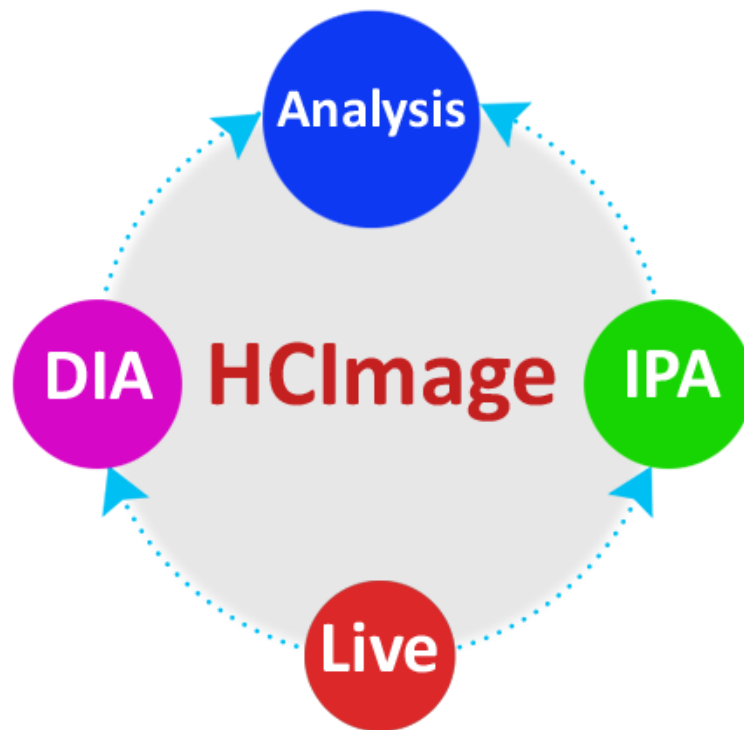


HCIimage

Getting Started Guide



Release) . \$
November 202&

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INSTALLATION

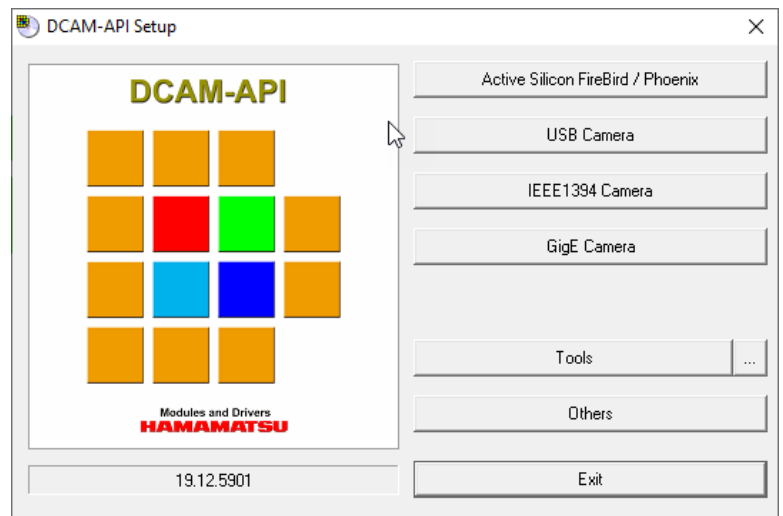
Install HCIImage

1. Insert the HCIImage installation DVD into the DVD-ROM drive. If autoplay is enabled, the HCIImage 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 HCIImage.
 12. Click the **HCIImage** icon on your Desktop to launch HCIImage.
 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 HCIImage installation DVD, expand the **Drivers folder**, open the **Cameras folder** and open the **DCAM folder**. If you downloaded HCIImage, 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.

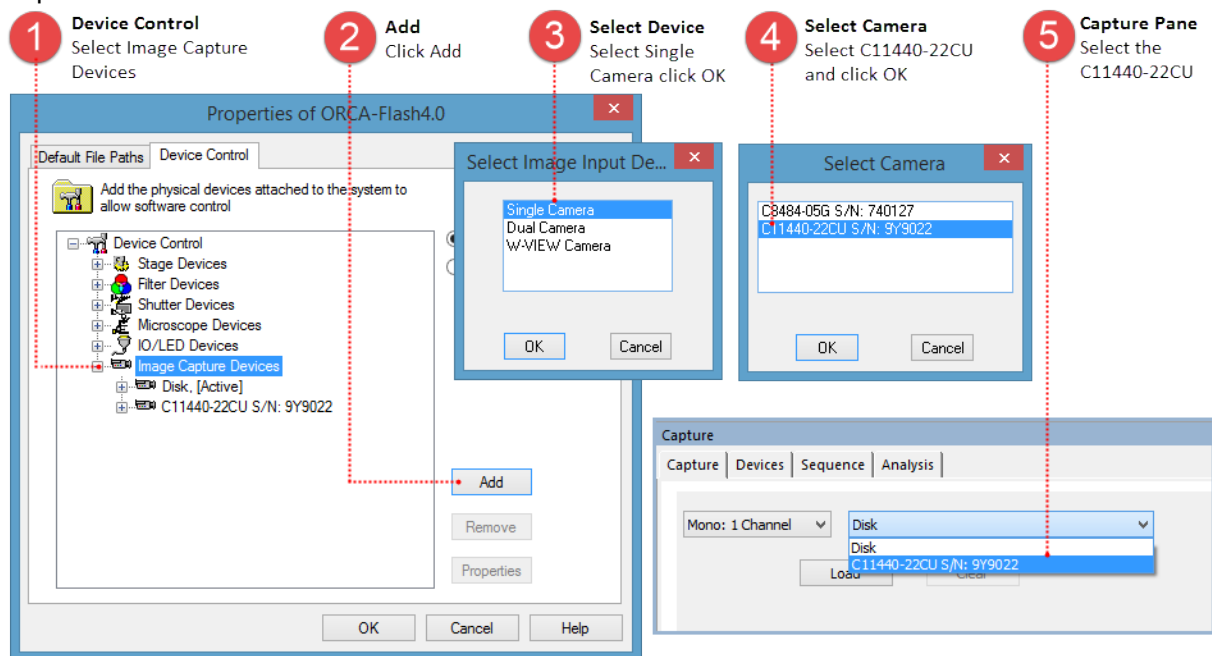


ADD DEVICES TO A PROFILE

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 HCIImage, 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 HCIImage DVD

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

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 HCIImage. Launch HCIImage, go to File and select Current Profile. In the Device Control tab, select Microscope Devices and click Add.

The figure shows four sequential screenshots of the 'Add MICROSCOPE Device' dialog box in HCIImage, illustrating the steps to add a microscope to a profile:

- 1 Device Control**: Select the microscope manufacturer and the model. The 'Controller' tab is active, showing a list of manufacturers (LEICA, NIKON, OLYMPUS, ZEISS, Hamamatsu AEUORIA, Yokogawa CSU) and a dropdown menu set to 'IX3'. The 'Select Components' section has 'Z Focus' checked.
- 2 Device List**: Select the device that is listed. The 'Device-List' tab is active, showing a list of installed devices with 'IX3 0' selected.
- 3 Device Control**: Select Auto and HCIImage will update the components list. The 'Controller' tab is active, showing the same list of manufacturers as in step 1, but the 'Select Components' section now has 'Z Focus' checked and 'XY Stage' unchecked.
- 4 Filter Setup**: Verify the settings for the individual components, modify if necessary and click OK. The 'Filter Setup' tab is active, showing a list of filter names (Filter cube1, Filter cube2, Filter cube3) and a 'Filter Pos Limit' of 8. The 'OK' button is highlighted.

Add a Filter Wheel and a Shutter

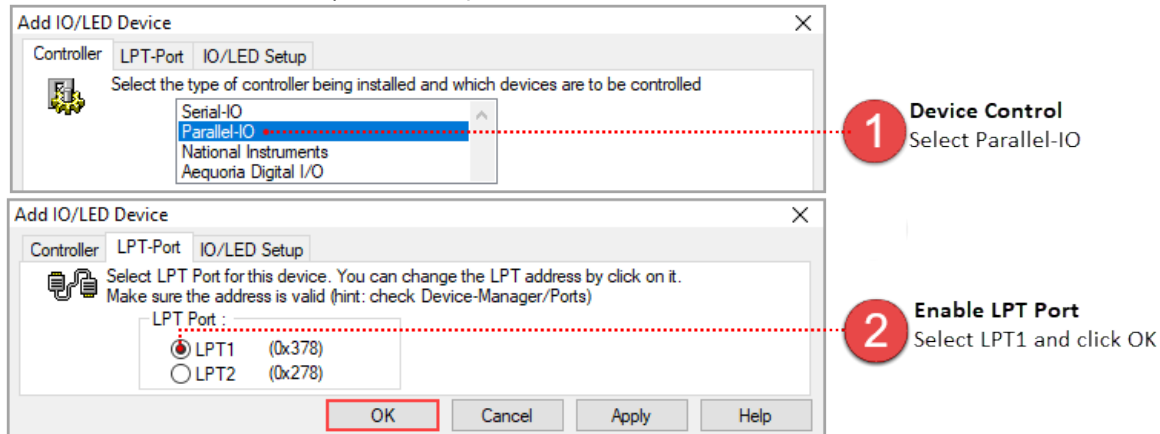
Launch HCIImage, go to File and select Current Profile. In the Device Control tab, select Filter Devices, click Add and follow the instructions below.

The image displays four sequential screenshots of the 'Add FILTER Device' dialog box, illustrating the configuration process for a filter wheel and shutter. Each screenshot is annotated with a red circle and a number, along with a red dotted line pointing to the relevant UI element.

