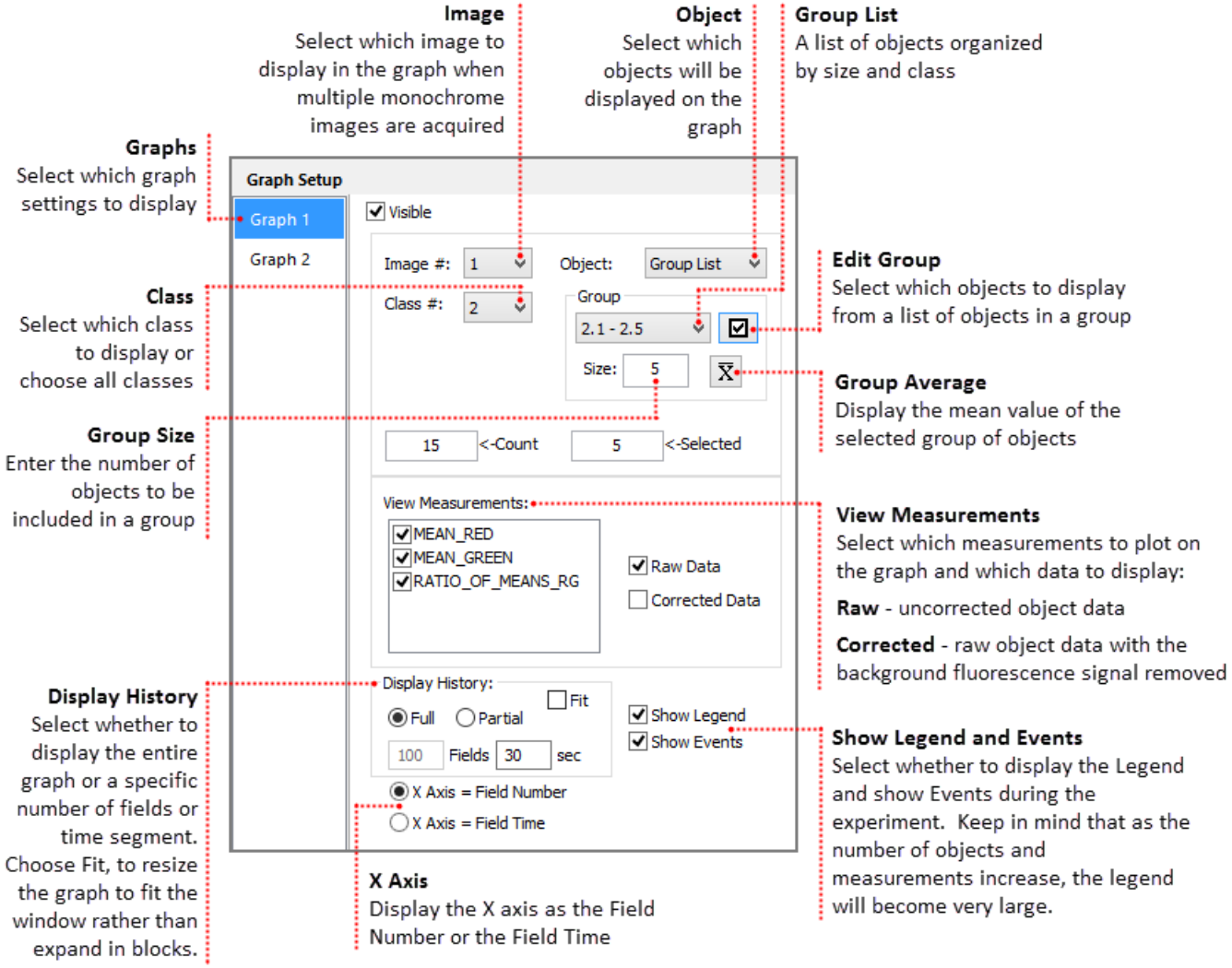


Note: When using the ORCA-Flash4.0 LT, ORCA-Flash4.0 V2/V3 or the ImagEM X2, select Measure in Electrons, to report the intensity measurement values in electrons. When Electrons is selected, measured data will ONLY be reported in electrons. Measurement names will be preceded by an "e" denoting the measurement is in electrons.

Custom measurements are available to deal with complex situations, use the built-in equation editor to apply standard measurements and mathematical functions to customize special measurements to suit specific applications. To create a custom measurement, click Select Measure and then click Custom to open the equation editor.

Graph Setup

The Graph Setup panel lets users decide the measurements that will be displayed during the experiment and how the graphs will be displayed. The user can choose to display the data from a single object, the average of all of the objects, or all of the objects. The data for all of the measurements in the View Measurements list will be collected and saved, regardless of whether they are graphed during the experiment. Also, keep in mind that depending on the number of objects and measurements selected, the graph will become very crowded and it may become hard to differentiate the objects.

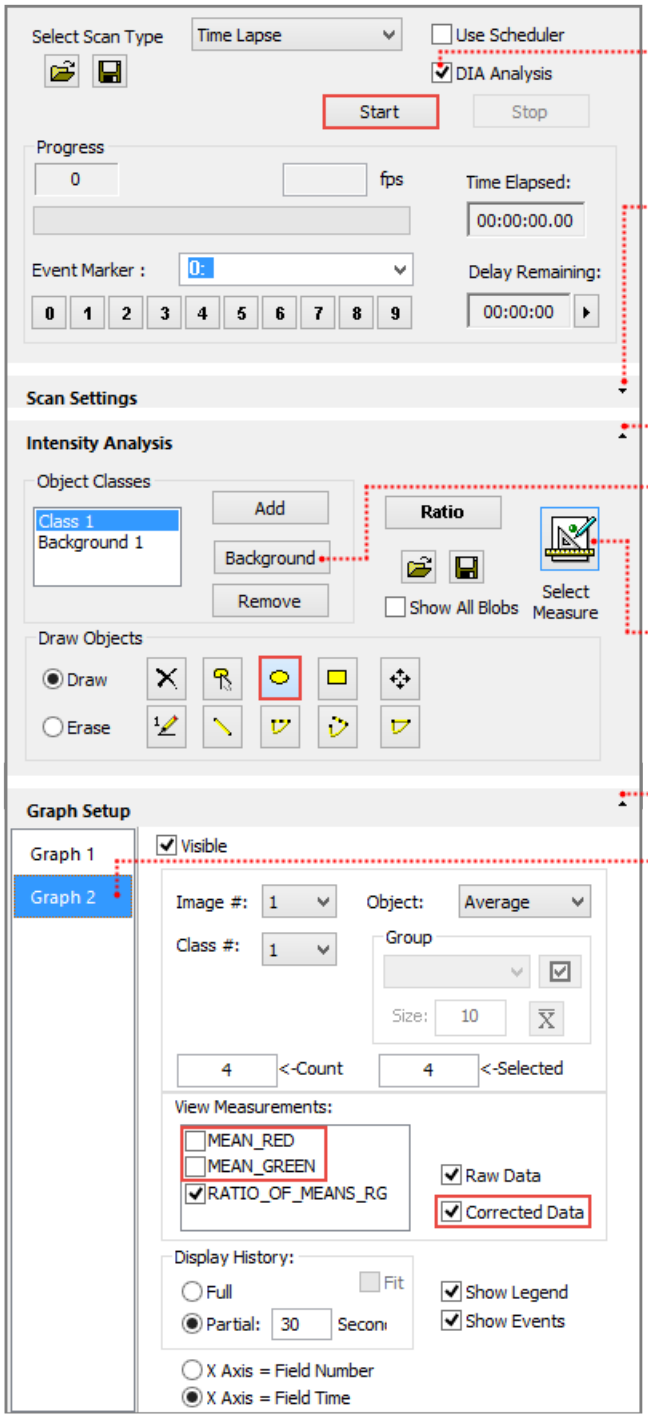


DIA ANALYSIS EXAMPLES

DIA Analysis can be run live, in real time, as well as on previously acquired data sets. Two examples are provided below, one for each type of situation.

DIA Example

The instructions below outline the steps for setting up a basic DIA Analysis experiment with a single class of objects and background correction. Configure the capture settings as needed for your sample. Two channel, Red and Green settings were used for this example.



1 **Enable DIA Analysis**
Select DIA Analysis

2 **Define Scan Settings**
Expand the Scan Settings panel, select Field Delay 1 and enter 5 s.

3 **Identify Objects of Interest**
Expand the Intensity Analysis panel, click on the ellipse icon and identify the cells of interest in the Image Display.

4 **Identify the Background**
Select Background, click on the ellipse icon and identify a background area in the image.

5 **Define the Measurements**
Click on the Select Measurements icon, select Mean Red, Mean Green, Ratio of the Means RG and click OK.

6 **Configure Graph 1 Settings**
Expand the Graph Setup panel and disable Ratio of Means RG.