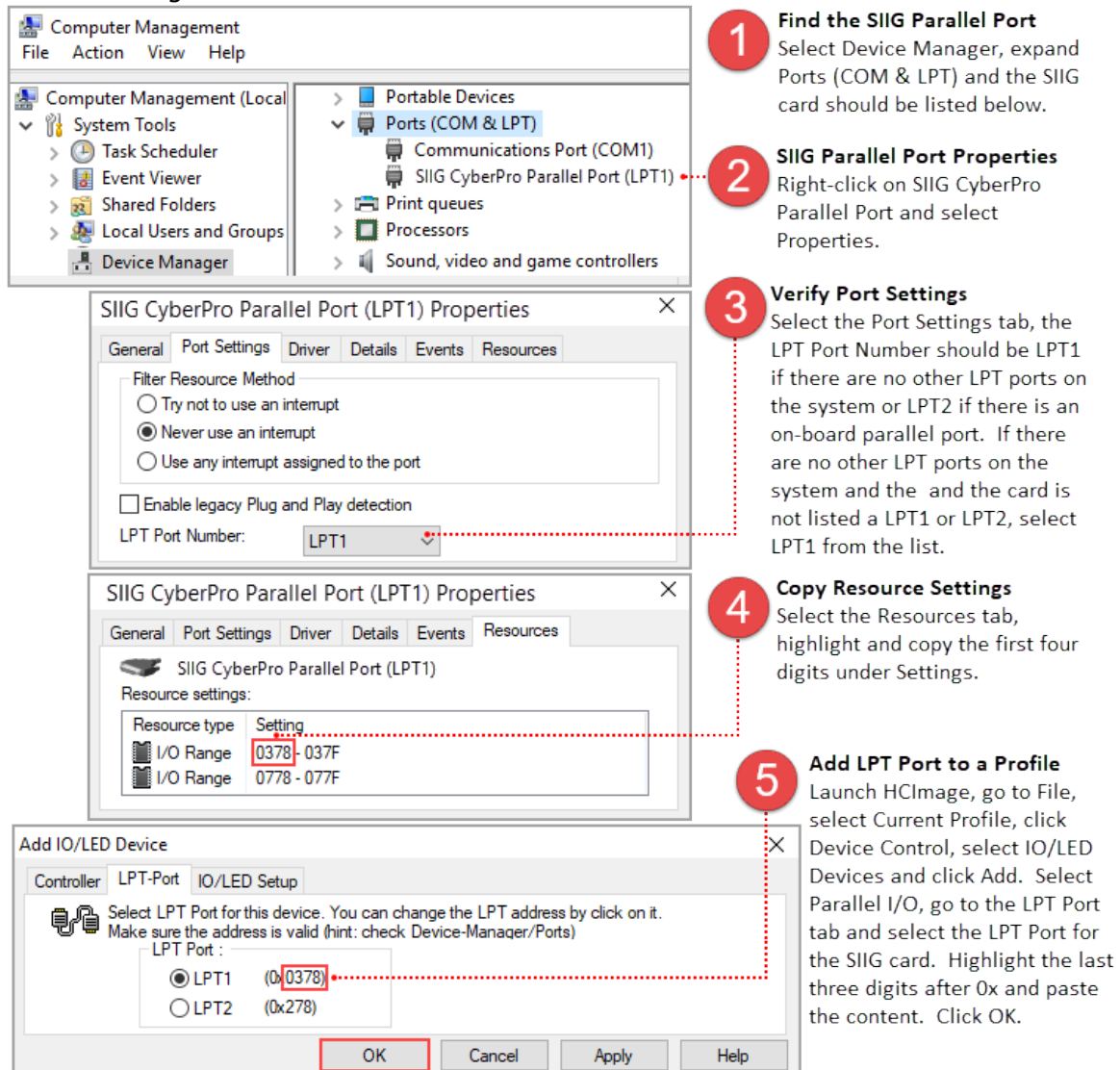
- 1 Device Control**: The 'Controller' tab is selected. A list of controllers is shown, with 'PRIOR Filter' selected. To the right, the 'Filter Wheel' section has 'Single' selected, and the 'Add Shutter' checkbox is checked.
- 2 Enable COM Port**: The 'COM-Port' tab is selected. The 'COM Port' dropdown is set to 'COM1'. The 'Communication settings' section shows 'Baud Rate: 9600', 'Data Bits: 8', 'Parity: None', and 'Stop Bits: 1'.
- 3 Filter Setup**: The 'Filter Setup' tab is selected. The 'Filter Wheel' section shows 'Customize Positions' with 'Wheel 1 Speed: 100' and 'Filter Pos Limit: 10'. A list of filter positions is shown, with '380 nm' selected. The 'Modify' button is highlighted.
- 4 Shutter Setup**: The 'Shutter Setup' tab is selected. The 'Shutter Settings' section shows 'Shutter 1: Active', 'Shutter 2: None', and 'Shutter 3: None'. The 'OK' button is highlighted.

Add a Parallel Port as an IO/LED Device

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




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.

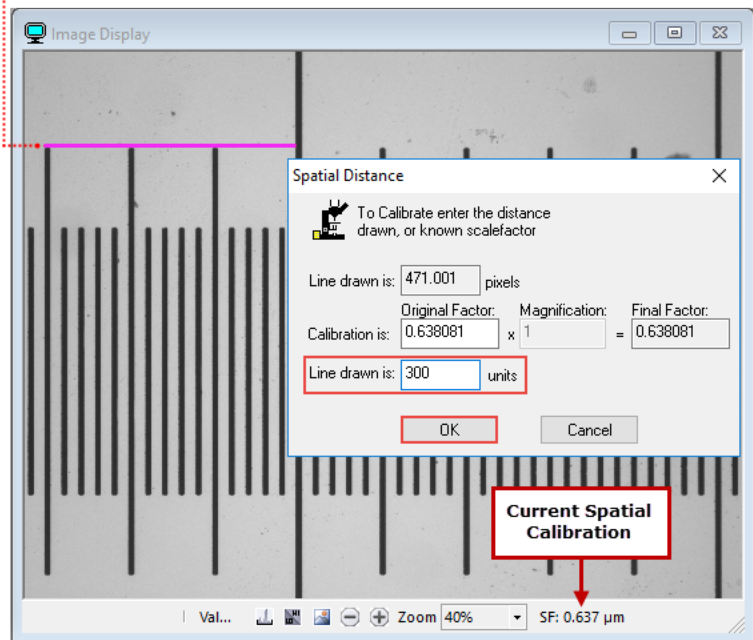
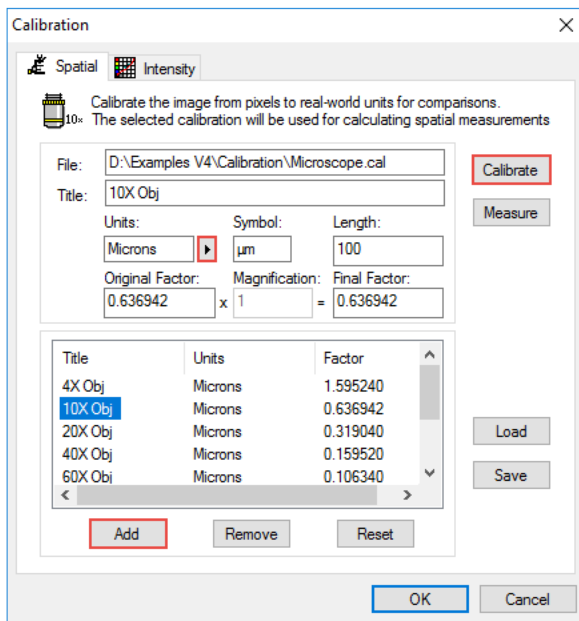


CALIBRATION

Calibrate an Image from Pixels to Microns

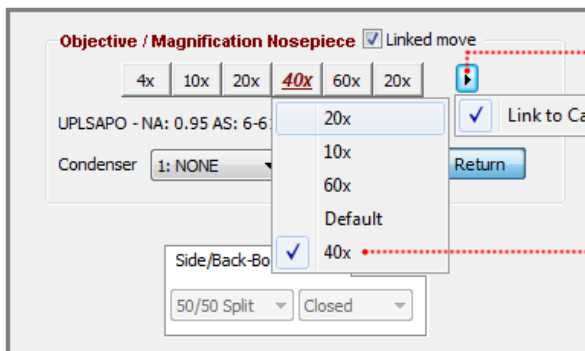
Open or capture an image with some known distance, for example a micrometer. Click on the Calibration Properties icon ( Calibration) on the Analysis toolbar and follow the instructions below.

- 1 Add Calibration**
Click Add and enter a title (e.g., 10x)
- 2 Select Units**
Select Microns from the Units list
- 3 Calibrate the Image**
Click Calibrate. Draw a line to span the distance to measure
- 4 Enter the Distance**
Enter the known distance and click OK



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.



- 1 Enable Link to Calibration**
Click and select Link to Calibration
- 2 Select Calibration**
Right-click on the 40x objective and select the 40x calibration

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

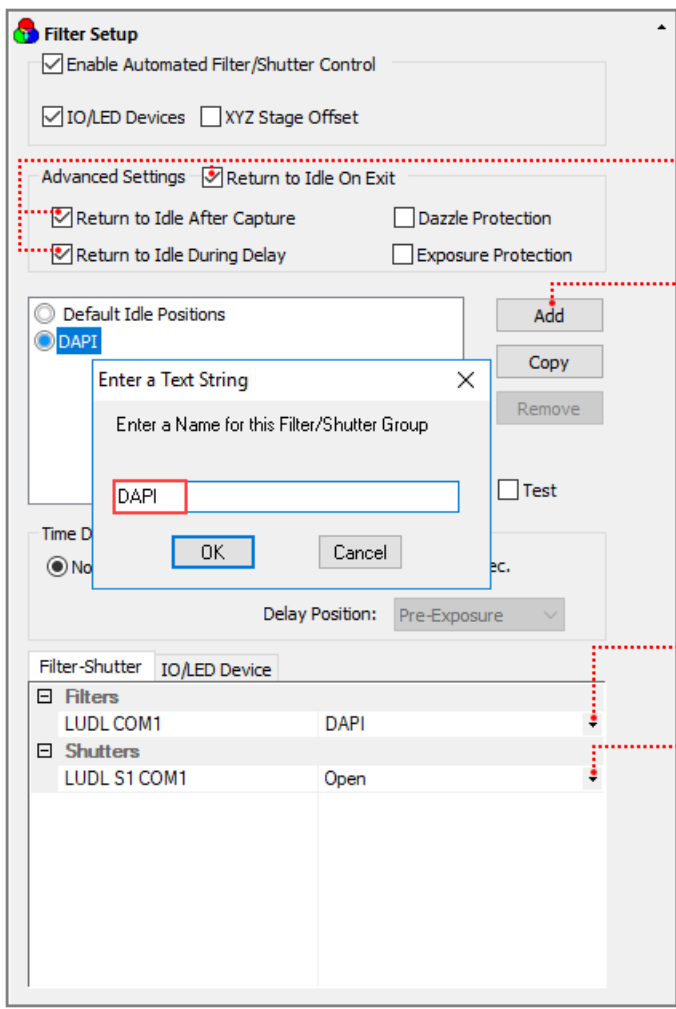
4 Object Centered
If the object moved back to the center of the top left crosshair, the calibration was successful, click OK. If the object did not move to the same location, check the camera orientation to the microscope and repeat Steps 1-3.

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.



The screenshot shows the 'Filter Setup' dialog box with the following settings and callouts:

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

The dialog box also shows the following settings:

- Enable Automated Filter/Shutter Control
- IO/LED Devices XYZ Stage Offset
- Return to Idle After Capture Dazzle Protection
- Return to Idle During Delay Exposure Protection
- Default Idle Positions DAPI
- Time Delay: No
- Delay Position: Pre-Exposure
- Filter-Shutter: IO/LED Device
- Filters: LUDL COM1 (DAPI)
- Shutters: LUDL S1 COM1 (Open)

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.

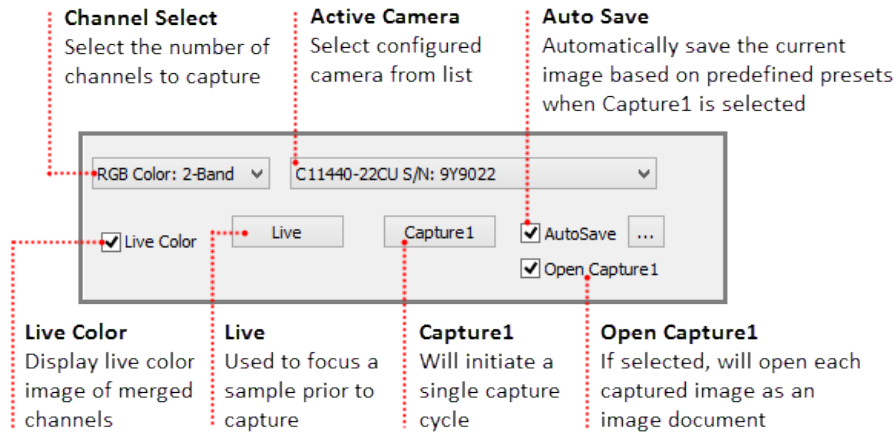
The screenshot shows the 'Filter Setup' dialog box with the following settings and callouts:

- 1 Enable IO/LED Devices**: Select IO/LED Devices (checked).
- 2 Enable Return to Idle Conditions**: Select Return to Idle on Exit, After Capture and During Delay (checked).
- 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

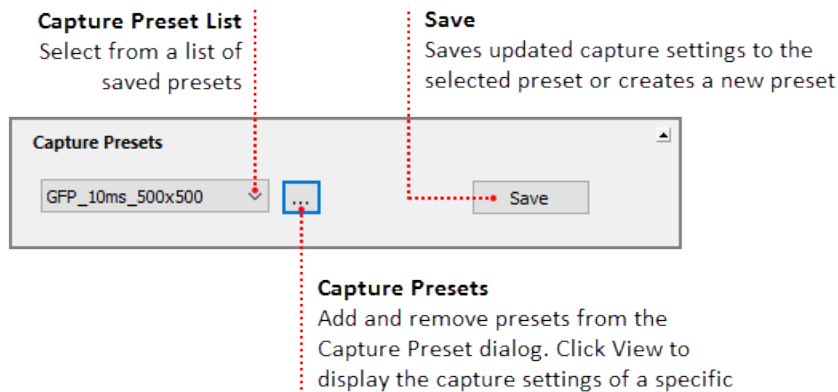
CAPTURE

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. HCIImage 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.

The screenshot shows the camera control software interface. The top section includes a dropdown for 'RGB Color: 3-Band' and a text field for 'C11440-42U S/N: 000030'. Below this are checkboxes for 'Live Color', 'Live', 'Capture1', 'AutoSave', and 'Open Capture1'. The 'Camera Control' section features an 'Auto Expose' button and a 'Gain' field. The bottom section displays three channels: Channel 1 (RED) with an exposure of 10.41 ms, Channel 2 (GREEN) with 11.97 ms, and Channel 3 (BLUE) with 13.27 ms. Each channel has a lock icon and a right-pointing arrow icon.

- 1 Select Capture Mode**
Select RGB Color: 3-Band
- 2 Select Filters**
Select Red for channel 1, Green for channel 2 and Blue for channel 3
- 3 Adjust Exposure**
Click Live and adjust the exposure manually or use Auto Expose
- 4 Capture a Color Image**
Click Capture1

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.

The screenshot shows the 'AutoSave Properties' dialog box. It has a 'Type' section with 'TIFF' selected and a checkbox for 'Use MPTIFF for multi-image Capture'. The 'Location' section includes a 'Folder' field with 'D:\Experiment Data\' and an ellipsis icon. The 'File Name' section has a 'Prefix' field with 'Image', a 'Start Number' field with '5', and a checkbox for 'Use Leading Zeros (ex: 00035)'. There is also a checkbox for 'Overwrite Existing Data'. At the bottom are 'OK' and 'Cancel' buttons.

- 1 TIFF or MPTIFF**
Enable to save as MPTIFF for multiple image capture versus individual TIFF images
- 2 Set Location**
Click the ellipsis icon and navigate to the destination directory
- 3 Set Default File Name**
Enter file name
- 4 Save Settings**
Click OK

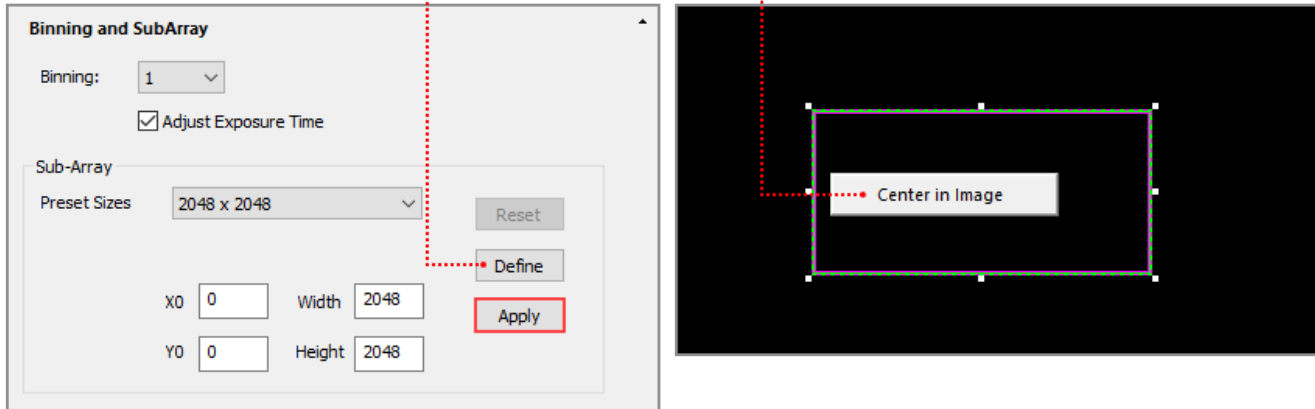
Define a Custom SubArray for Maximum Speed

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.

1 Define SubArray
Click Define and draw the area on the image

2 Center on Sensor
Right-click and select Center on Image

3 Apply SubArray
Click Apply



Note: Centering the subarray for maximum speed is only required for the ORCA-Flash 4.0 series cameras.

Control an LED using Output Trigger from the Camera

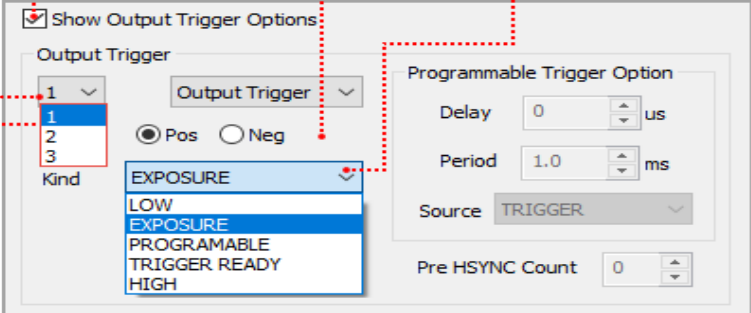

Some cameras provide a range of output trigger signals to synchronize with an external instrument where the camera becomes the master and the external instrument becomes the slave.

Timing Output
SMA connectors on the back of the camera

Show Output Trigger Options
Expand output trigger panel

Polarity
Positive or Negative

Trigger Output
Three output trigger functions plus a continuous fixed High or Low output



1
2
3

Kind

EXPOSURE
LOW
EXPOSURE
PROGRAMMABLE
TRIGGER READY
HIGH

Output Trigger

Pos Neg

Programmable Trigger Option

Delay 0 us

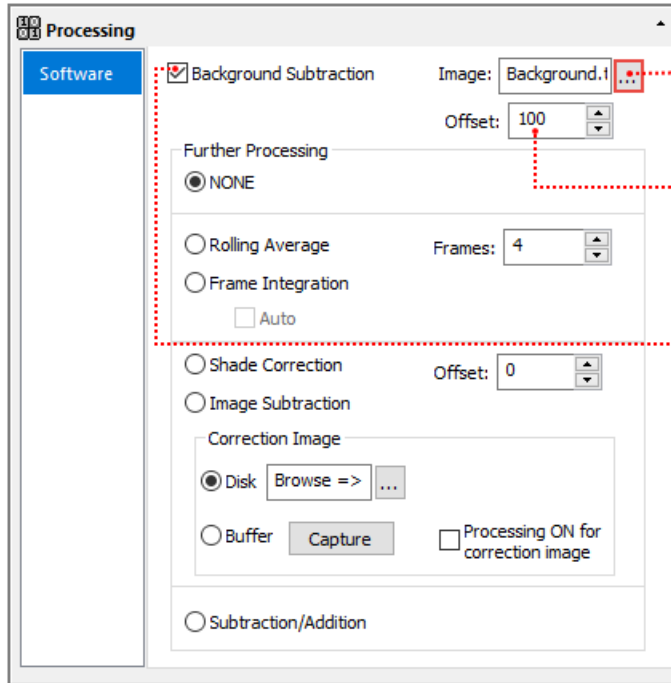
Period 1.0 ms

Source TRIGGER

Pre HSYNC Count 0

How to Setup a Background Subtraction

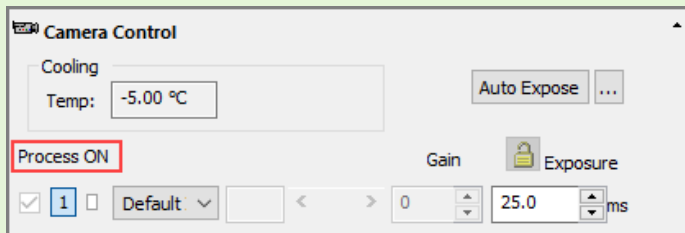
Typically used in fluorescence microscopy, a background subtraction can be used when the image presents a dark non-uniform background. To perform a background subtraction click Live, bring the sample into focus and then move the stage off of the sample so that only the background is visible. Next, follow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



The screenshot shows the 'Processing' window with the 'Software' tab selected. The 'Background Subtraction' checkbox is checked. The 'Image' field is set to 'Background.1'. The 'Offset' is set to 100. Under 'Further Processing', 'NONE' is selected. 'Frames' is set to 4. Under 'Correction Image', 'Disk' is selected with a 'Browse =>' button. 'Buffer' is selected with a 'Capture' button. 'Processing ON for correction image' is unchecked. 'Subtraction/Addition' is selected.