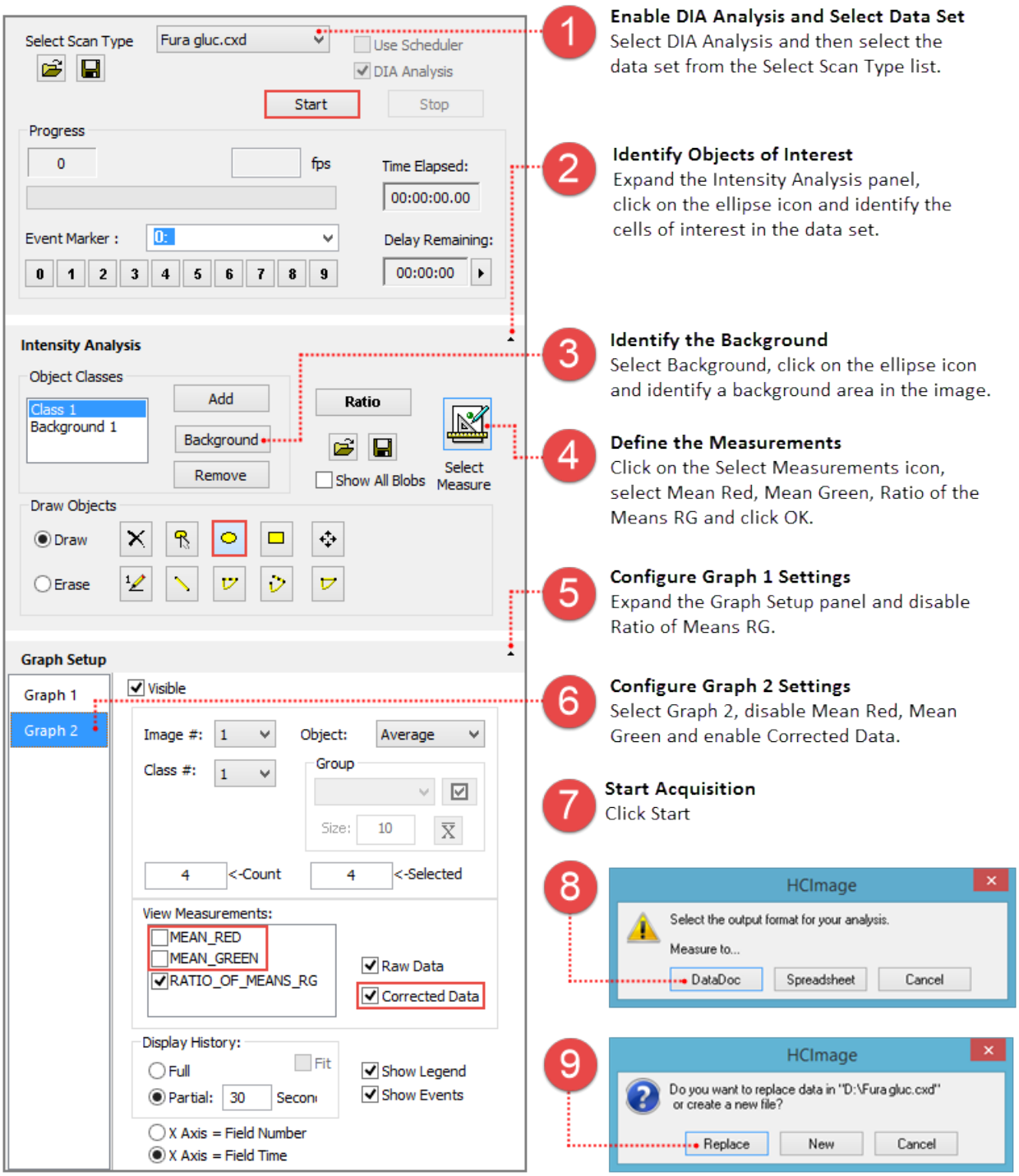
7 **Configure Graph 2 Settings**
Select Graph 2, disable Mean Red, Mean Green and enable Corrected Data.

8 **Start Acquisition**
Click Start

DIA Post Acquisition Example

DIA Analysis can be run on previously acquired image sequences. The example below includes a single class of objects with background subtraction. Open the data document to analyze, go to the Sequence pane and follow the instructions below.

Note: In addition to data documents (cxd), DIA Analysis can run directly from multi-page tiff and dcimg files.



1 Enable DIA Analysis and Select Data Set
Select DIA Analysis and then select the data set from the Select Scan Type list.

2 Identify Objects of Interest
Expand the Intensity Analysis panel, click on the ellipse icon and identify the cells of interest in the data set.

3 Identify the Background
Select Background, click on the ellipse icon and identify a background area in the image.

4 Define the Measurements
Click on the Select Measurements icon, select Mean Red, Mean Green, Ratio of the Means RG and click OK.

5 Configure Graph 1 Settings
Expand the Graph Setup panel and disable Ratio of Means RG.

6 Configure Graph 2 Settings
Select Graph 2, disable Mean Red, Mean Green and enable Corrected Data.

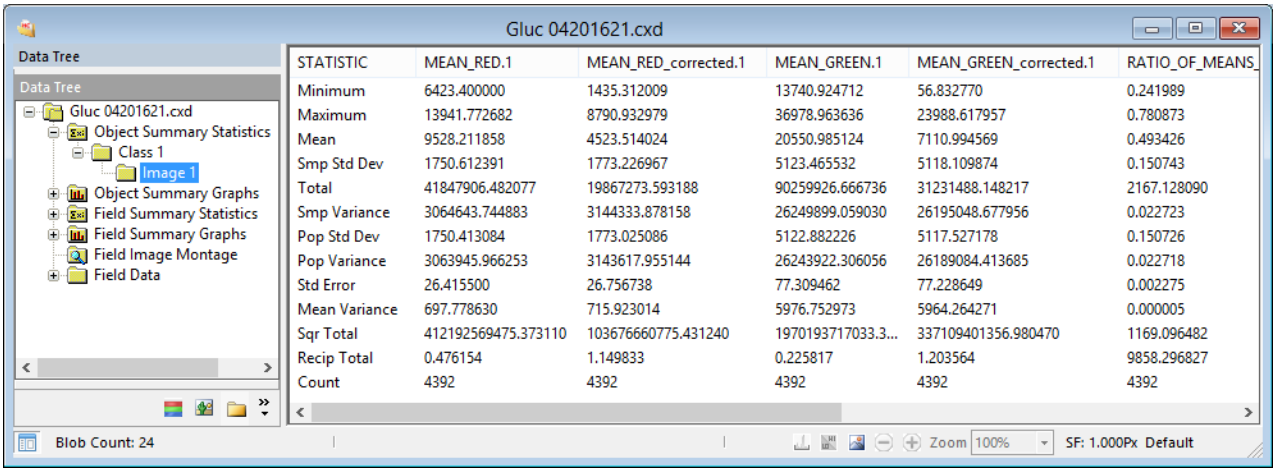
7 Start Acquisition
Click Start

8

9

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 deselected 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.



STATISTIC	MEAN_RED.1	MEAN_RED_corrected.1	MEAN_GREEN.1	MEAN_GREEN_corrected.1	RATIO_OF_MEANS_
Minimum	6423.400000	1435.312009	13740.924712	56.832770	0.241989
Maximum	13941.772682	8790.932979	36978.963636	23988.617957	0.780873
Mean	9528.211858	4523.514024	20550.985124	7110.994569	0.493426
Smp Std Dev	1750.612391	1773.226967	5123.465532	5118.109874	0.150743
Total	41847906.482077	19867273.593188	90259926.666736	31231488.148217	2167.128090
Smp Variance	3064643.744883	3144333.878158	26249899.059030	26195048.677956	0.022723
Pop Std Dev	1750.413084	1773.025086	5122.882226	5117.527178	0.150726
Pop Variance	3063945.966253	3143617.955144	26243922.306056	26189084.413685	0.022718
Std Error	26.415500	26.756738	77.309462	77.228649	0.002275
Mean Variance	697.778630	715.923014	5976.752973	5964.264271	0.000005
Sqr Total	412192569475.373110	103676660775.431240	1970193717033.3...	337109401356.980470	1169.096482
Recip Total	0.476154	1.149833	0.225817	1.203564	9858.296827
Count	4392	4392	4392	4392	4392

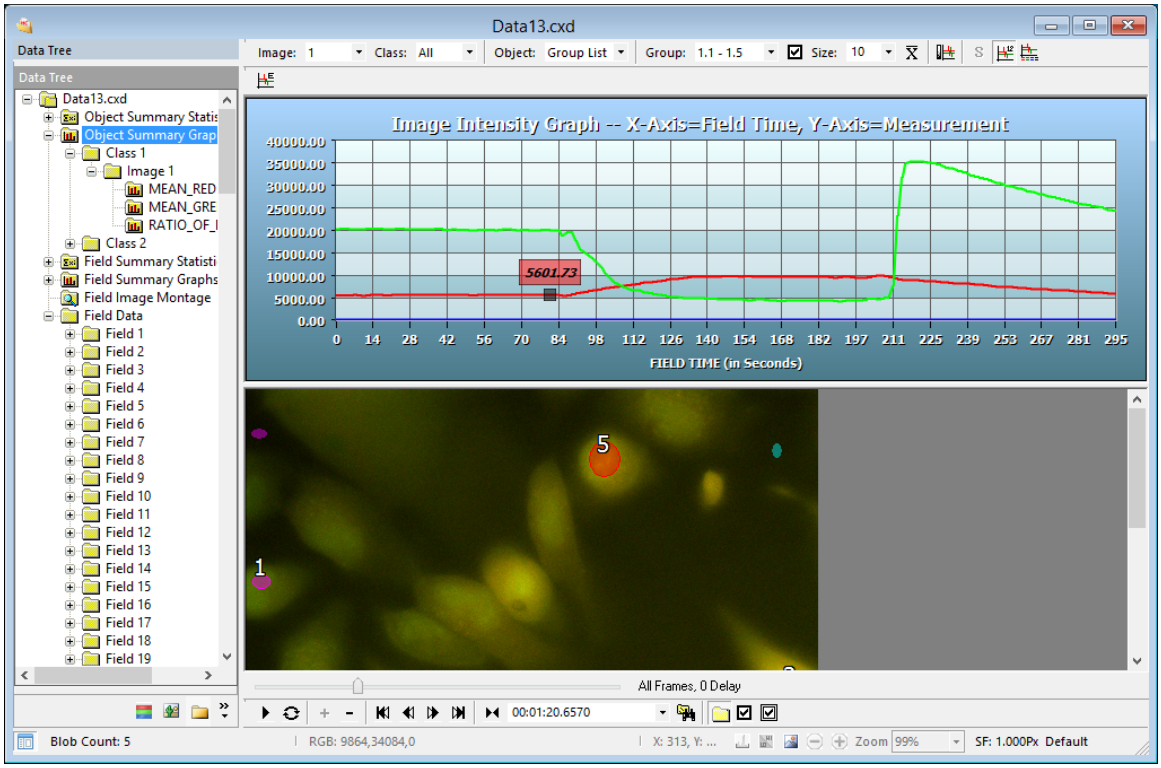
Statistical Measurements

The Statistics computed for Object and Field Measurements are derived as follows:

Statistic	Note	Formula
Count of Items	total number of items considered in the data set	Count = N
Minimum	minimum occurring value in the data set	Min (x)
Maximum	maximum occurring value in the data set	Max (x)
Total Value	sum of all values in the data set	Total value = (Σx)
Mean	total value divided by count of items	$\mu = (\Sigma x)/N$
Sample Variance	used to characterize incomplete samples	$s^2 = (\Sigma x^2 - (\Sigma x)^2/N)/(N-1)$
Sample Standard Deviation	used to characterize incomplete samples	$s = \sqrt{(s^2)}$
Population Variance	used to characterize complete samples	$\sigma^2 = (\Sigma x^2 - (\Sigma x)^2/N)/N$
Population Standard Deviation	used to characterize complete samples	$\sigma = \sqrt{(\sigma^2)}$
Standard Error of the Mean	experimental uncertainty of an averaged measurement	$SE_\mu = s / \sqrt{N}$
Total of Values Squared	sum of squares	Σx^2
Total of Reciprocal Values	sum of reciprocals	$\Sigma 1/x$

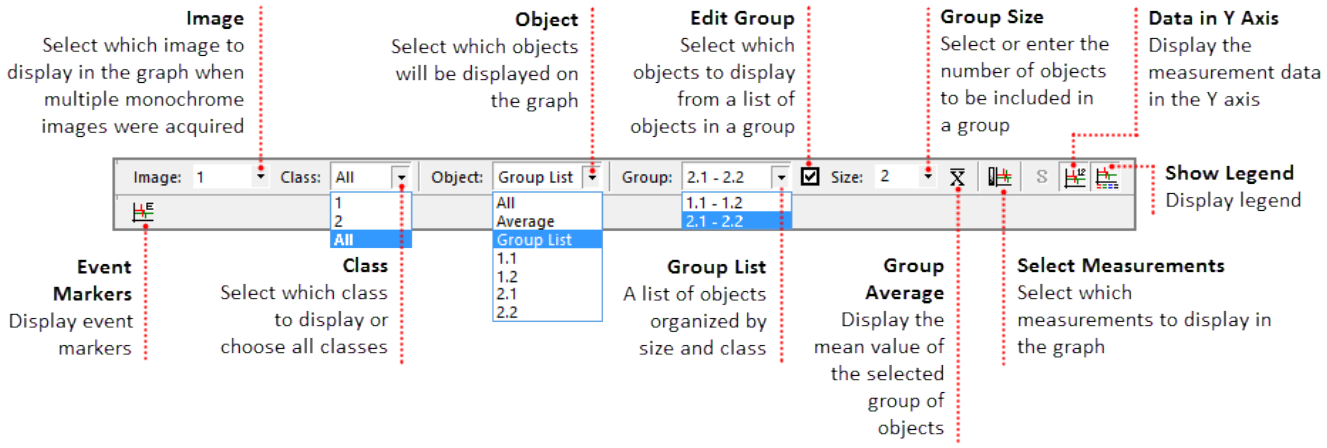
Object Summary Graphs

Object Measurements can be plotted for each object in the data document. The Object Summary Graphs show Object Measurement data of all fields. The interactive graph lets the user customize the display. Use the graph toolbar to select the measurements and which class and objects to display. Use the right-click menu to customize the look and feel of the graph by changing the title and legend fonts or adjusting background and border colors. The right-click menu also allows users to display the X axis as number of fields or field time.

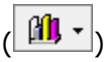


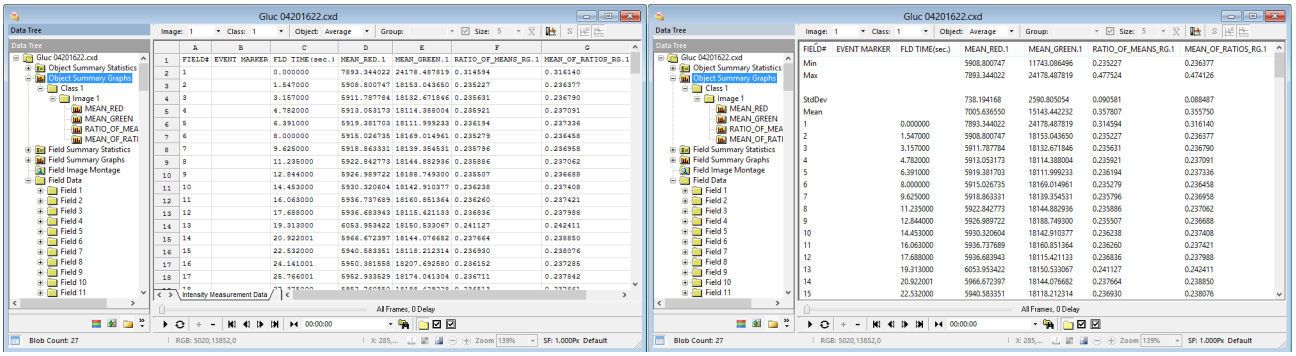
Object Summary Graph Toolbar

The toolbar provides multiple options for managing how the data is displayed.



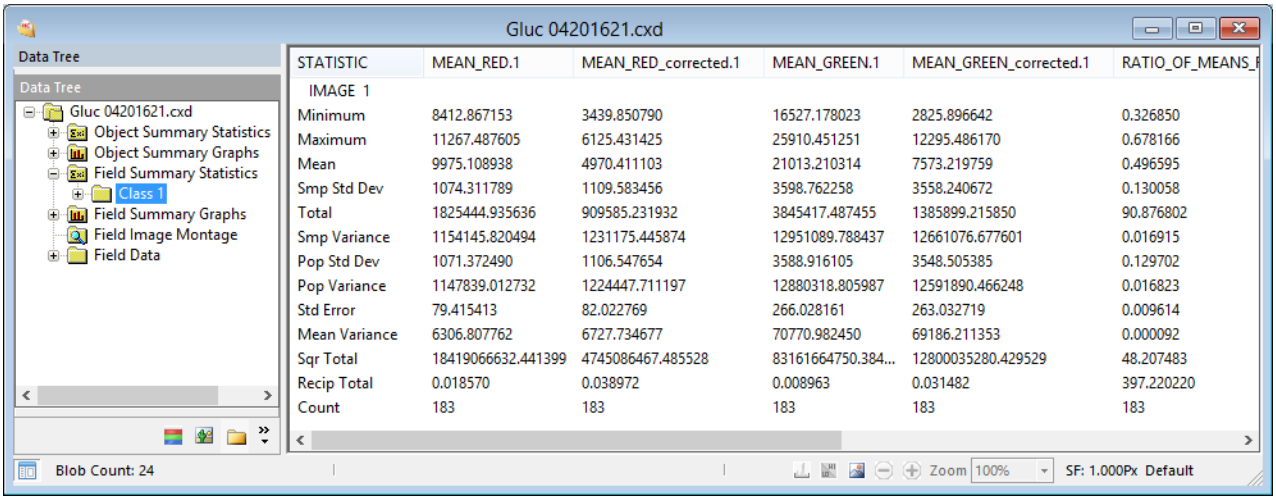
Object Summary Data

In addition to the Object Summary graphs, the intensity measurement data can also be displayed using a Spreadsheet View and a Table View. To change the view, go to the Image Data Views toolbar, click on the Current View icon () and select either Spreadsheet View or Table 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.