- 1 Correction Image**
Select Buffer and click Capture
- 2 Camera Offset**
Enter 100
- 3 Operation**
Select Background Subtraction

Hint: HcImage remembers the capture settings from the previous session, if background subtraction was left enabled, Process ON will be displayed in the Camera Control panel. The display image may appear distorted or black.



The screenshot shows the 'Camera Control' window. The 'Cooling' section has 'Temp: -5.00 °C' and an 'Auto Expose ...' button. The 'Process ON' button is highlighted with a red box. Below it, there are checkboxes for '1' and 'Default', and a 'Gain' section with a lock icon and 'Exposure' set to '25.0 ms'.

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.

Scan Settings
Save and load scan settings

Scan Type
Select acquisition type from list

Progress
Displays the number of images

Frame Rate
Displays the current speed in frames per second

Elapsed Time
Time from the start of the acquisition (hh:mm:ss.ms)

Event Markers
Annotate the time when a significant occurred

The screenshot shows a 'Select Scan Type' dropdown menu with 'Time Lapse' selected. Below it are 'Start' and 'Stop' buttons. A 'Progress' section shows '231' images and a green progress bar. A 'Frame Rate' display shows '43.01 fps'. A 'Time Elapsed' display shows '00:00:05.35'. An 'Event Marker' section shows '0' with a dropdown arrow. At the bottom, there are buttons for '0' through '9' and a 'Delay Remaining' display showing '00:00:00' with a right arrow.

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.

AutoSave
Define where and how to store acquired data

Speed
Select maximum speed or define a capture interval

Storage Type
Write data directly to disk (Slow) or stream into memory (Fast)

Display
Select a live display or to review acquired images

Control
Define acquisition endpoint by user control, frame number or time duration

Tooltip
Hovering over the delay time will display the units of time

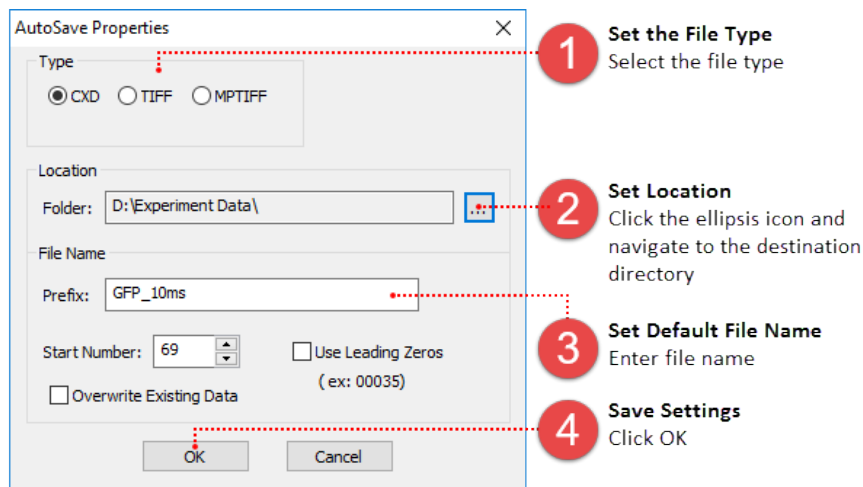
RAM Limit
Define the amount of available RAM for streaming

Temporary Buffer
Stream data to memory with the option to delete or save to a CXD, TIFF or MPTIFF

The screenshot shows the 'Scan Settings' panel. It has an 'AutoSave' checkbox checked with a dropdown arrow. Under 'Storage Type', there are radio buttons for 'CXD', 'TIFF', and 'MPTIFF'. Under 'Display', there are radio buttons for 'Live Image' and 'Review'. Under 'Speed', there is an 'Enable Maximum' checkbox checked. Below it are radio buttons for '0 Delay', 'Field Delay 1', and 'Field Delay 2'. The 'Field Delay 1' and '2' fields show '0.0 sec'. Under 'Control', there are radio buttons for 'Continuous', 'End Frame', and 'End Time'. The 'End Frame' field shows '2556' and the 'End Time' field shows '0.0 sec'. Under 'Storage Type', there are radio buttons for 'to Disk', 'to Memory (2555)', and 'to Temporary Buffer'. The 'to Memory (2555)' option has a 'RAM...' button next to it. A tooltip is shown over the '0.0 sec' field, containing the text: 'Type "u", "m", "s", "t" to change Units. u=microsec, m=millisec, s=sec, t=min'.

How to Use AutoSave

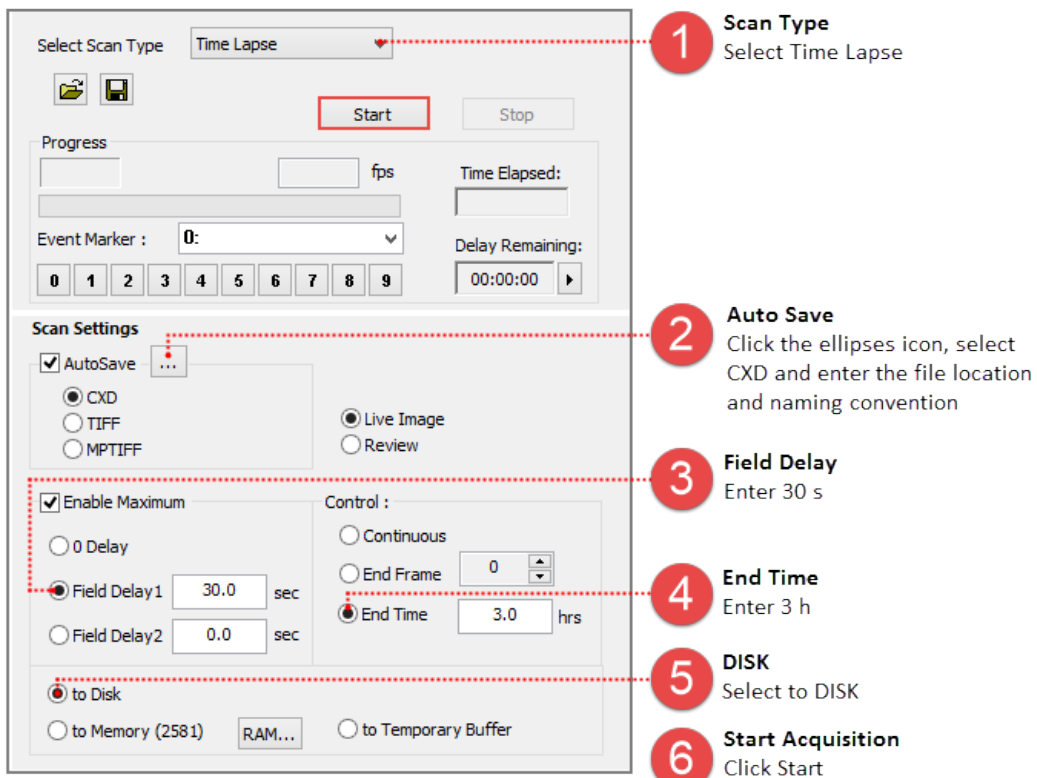
In the AutoSave Properties dialog, the user can determine how and where to store the acquired data. Image data can be saved as a CXD, TIFF or MPTIFF. The example below provides a description of the Auto Save Properties dialog.



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 you 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.

The image shows two windows from a software application. The top window is the 'Time Lapse' control panel, and the bottom window is the 'Save Buffered Images' dialog box. Red dashed lines and numbered callouts (1-8) indicate the steps for setting up a time lapse to be saved to the temporary buffer.

- 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 you are satisfied with capture settings 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 Continuous
Select Continuous

5 Memory
Select to Memory

6 Start Acquisition
Click Start

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+](#).

Control
Enter the number of frames to acquire and the approximate end time is displayed to the right

Stream Type
Stream directly to HDD or into memory with option to use Circular Buffer

AutoSave/AutoConvert
Define how streamed data is handled

DCIMG Location
Set a file location for streaming data to DISK

Display
Select a live display or to review acquired images

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.

The screenshot shows the software interface for streaming to disk. It includes a 'Select Scan Type' dropdown menu set to 'High Speed Streaming', a 'Start' button, and a 'Progress' section with a '0' value and 'fps' label. Below this is an 'Event Marker' dropdown and a 'Delay Remaining' timer set to '00:00:00'. The 'Scan Settings' section is expanded, showing 'Frame Count' set to '1000' and 'Best Time' as '9.9003 sec'. Underneath, there are options for 'DISK' (selected) and 'RAM', with a file path 'D:\Experiment Data\DCIMG\rec*.dcimg' and a 'Circular Buffer' checkbox. At the bottom, the 'AutoConvert' checkbox is checked, and file format options include 'CXD' (selected), 'TIFF', 'MPTIFF', 'Live Image', and 'Review'. Five numbered callouts point to these specific settings: 1. Select Scan Type, 2. Enter Frame Count, 3. Select Stream Type, 4. Auto Convert File Type, and 5. Start Streaming.