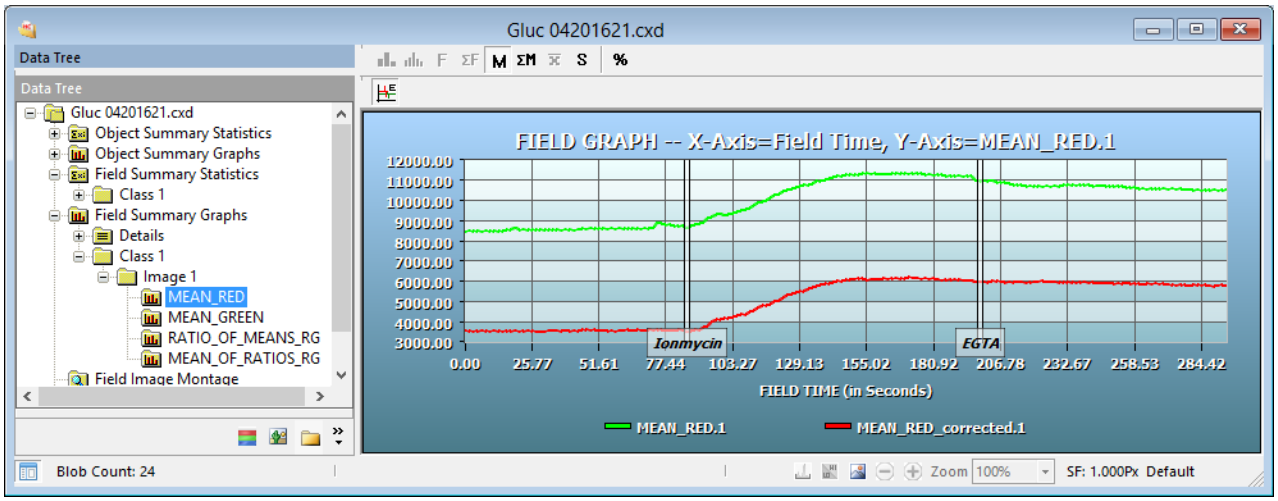


STATISTIC	MEAN_RED.1	MEAN_RED_corrected.1	MEAN_GREEN.1	MEAN_GREEN_corrected.1	RATIO_OF_MEANS_F
IMAGE 1					
Minimum	8412.867153	3439.850790	16527.178023	2825.896642	0.326850
Maximum	11267.487605	6125.431425	25910.451251	12295.486170	0.678166
Mean	9975.108938	4970.411103	21013.210314	7573.219759	0.496595
Smp Std Dev	1074.311789	1109.583456	3598.762258	3558.240672	0.130058
Total	1825444.935636	909585.231932	3845417.487455	1385899.215850	90.876802
Smp Variance	1154145.820494	1231175.445874	12951089.788437	12661076.677601	0.016915
Pop Std Dev	1071.372490	1106.547654	3588.916105	3548.505385	0.129702
Pop Variance	1147839.012732	1224447.711197	12880318.805987	12591890.466248	0.016823
Std Error	79.415413	82.022769	266.028161	263.032719	0.009614
Mean Variance	6306.807762	6727.734677	70770.982450	69186.211353	0.000092
Sqr Total	18419066632.441399	4745086467.485528	83161664750.384...	12800035280.429529	48.207483
Recip Total	0.018570	0.038972	0.008963	0.031482	397.220220
Count	183	183	183	183	183

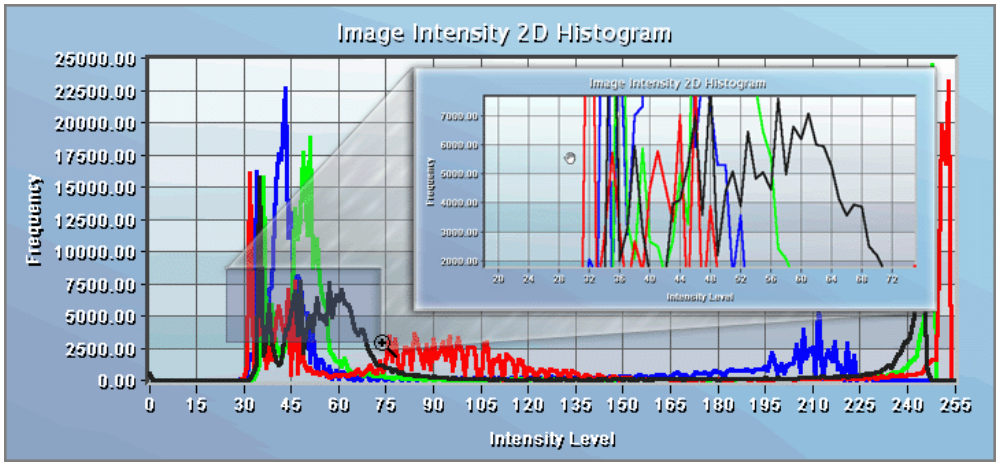
Opening the Field Summary Statistics node will display a node for each Measurement Class present. Selecting the Field Summary Statistics node will display all Field Measurement Classes. Under the Field Summary Statistics node is a node for each Measurement Class. Selecting the Class node will display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

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.



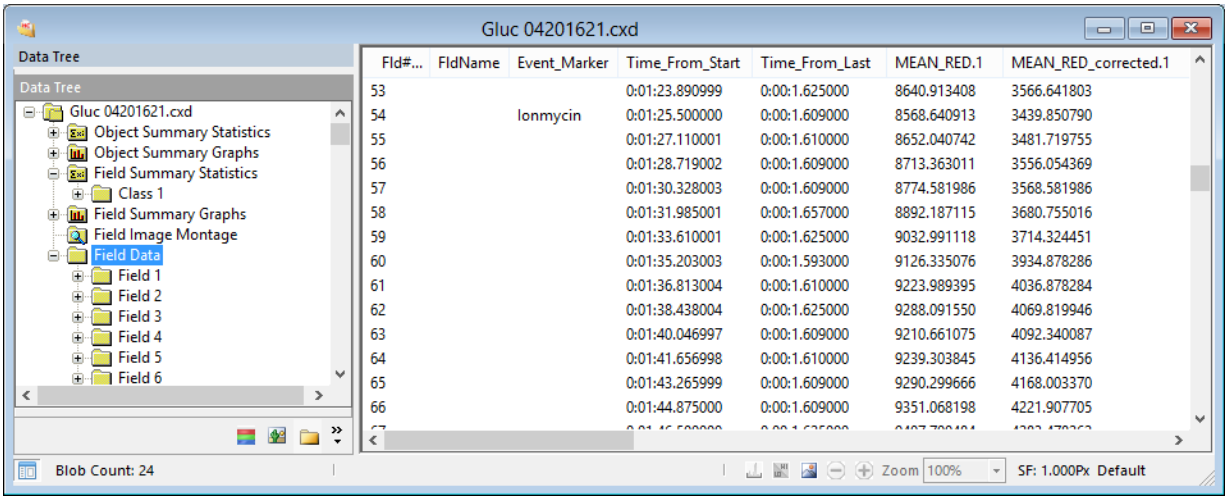
The Field Summary graphs are an easy to use interactive way of displaying and viewing data. First the Mean Red (Corrected 340nm) intensity is measured over time. Second there are two Event Markers that indicate specific points during the experiment that an event happened. In this case, we know the name of the reagents that were added and the time they were added. The Event Markers may be toggled on/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. 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. 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. Use the right-click menu to customize the look and feel of the graph by changing the title and legend fonts or adjusting background and border colors. The right-click menu also allows users to display the X axis as number of fields or field time.



Field Data

Field Data contains information for each field about when and where the image was captured. The data can be viewed in a Table View or Spreadsheet View and copied to the Windows Clipboard. These details can include:

- X,Y,Z Stage Position Microns
- Image Width in pixels
- Image Height in pixels
- Image Depth in bits per pixel
- Time From the Start (Hours:Minutes:Seconds.Hundredths)
- Time From Last (Hours:Minutes:Seconds.Hundredths)
- Computer-controlled Wavelength used (in nanometers)
- Group Number
- Group Index
- Group Size



IPA OVERVIEW

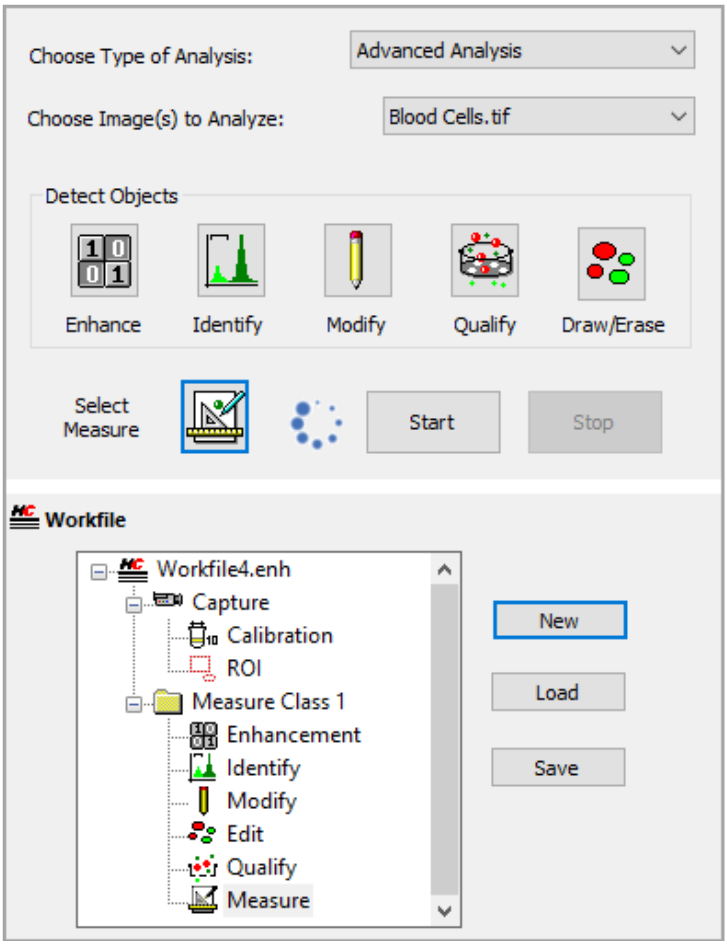
Image Processing and Analysis provide an extensive selection of image processing and image analysis tools to enable quantitative analysis on a wide range of complex image sequences. Imaging tools are selected using customized icons to derive workfiles (macros), which are saved and can be used multiple times. Images are saved with measured data allowing dynamic interaction between images, objects, graphs and tables to provide instant user feedback.