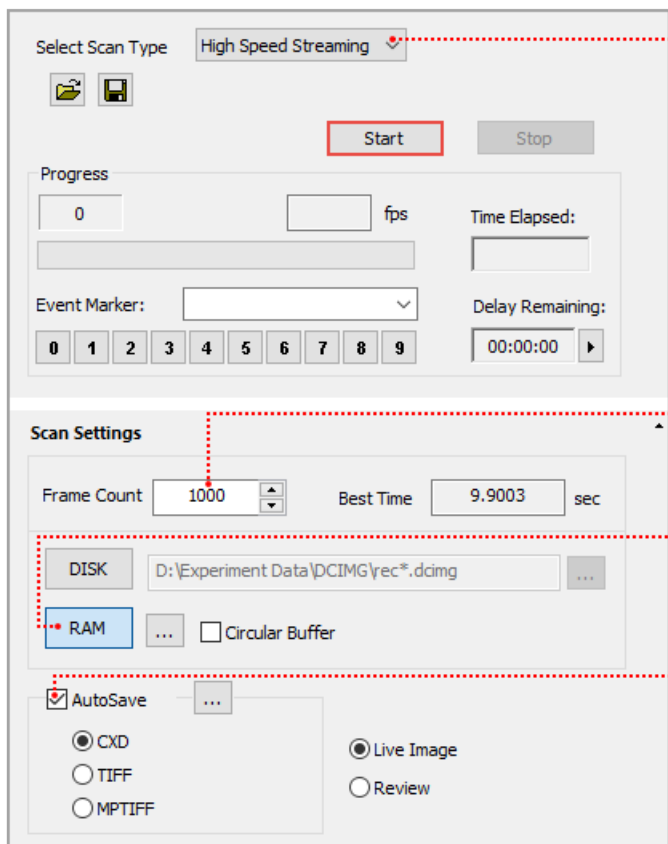
- 1 Select Scan Type**
Select High Speed Streaming
- 2 Enter Frame Count**
Enter the number of images to acquire
- 3 Select Stream Type**
Select DISK
- 4 Auto Convert File Type**
Enable AutoConvert and select file type
- 5 Start Streaming**
Click Start

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 you 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.



The screenshot shows the software's AutoSave Properties dialog. It is divided into several sections: 'Select Scan Type' (set to 'High Speed Streaming'), 'Progress' (0 fps, 00:00:00 Time Elapsed), 'Event Marker' (0-9 buttons), 'Scan Settings' (Frame Count: 1000, Best Time: 9.9003 sec), 'Storage' (DISK and RAM options), and 'AutoSave' (checked, with file type options: CXD, TIFF, MPTIFF, Live Image, Review). Five red dashed lines with numbered callouts point to specific elements: 1. 'High Speed Streaming' dropdown; 2. 'Frame Count' input field; 3. 'RAM' radio button; 4. 'AutoSave' checkbox and file type options; 5. 'Start' button.

- 1 Select Scan Type**
Select High Speed Streaming
- 2 Enter Frame Count**
Enter the number of images to acquire
- 3 Select Stream Type**
Select RAM
- 4 Auto Save File Type**
Enable AutoSave and select file type
- 5 Start Streaming**
Click Start

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 accessed by clicking DIA Analysis in the Sequence Pane. This functionality is only available in HCIImage DIA and HCIImage 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
Save and load scan settings

DIA Analysis
Enable the Intensity Analysis and Graph Setup panels

Progress
Displays the number of images acquired

Frame Rate
Displays the current speed in frames per second

Elapsed Time
Time from the start of the acquisition (hh:mm:ss.ms)

Event Markers
Annotate the time when a significant occurred

UI elements shown: Select Scan Type (Time Lapse), Use Scheduler (checkbox), DIA Analysis (checkbox), Start/Stop buttons, Progress bar (78), 7.81 fps, Time Elapsed: 00:00:09.87, Event Marker: 0: 2 μm Bradykinin, Delay Remaining: 00:00:00, and a numeric keypad (0-9).

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.

AutoSave
Define where and how to store acquired data

Display
Select a live display or to review acquired images

Speed
Select maximum speed or define a capture interval

Control
Define acquisition endpoint by user control, frame number or time duration

Storage Type
Write data directly to disk (Slow) or stream into memory (Fast)

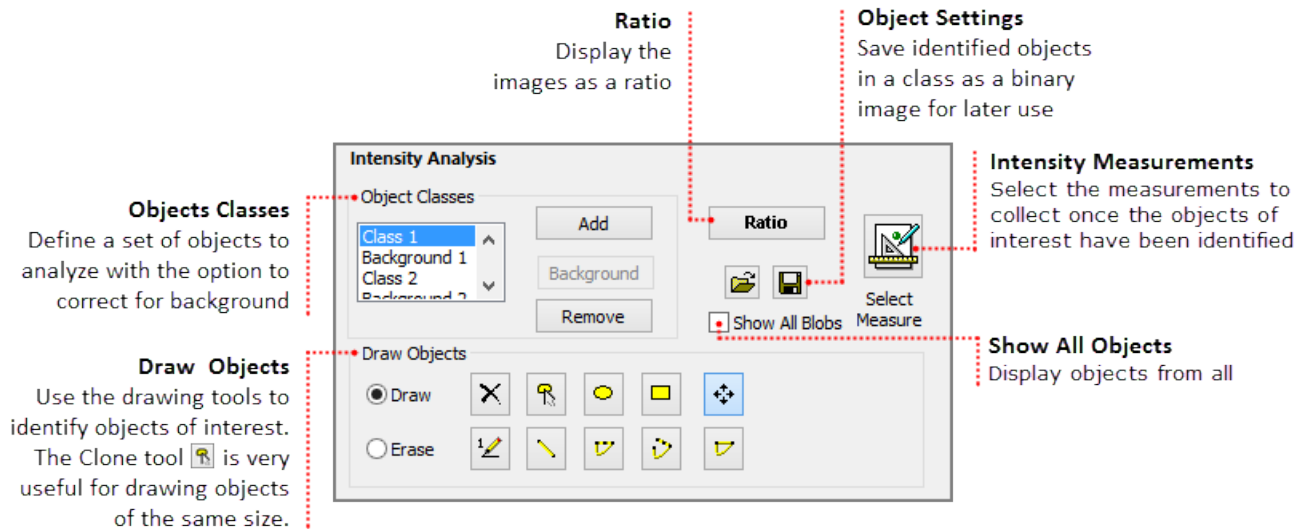
RAM Limit
Define the amount of available RAM for streaming

Tooltip
Hovering over the delay time will display the units of time

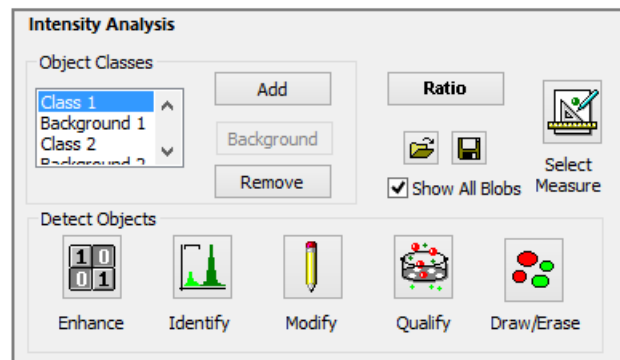
UI elements shown: AutoSave (checkbox), CXD (radio), TIFF (radio), MPTIFF (radio), Live Image (radio), Review (radio), Enable Maximum (checkbox), 0 Delay (radio), Field Delay1 (1.0 sec), Field Delay2 (5.0 sec), Control (radio), Continuous (radio), End Frame (16937), End Time (0.0 sec), to Disk (radio), to Memory (16961) (radio), RAM... button, and a tooltip: Type "u", "m", "s", "t" to change Units u=microsec, m=millisec, s=sec, t=min.

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**.

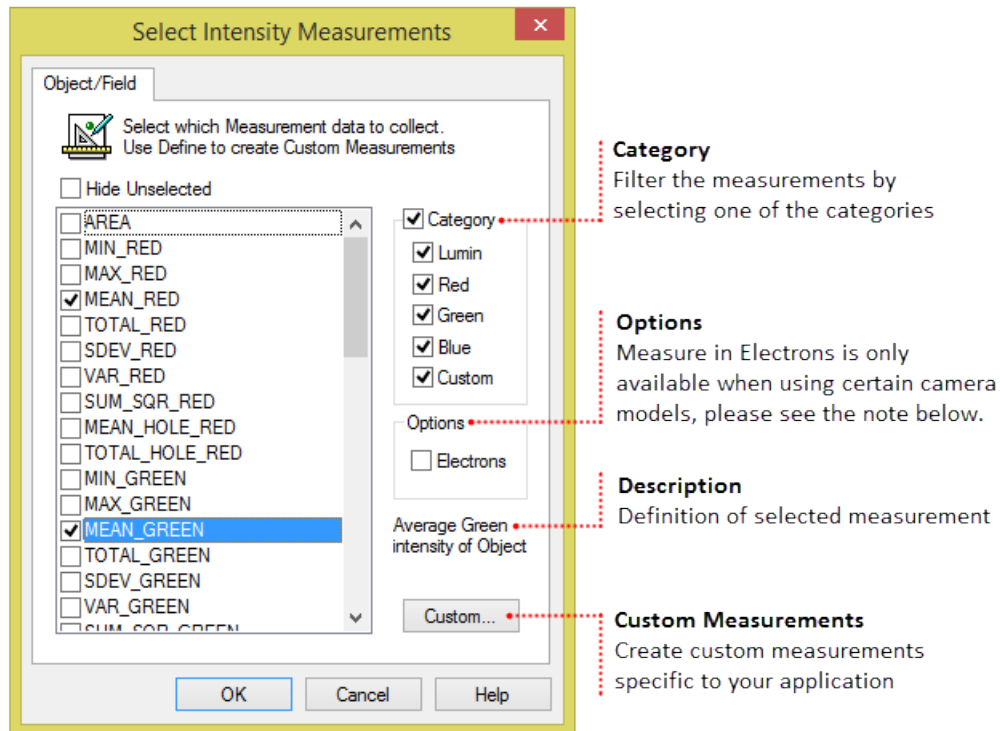


In the Advanced Mode, the user has the ability to identify a large number of objects, as well as, identify hard to detect objects because of defects in the image. The tools are grouped by function as described below:

- **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.

Image
Select which image to display in the graph when multiple monochrome images are acquired

Object
Select which objects will be displayed on the graph

Group List
A list of objects organized by size and class

Graphs
Select which graph settings to display

Class
Select which class to display or choose all classes

Group Size
Enter the number of objects to be included in a group

Edit Group
Select which objects to display from a list of objects in a group

Group Average
Display the mean value of the selected group of objects

View Measurements
Select which measurements to plot on the graph and which data to display:
Raw - uncorrected object data
Corrected - raw object data with the background fluorescence signal removed

Show Legend and Events
Select whether to display the Legend and show Events during the experiment. Keep in mind that as the number of objects and measurements increase, the legend will become very large.

Display History
Select whether to display the entire graph or a specific number of fields or time segment. Choose Fit, to resize the graph to fit the window rather than expand in blocks.