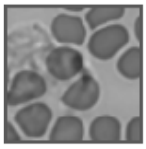
Understanding the Workspace

The Image Processing and Analysis functionality is accessed through the Analysis pane by selecting Advanced Analysis from the Choose Type of Analysis list. In addition to the Advanced Analysis, Single Image Measure and Sequence Intensity Analysis are also available. For Single Image Measure and Sequence Intensity Analysis the user can select from Simple Analysis and Advanced Analysis modes by going to View in the menu bar, then highlight Analysis Mode and select Simple or Advanced. The Simple modes provides a variety of drawing tools that can be used to identify objects of interest. The Advanced mode described in the example below.

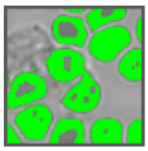
Advanced Analysis

For Advanced Analysis, the measurement algorithm is set up by configuring an icon-driven workfile. This is done by adding steps in an interactive process and observing the effects on the identified image objects as the various steps are added and modified as shown below. The procedure is methodical, where the operator selects each option interactively. The steps used can be saved in a workfile (.enh) for later reuse, review, or modification.

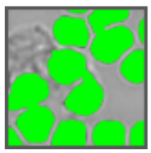




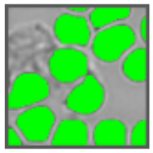
Enhance the image using image processing filters



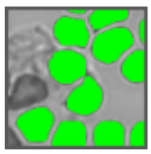
Identify objects of interest with a binary image layer



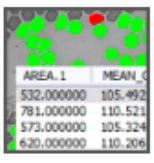
Modify the binary layer to improve object size, shape and connectivity



Draw/Erase objects using drawing tools



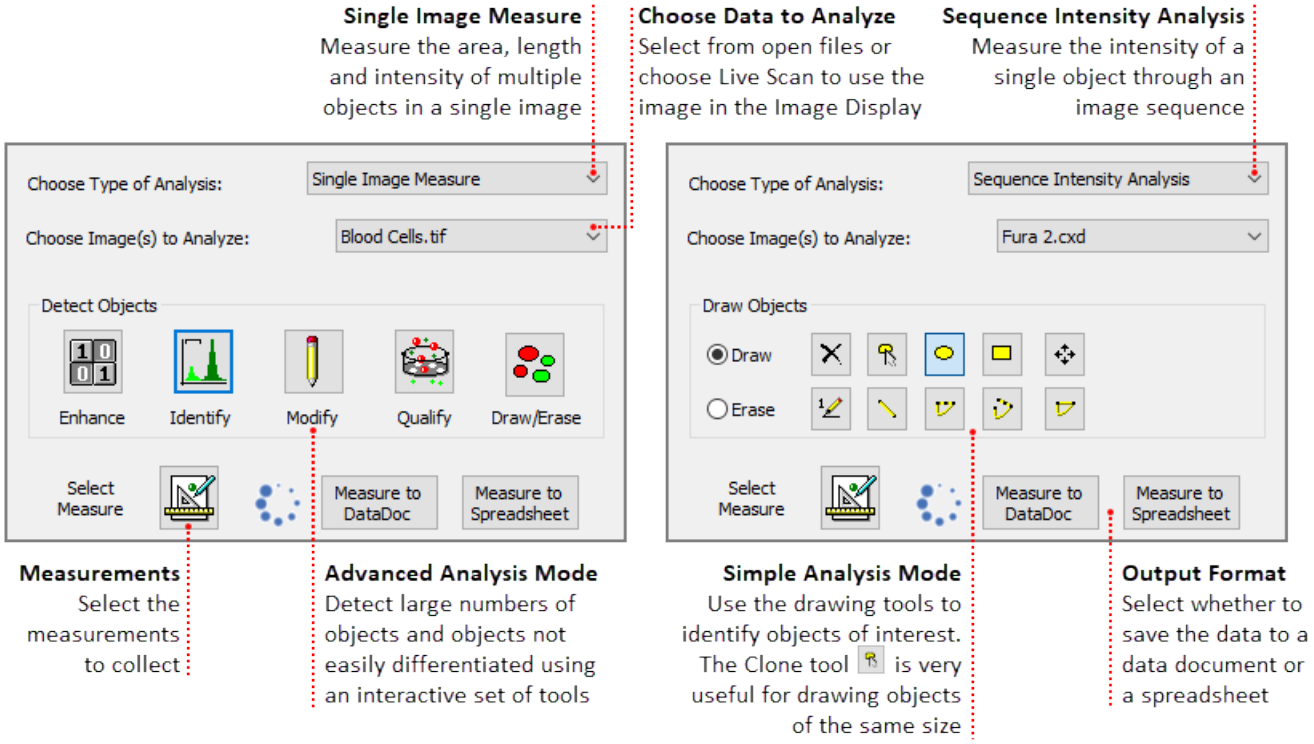
Qualify objects based on size, shape, position, etc.



Measure multiple objects in a single image

Single Image measure

For Single Image Measure, measure the size, shape, intensity, position or create a custom measurement of multiple objects in a single image. The image can be from the Live Scan (image display), part of an image sequence, or a single standalone image. The object of interest can identified using the Advanced Analysis mode or drawn using the Simple Analysis mode (both shown below). The Simple mode is active by default but can be changed by clicking View on the Menu bar, highlighting Analysis Mode and selecting Advanced. The measured data can be saved to data document (.cxd) or to a spreadsheet.



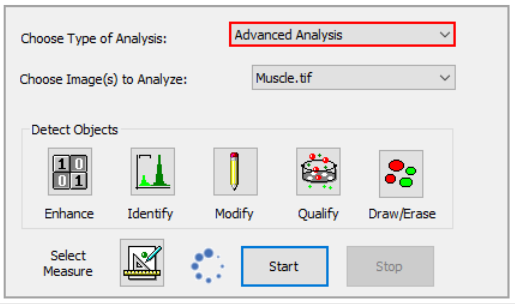
Sequence Intensity Analysis

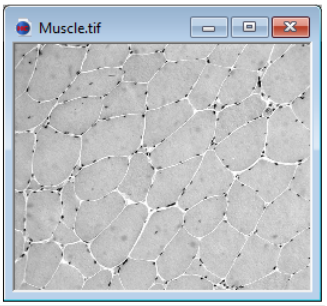
For Sequence Intensity Analysis, measure the intensity of a single object over time in an image sequence. The object of interest can identified using the Advanced Analysis mode or drawn using the Simple Analysis mode (both shown above). The Simple mode is active by default but can be changed by clicking View on the Menu bar, highlighting Analysis Mode and selecting Advanced. If multiple areas are drawn or identified, they are treated as a single object. The measured data can be saved to data document (.cxd) or to a spreadsheet.

IPA EXAMPLES

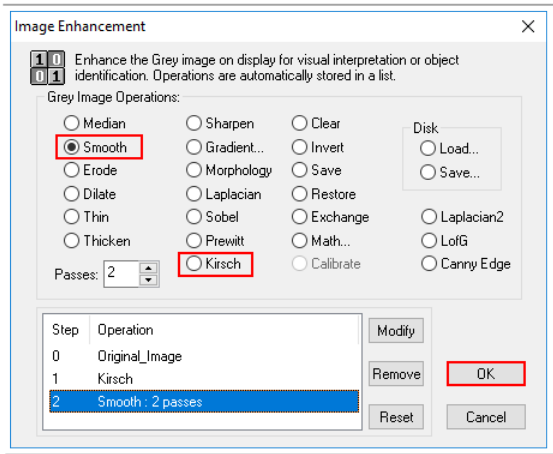
Advanced Analysis - Muscle Fiber Example

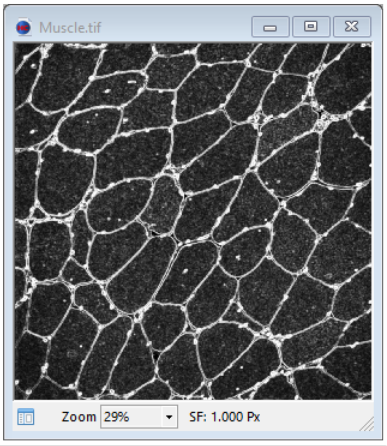
The example below explains how to create a Workfile for measuring the size and shape of muscle fibers.



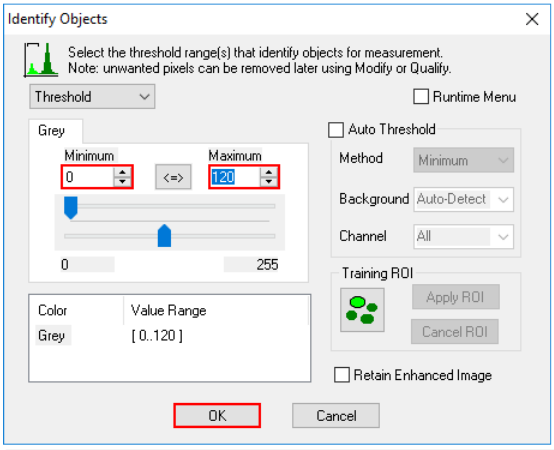


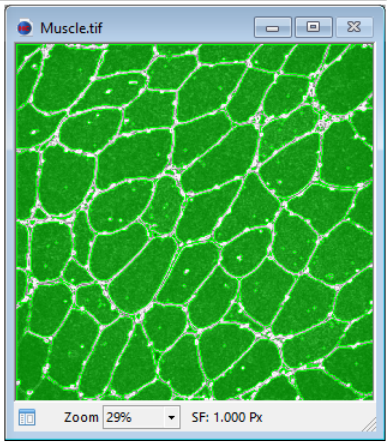
1 **Enable Advanced Analysis**
Open the Muscle.tiff and select Advanced Analysis from the drop-menu.



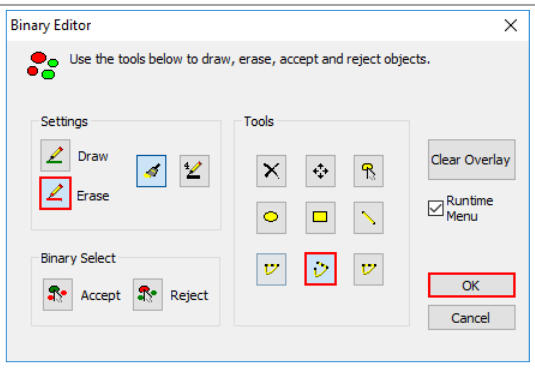


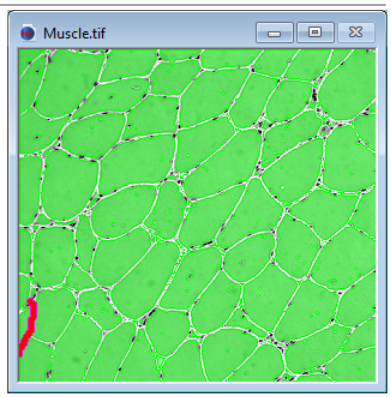
2 **Enhance the Image**
Click the Enhance icon, apply a Kirsh and two passes of the Smooth filter to the image. Click OK.



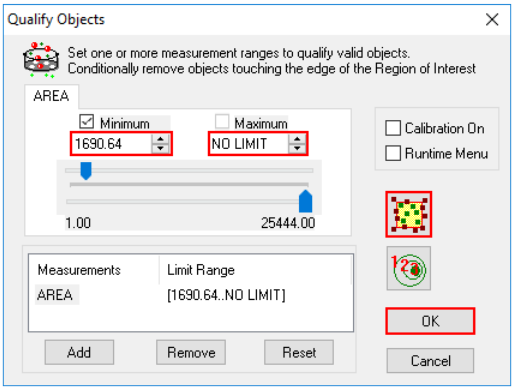


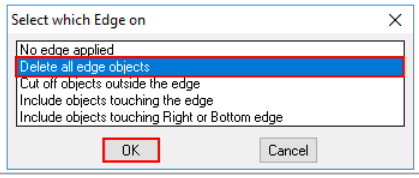
3 **Identify Objects of Interest**
Click the Identify icon, adjust the min to 0 and the max to 120, covering the muscle fiber with a green overlay and click OK.

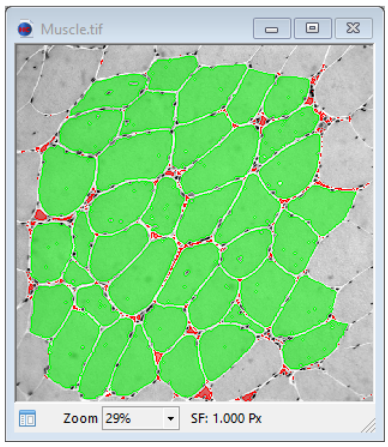




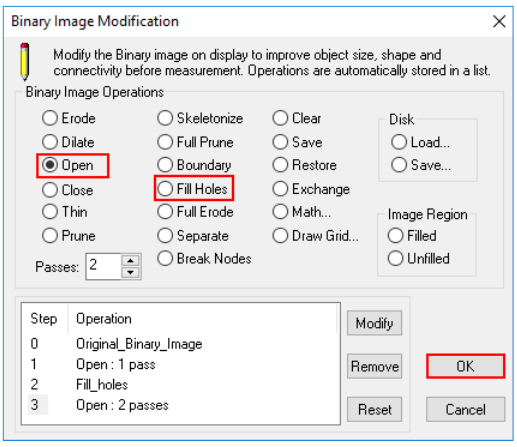
4 **Separate Muscle Fibers**
Click the Draw/Erase icon, click Erase, select the Open Polygon tool and erase any connections between fibers by drawing a line across the connection and right-click to apply. Click OK.

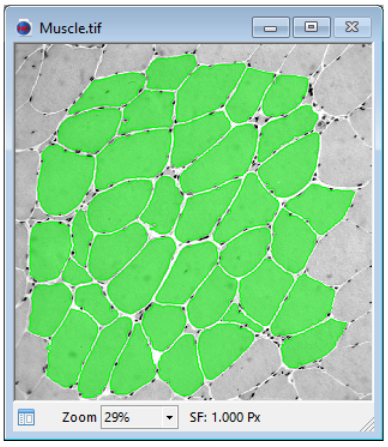




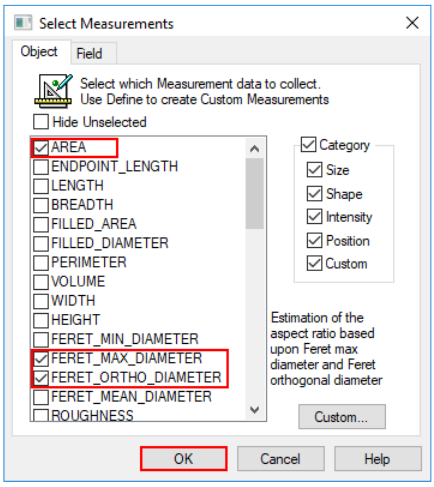


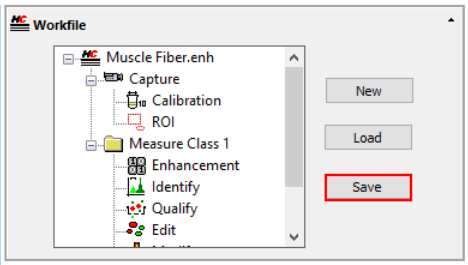
5 Remove Unwanted Objects
Click the Qualify icon, click the Edge Objects icon, select Delete all edge objects and click OK. Adjust the min area to 1690 to remove the smaller objects and click OK.





6 Modify the Binary Image
Click the Modify icon, apply an Open, select Fill Holes, apply Open two passes and click OK.



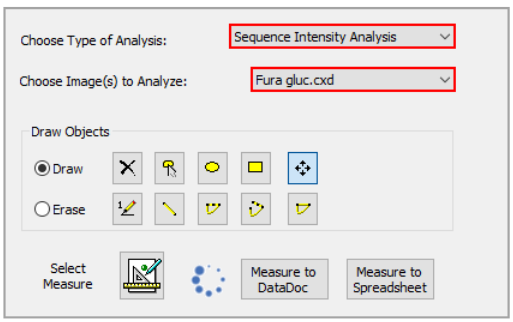


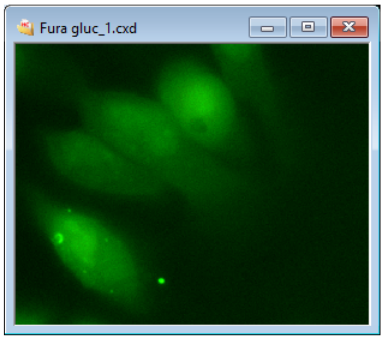
7 Select Measurements
Click the Measure icon, select Area, Feret Max Diameter, Feret Ortho Diameter, Feret Elongation and Feret Aspect Ratio. Click OK.