X Axis
Display the X axis as the Field Number or the Field Time

Graph Setup Panel Details:
 - Visible:
 - Image #: 1
 - Class #: 2
 - Object: Group List
 - Group: 2.1 - 2.5
 - Size: 5
 - View Measurements: MEAN_RED, MEAN_GREEN, RATIO_OF_MEANS_RG, Raw Data, Corrected Data
 - Display History: Full, Partial, Fit, 100 Fields, 30 sec
 - X Axis: X Axis = Field Number, X Axis = Field Time
 - Count: 15, Selected: 5

DIA ANALYSIS EXAMPLES

DIA Analysis can be run live, in real time, as well as on previously acquired data sets. The following examples provide instructions for various situations.

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.

The screenshot shows the software interface for DIA Analysis, divided into several panels. Red dashed lines and numbered callouts (1-8) point to specific settings and actions:

- 1 Enable DIA Analysis:** The **DIA Analysis** checkbox is checked in the top right.
- 2 Define Scan Settings:** The **Scan Settings** panel is expanded, and the **Field Delay 1** is set to 5 seconds.
- 3 Identify Objects of Interest:** The **Intensity Analysis** panel is expanded, and an ellipse icon is used to identify cells of interest in the image display.
- 4 Identify the Background:** The **Ratio** button is selected, and an ellipse icon is used to identify a background area in the image.
- 5 Define the Measurements:** The **Select Measurements** icon is clicked, and **Mean Red**, **Mean Green**, and **Ratio of the Means RG** are selected.
- 6 Configure Graph 1 Settings:** The **Graph Setup** panel is expanded, and the **Ratio of Means RG** checkbox is disabled.
- 7 Configure Graph 2 Settings:** **Graph 2** is selected, **Mean Red** and **Mean Green** checkboxes are disabled, and **Corrected Data** is enabled.
- 8 Start Acquisition:** The **Start** button is clicked.

DIA Post Acquisition Example

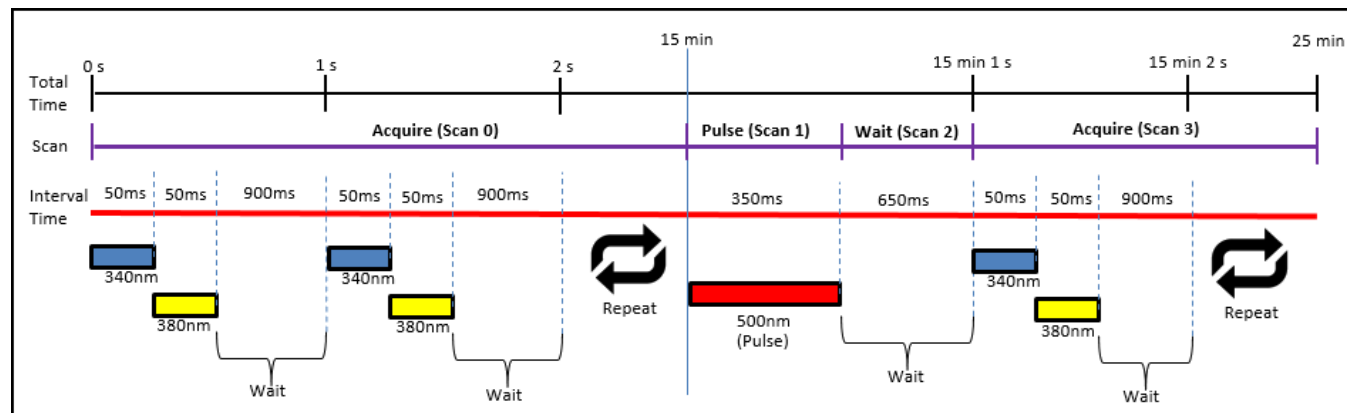
DIA Analysis can be run on previously acquired image sequences. The example below includes a single class of objects with background subtraction. Open the data document to analyze, go to the Sequence pane and follow the instructions below.

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**
Select the output format for your analysis.
Measure to...
DataDoc Spreadsheet Cancel
- 9**
Do you want to replace data in "D:\Fura gluc.cxd" or create a new file?
Replace New Cancel

DIA Scheduler Multi-channel Example

The diagram below provides a partial timeline of the experiment. Each component is broken down and plotted as interval time (red line), with the individual intervals labeled by action. Components are grouped into scans (purple line) and labeled by type. This example provides instructions for creating the schedule and defining the scans, identifying objects of interest, selecting measurement and graph setup have already been covered and are not included in the DIA Scheduler example.



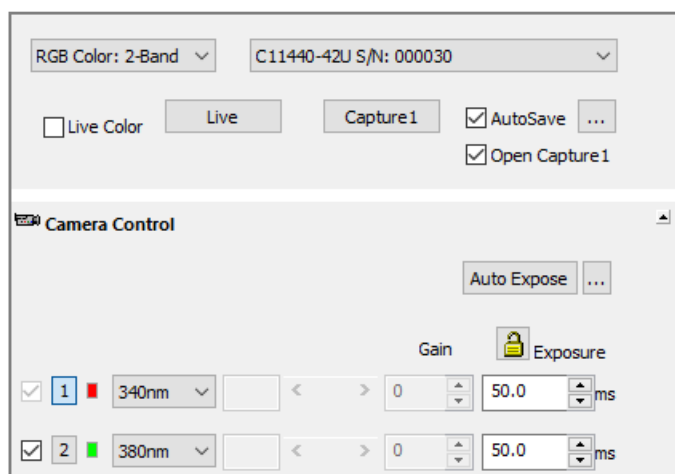
The schedule for the experiment consists of four scans, the scans are defined below:

1. **Scan 0** (Acquire) 340 nm and 380 nm with 50 ms exposures and a field delay of 900 ms for 15 minutes
2. **Scan 1** (Pulse) excite at 500 nm for 350 ms single pulse
3. **Scan 2** (Wait) delay of 650 ms
4. **Scan 3** (Acquire) 340 nm and 380 nm with 50 ms exposures and a field delay of 900 ms for 10 minutes

Create the Schedule

Before setting up the scans we need to enter the capture settings and configure the TTL settings for triggering pulses. For this example, the light source is controlled using TTL, where pin 2 (340 nm), pin 3 (380 nm), and pin 4 (500 nm).

Capture Settings



- 1 **Define Capture Settings**
Enable RGB Color: 2-Band, select 340 nm and 380 nm filters and enter a 50 ms exposure for each channel.

I/O Setup

Click the **I/O Setup** button in the Devices pane, the TTL trigger outputs are identified as Scan Pulse On, 0-3. If the I/O Setup button is not visible you will need to add an I/O device in the Profile.

Input and Output Job Settings

Link each I/O pin to a specific Capture Event or an Event Marker key. In Event Markers tab, you can customize names by highlighting the individual and press F2 key.

Enable I/O During Capture
 Capture Trigger/Signal
 Event Marker

Capture Inputs	Input Pins	State	Frequency
Sequence Start	None	None	None
Sequence Stop	None	None	None
0: Scan Start	None	None	None
1: Scan Start	None	None	None
2: Scan Start	None	None	None
3: Scan Start	None	None	None
4: Scan Start	None	None	None

Capture Outputs	Output Pins	State
Sequence On	None	None
Field On	None	None
Exposure On	None	None
0: Scan Pulse On	LPT1 P4-	High
1: Scan Pulse On	None	None
2: Scan Pulse On	None	None
3: Scan Pulse On	None	None

OK Cancel

- Define TTL Output**
For 0: Scan Pulse On, click None under Output Pins and select LPT1 P4.

Create Scans

Go to the Sequence pane, select Use Scheduler and DIA Analysis and follow the instructions below.

Select Scan Type: Time Lapse

Use Scheduler
 DIA Analysis

Frame Bundle: 2

Progress: 0 fps, Time Elapsed: 00:00:00.00

Event Marker: 0

Schedule Loop: 1

Name	Type	Duration	Progress	Status
Scan 0	Time	900.00 s	% 0	Not Started
Scan 1	TTL Out	0.35 s	% 0	Not Started
Scan 2	Delay	0.65 s	% 0	Not Started
Scan 3	Time	900.00 s	% 0	Not Started

- Define Scan 0 - Acquire**
Double-click Time and enter the settings below. Right-click and select Add Subscan.

Edit SubScan

Setup

Field Delay: 900.0 ms

End Frame: 1

End Time: 15.0 min

to Disk

to Memory (214) RAM...

OK Cancel

- Define Scan 1 - Pulse**
Select TTL Out from the list, double-click TTL Out and enter the settings below. Add the next Subscan.

Edit SubScan

TTL Out: 0

Asynchronous

Wait: 0.0 sec before 1st Pulse

Setup

Pulse Interval: 0.0 sec

End Count: 1

Pulse Duration: 350.0 ms

End Time: 0.0 sec

OK Cancel

- Define Scan 3 - Acquire**
Double-click Time and enter the settings below.

Edit SubScan

Setup

Field Delay: 900.0 ms

End Frame: 1

End Time: 10.0 min

to Disk

to Memory (261) RAM...

OK Cancel

- Define Scan 2 - Wait**
Select Delay from the list, double-click Delay and enter the settings below. Add the next Subscan.

Edit SubScan

Setup

Delay: 650.0 ms

End Frame: 1

End Time: 0.0 sec

OK Cancel

DIA Scheduler Monochrome Example

This example provides instructions for defining the scans, identifying objects of interest, selecting measurement and graph setup have already been covered and are not included in the DIA Scheduler example.

Create Scans

Go to the Sequence pane, select Use Scheduler and DIA Analysis and follow the instructions below.

The screenshot shows the DIA Scheduler interface. At the top, 'Select Scan Type' is set to 'Time Lapse', with 'Use Scheduler' and 'DIA Analysis' checked. Below this are 'Frame Bundle' (set to 2) and 'Start/Stop' buttons. The 'Progress' section shows '0' frames at '00:00:00.00' time elapsed. The 'Event Marker' is set to '0'. The 'Schedule' section shows 'Loop: 5' and a table of scans:

Name	Type	Duration	Progress	Status
Scan 0	Time	120.00 s	% 0	Not Started
Scan 1	Delay	60.00 s	% 0	Not Started
Scan 2	Time	60.00 s	% 0	Not Started

The 'Scan Settings' section at the bottom has 'AutoSave' checked. Under 'To a Single File', 'Average each Loop' is selected. Other options include 'CXD', 'TIFF', 'MPTIFF', 'Live Image', and 'Review'.