8 Save and Run the Workfile
Click the Save button and click Start.

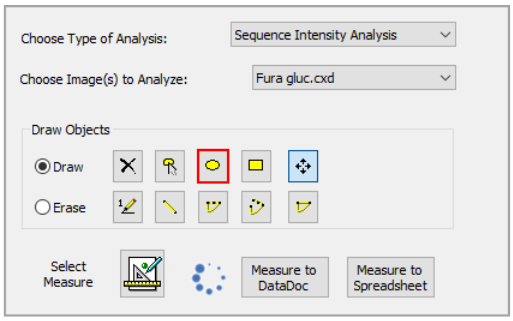
Sequence Intensity Analysis - Simple Mode

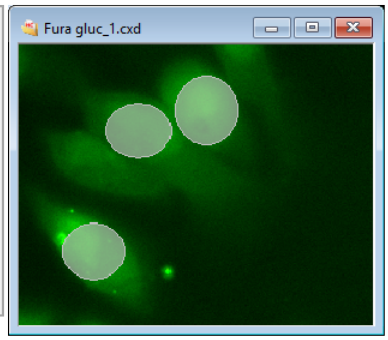
Sequence Intensity Analysis will measure the intensity of a single object in the image sequence. If multiple areas are drawn or identified, they are treated as a single object. HCImage has two modes, the Simple mode is active by default but can be changed by clicking View on the menu bar, then highlighting Analysis Mode and selecting Advanced.





1 Enable Sequence Intensity Analysis
Open the dataset and select Sequence Intensity Analysis from the drop-menu.

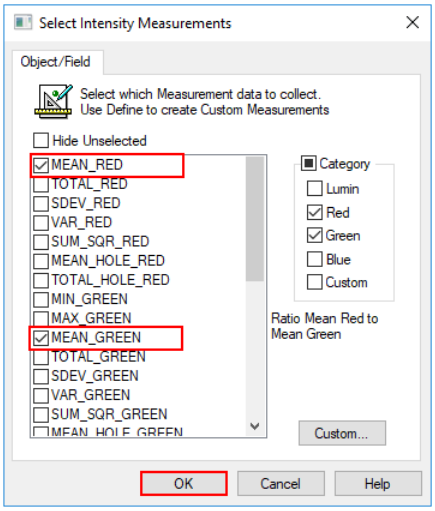


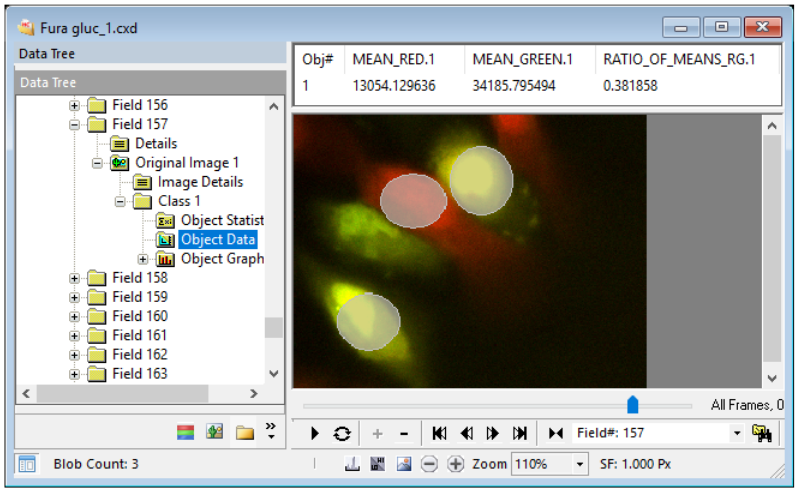


2 Draw Object
Click the Ellipse icon and manually identify the object of interest.
Tooltip
Press SHIFT to draw a circle.

3 Analyze Objects of Interest
Click the Measure icon, select measurements, click OK and select Measure to DataDoc 

.

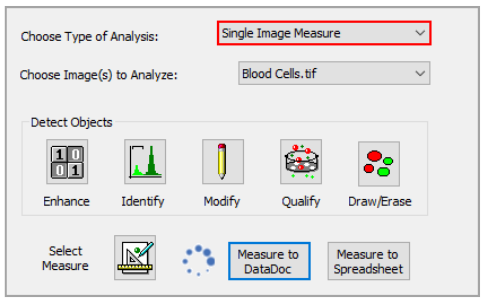


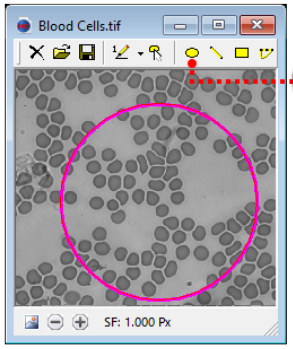


Note: Change the overlay color to silver instead of green in the **Change Overlay Color** icon. Use the translucency slider to adjust the overlay transparency or hide it by selecting **View Overlay**. Click **Clear** to delete the overlay.

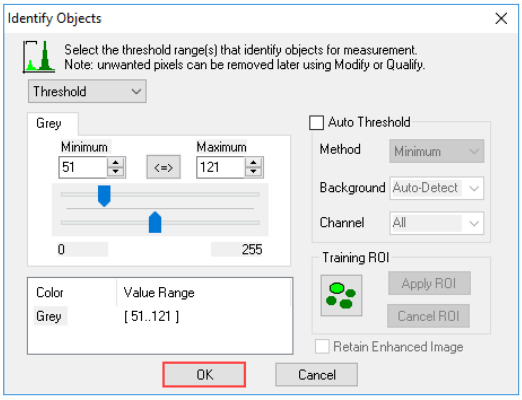
Single Image - Measure Analyze Objects Inside of a ROI

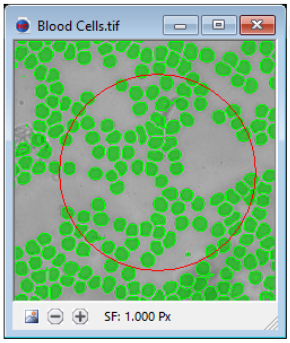
Enable the Advanced Analysis mode by clicking View on the Menu bar, then highlighting Analysis Mode and selecting Advanced. Open an image, go to the Analysis pane and select Single Image Measure from the drop-down list. Click on the image and follow the instructions below.



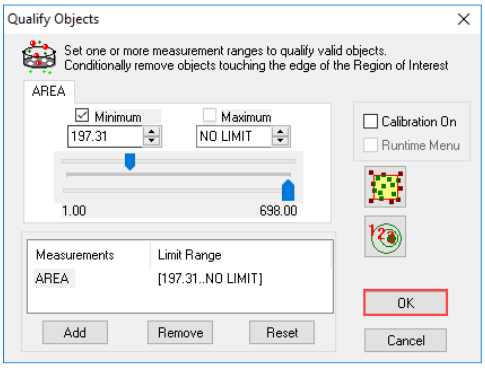


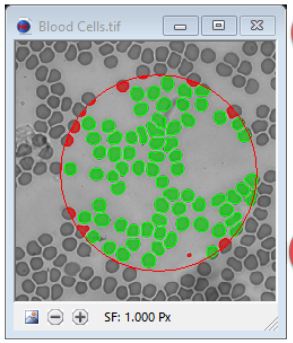
1 **Define Region of Interest**
Activate the Image region layer, click the ellipse icon and draw region of interest





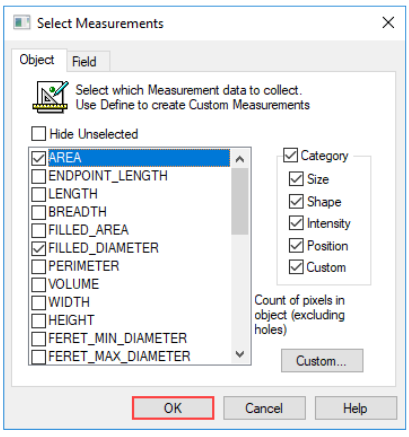
2 **Identify Objects of Interest**
Click the Identify icon, adjust the Min and Max sliders until the objects of interest are covered by the green overlay and click OK

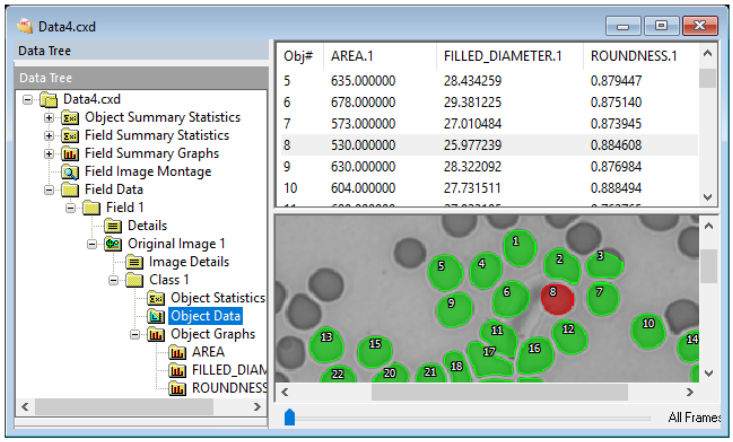




3 **Remove Unwanted Objects**
Click the Qualify icon, use the Min and Max sliders to reject objects and click OK

4 **Analyze Objects of Interest**
Click the Measure icon, select measurements, click OK and select Measure to DataDoc