- Define Scan Settings**
Select Average each Loop.

- Define Loops**
Set the numbers of Loops to five.

- Define Scan 0 - Acquire**
Double-click Time and enter the settings below. Right-click and select Add Subscan.

The 'Edit SubScan' dialog for Scan 0 shows 'Setup' with 'Field Delay' set to 5 sec. 'End Frame' is set to 1 and 'End Time' is set to 5 min. The 'to Memory (685)' option is selected.

- Define Scan 1 - Wait**
Select Delay from the list, double-click Delay and enter the settings below. Add the next Subscan.

The 'Edit SubScan' dialog for Scan 1 shows 'Setup' with 'Delay' set to 1 min. 'End Frame' is set to 1 and 'End Time' is set to 0.0 sec. The 'to Memory (685)' option is selected.

- Define Scan 2 - Acquire**
Double-click Time and enter the settings below.

The 'Edit SubScan' dialog for Scan 2 shows 'Setup' with 'Field Delay' set to 0.0 sec. 'End Frame' is set to 1 and 'End Time' is set to 1 min. The 'to Memory (685)' option is selected.

VIEWING THE 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 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

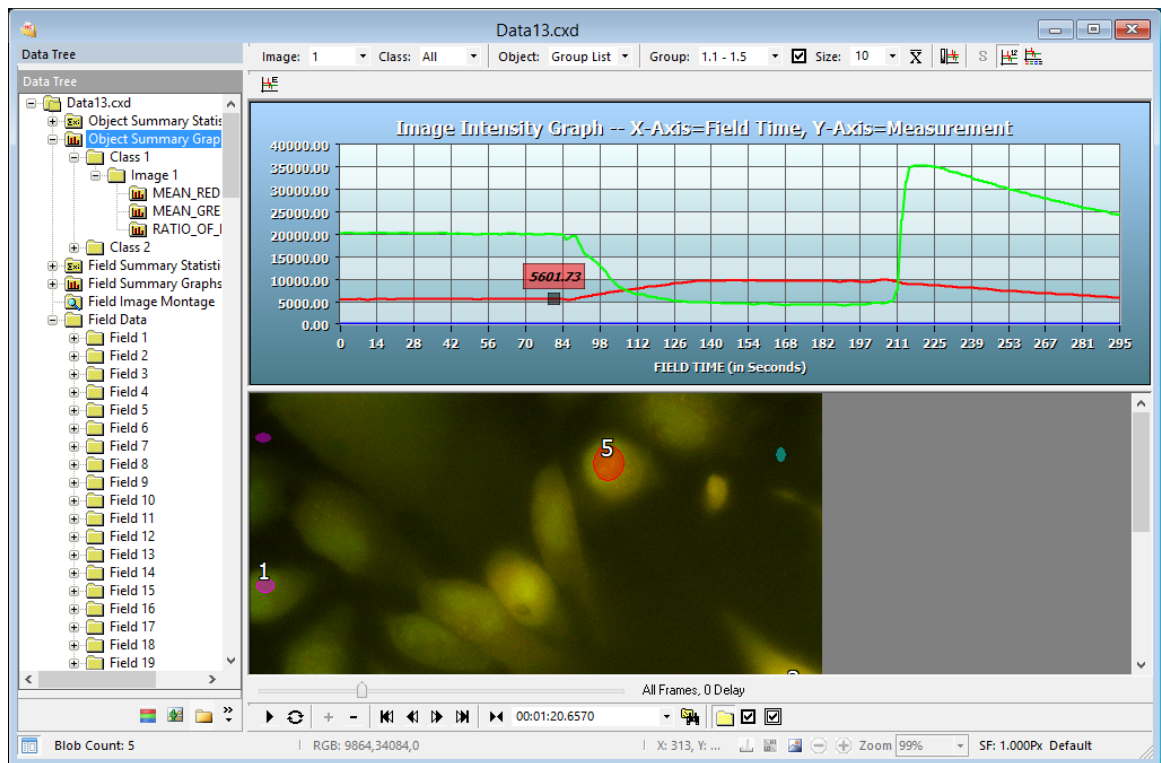
Statistical Measurements

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.

Image
Select which image to display in the graph when multiple monochrome images were acquired

Object
Select which objects will be displayed on the graph

Edit Group
Select which objects to display from a list of objects in a group

Group Size
Select or enter the number of objects to be included in a group

Data in Y Axis
Display the measurement data in the Y axis

Show Legend
Display legend

Event Markers
Display event markers

Class
Select which class to display or choose all classes


Group List
A list of objects organized by size and class

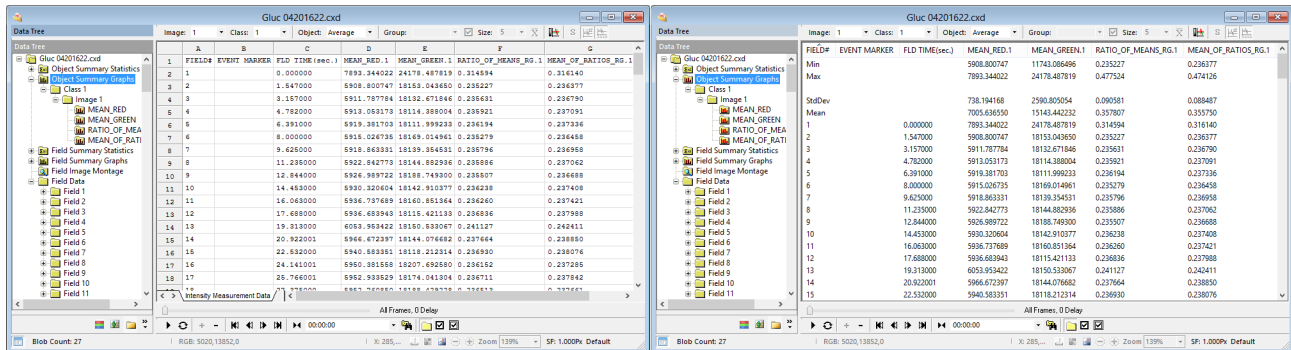
Group Average
Display the mean value of the selected group of objects

Select Measurements
Select which measurements to display in the graph

Object Summary Data

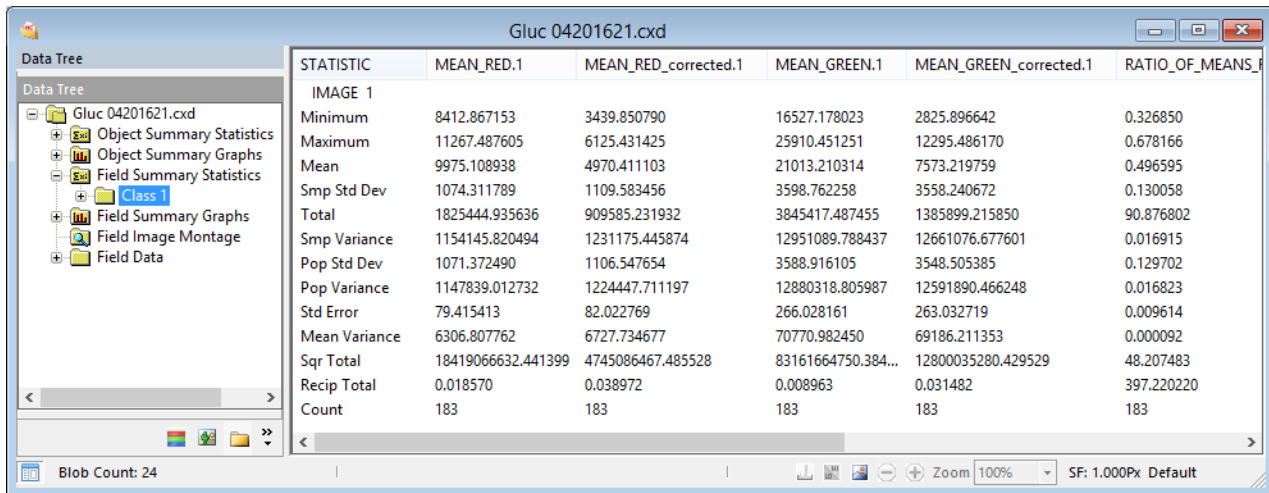
In addition to the Object Summary graphs, the intensity measurement data can also be displayed using a Spreadsheet View and a Table View. To change the view, go to the Image Data Views

toolbar, click on the Current View icon () and select either Spreadsheet View or Table View.



Field Summary Statistics

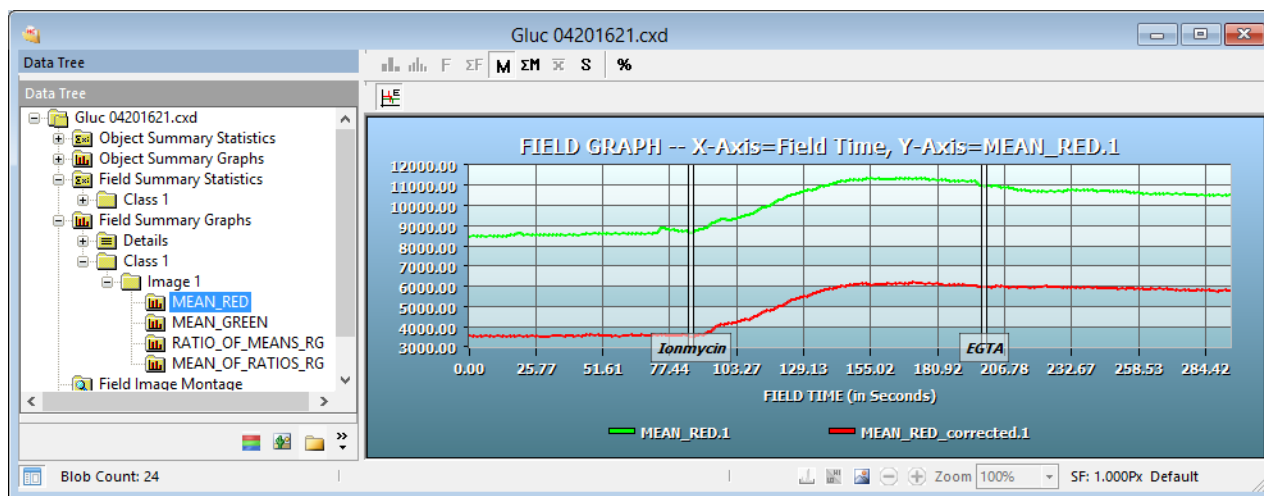
Field Summary Statistics are collected for each of the Field Measurements made for each Measurement Class. As Field Measurements in a Workfile may be selected and deselected during data collection the Count value may vary between Field Measurements. Each Statistic is computed according to the actual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




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.