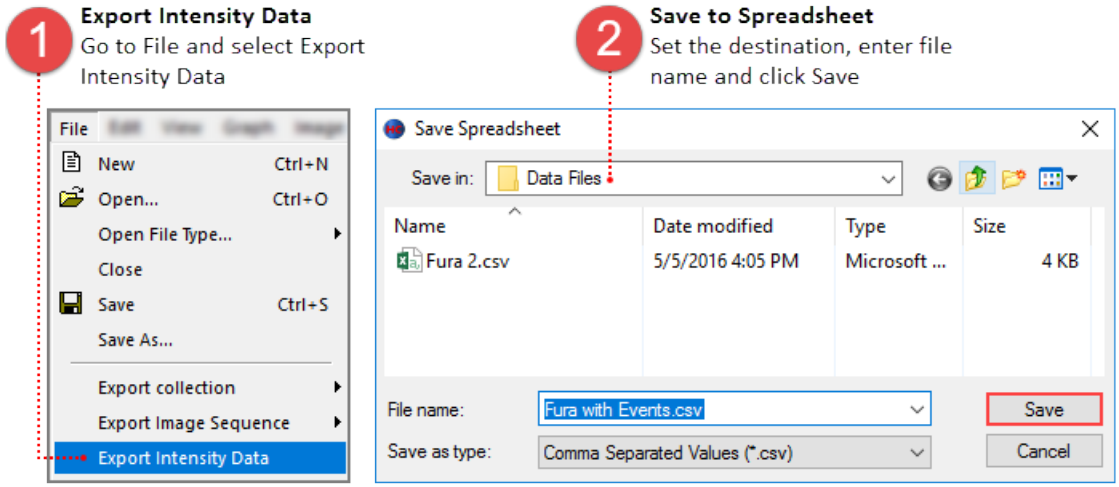




EXPORT THE DATA

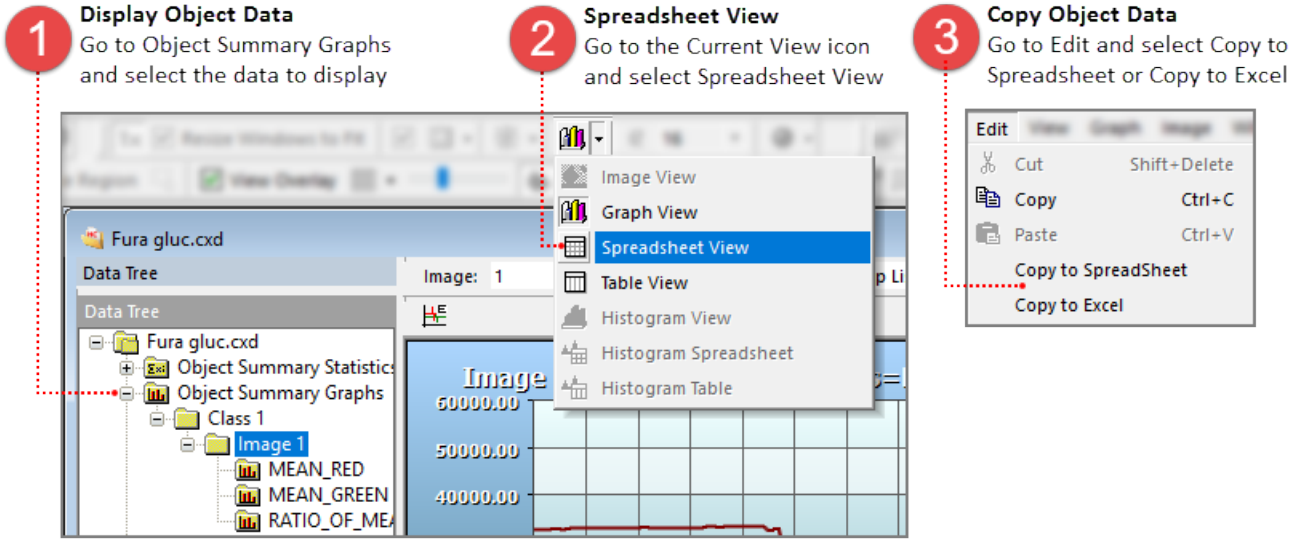
Export Intensity Data

Use this method to export all of the collected intensity data from the data document to a spreadsheet. This includes the object and field data as well as the object and field summary statistics. With the data document open follow the steps below to export the intensity data to a spreadsheet.



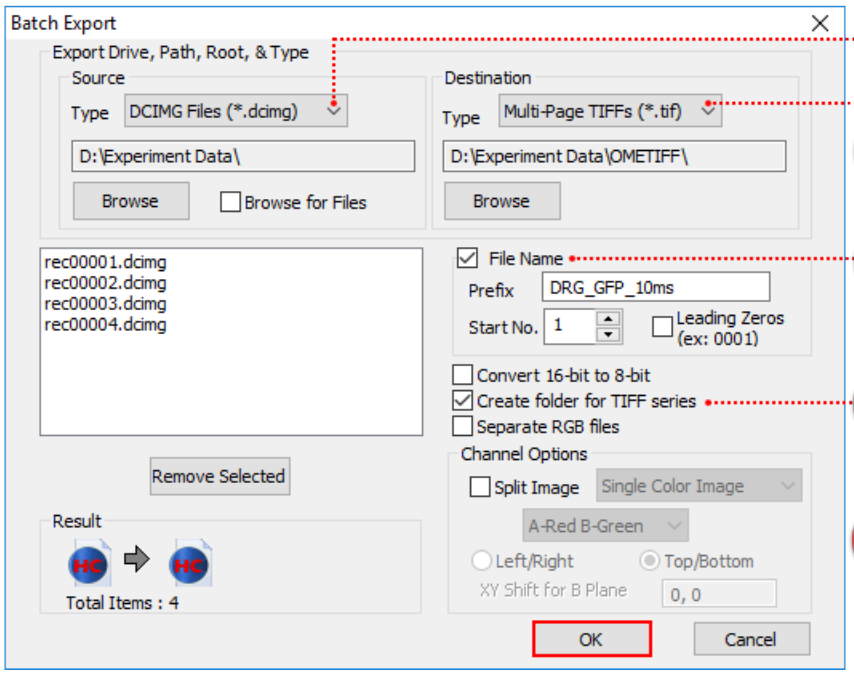
Copy to Spreadsheet or Excel

To copy only the data from a specific graph, select the graph and follow the instructions below.



Batch Export DCIMG to MPTIFF

In the File menu select Batch Export and follow the instructions below. The exported files are not automatically opened in the software.



1 **Enter Source Location**
Type: Select DCIMG Files
Browse: Go to the file directory

2 **Enter Destination Location**
Type: Select Multi-Page TIFF Files
Browse: Go to output directory

3 **Define Output File Name**
Define the file naming convention

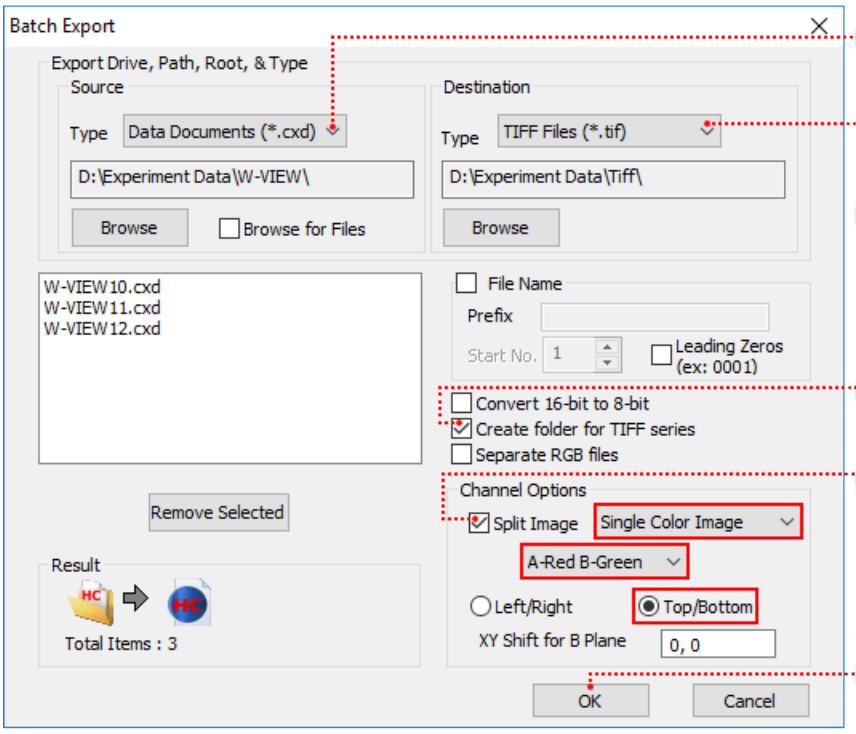
4 **Enable Create Series Folder**
Select Create folder for TIFF series

5 **Export to MPTIFF**
Click OK

Note: MPTIFF files have a 65,000 image limit and 4 GB size limit. For image sequences having more than 65,000 images or larger than 4 GB, multiple MPTIFF files will be saved and numbered sequentially.

Batch Export W-VIEW Images

In the File menu select Batch Export and follow the instructions below.



1 **Enter Source Type and Location**
Type: Data Select Data Documents
Browse: Go to the file directory

2 **Enter Destination Type and Location**
Type: Select TIFF Files
Browse: Go to the output directory

3 **Enable Create Series Folder**
Select Create folder for TIFF series

4 **Define Channel Options**
Enable Split Image and select Single Color Image, A-Red B-Green and Top/Bottom

5 **Export to TIFF**
Click OK