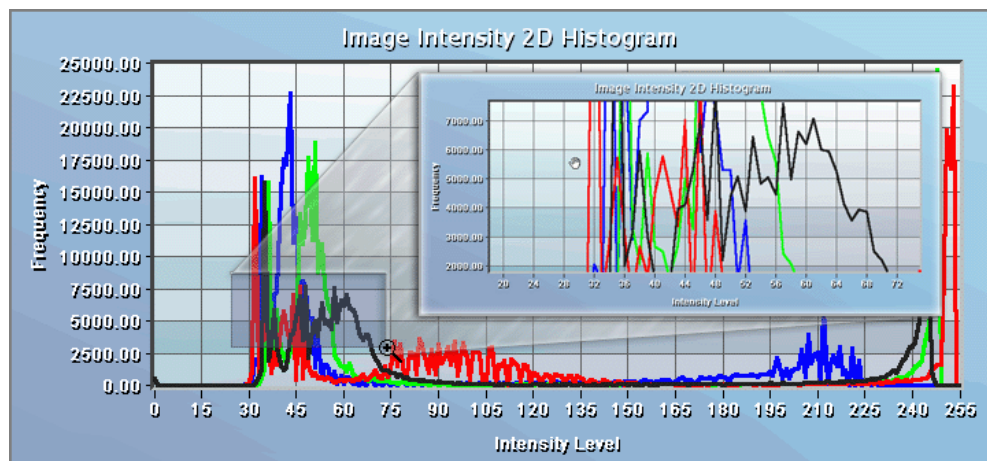
Field Summary Graphs

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

Fld#...	FldName	Event_Marker	Time_From_Start	Time_From_Last	MEAN_RED.1	MEAN_RED_corrected.1
53			0:01:23.890999	0:00:1.625000	8640.913408	3566.641803
54	lonmycin		0:01:25.500000	0:00:1.609000	8568.640913	3439.850790
55			0:01:27.110001	0:00:1.610000	8652.040742	3481.719755
56			0:01:28.719002	0:00:1.609000	8713.363011	3556.054369
57			0:01:30.328003	0:00:1.609000	8774.581986	3568.581986
58			0:01:31.985001	0:00:1.657000	8892.187115	3680.755016
59			0:01:33.610001	0:00:1.625000	9032.991118	3714.324451
60			0:01:35.203003	0:00:1.593000	9126.335076	3934.878286
61			0:01:36.813004	0:00:1.610000	9223.989395	4036.878284
62			0:01:38.438004	0:00:1.625000	9288.091550	4069.819946
63			0:01:40.046997	0:00:1.609000	9210.661075	4092.340087
64			0:01:41.656998	0:00:1.610000	9239.303845	4136.414956
65			0:01:43.265999	0:00:1.609000	9290.299666	4168.003370
66			0:01:44.875000	0:00:1.609000	9351.068198	4221.907705

IPA OVERVIEW

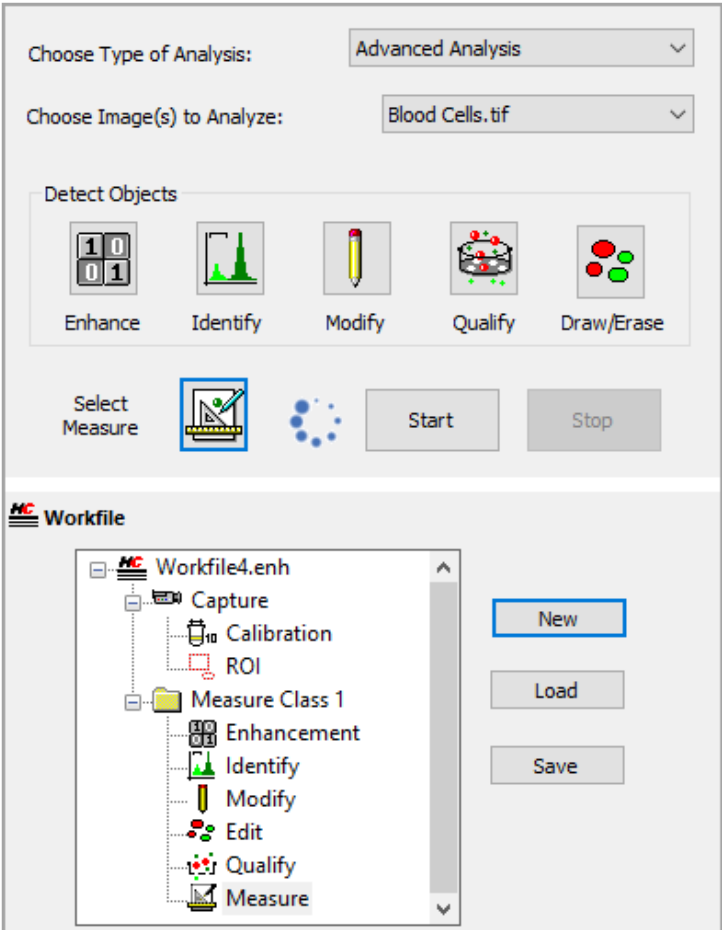
Image Processing and Analysis provide an extensive selection of image processing and image analysis tools to enable quantitative analysis on a wide range of complex image sequences. Imaging tools are selected using customized icons to derive workfiles (macros), which are saved and can be used multiple 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.



The screenshot displays the software's Advanced Analysis workflow. At the top, 'Choose Type of Analysis' is set to 'Advanced Analysis' and 'Choose Image(s) to Analyze' is 'Blood Cells.tif'. The 'Detect Objects' section includes icons for Enhance, Identify, Modify, Qualify, and Draw/Erase. Below these are 'Select Measure', a drawing tool icon, and 'Start' and 'Stop' buttons. The 'Workfile' pane shows a tree view for 'Workfile4.enh' with steps: Capture, Calibration, ROI, Measure Class 1, Enhancement, Identify, Modify, Edit, Qualify, and Measure. To the right, five image thumbnails illustrate the process: 1. Original grayscale image. 2. Binary image with green objects. 3. Binary image with modified object shapes. 4. Binary image with objects drawn/erased. 5. Final image with a table of measurements.

AREA.1	MEAN.C
532.000000	105.492
781.000000	110.521
573.000000	105.324
420.000000	110.206

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

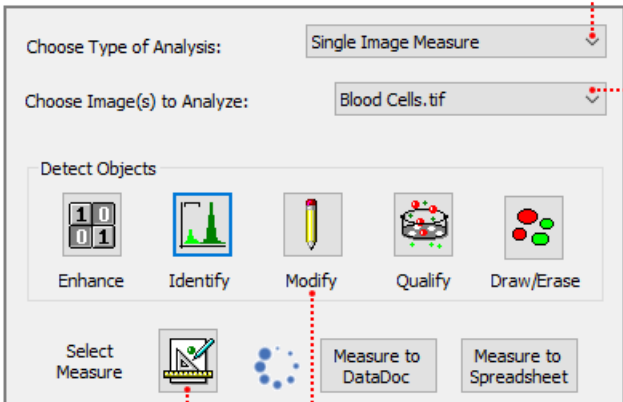
Qualify objects based on size, shape, position, etc.

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 be 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.

Single Image Measure
Measure the area, length and intensity of multiple objects in a single image

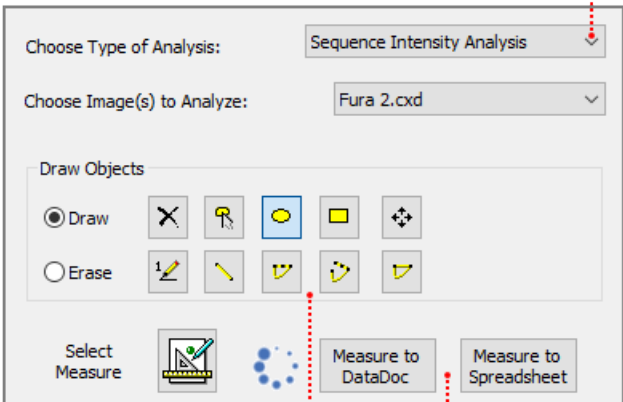


Measurements
Select the measurements to collect

Advanced Analysis Mode
Detect large numbers of objects and objects not easily differentiated using an interactive set of tools

Choose Data to Analyze
Select from open files or choose Live Scan to use the image in the Image Display

Sequence Intensity Analysis
Measure the intensity of a single object through an image sequence

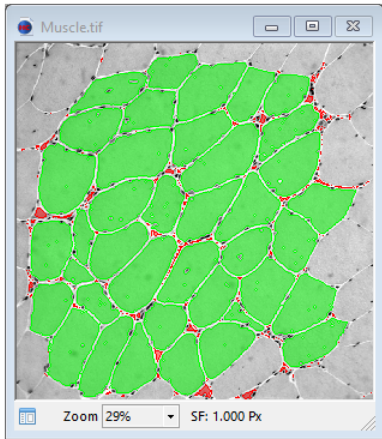
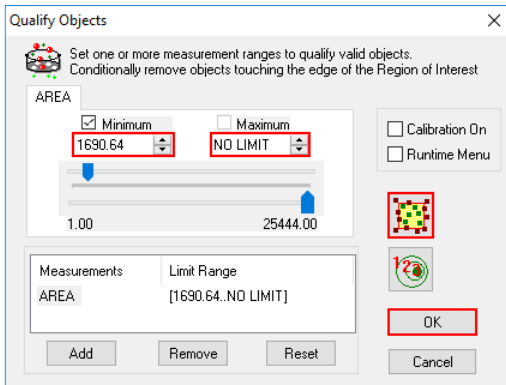


Simple Analysis Mode
Use the drawing tools to identify objects of interest. The Clone tool is very useful for drawing objects of the same size

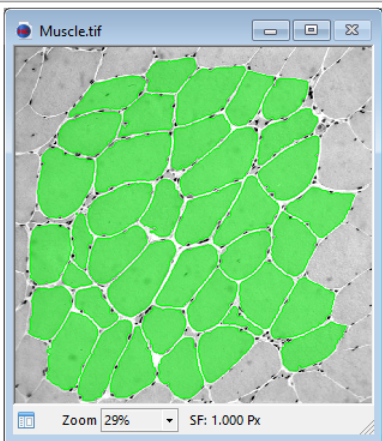
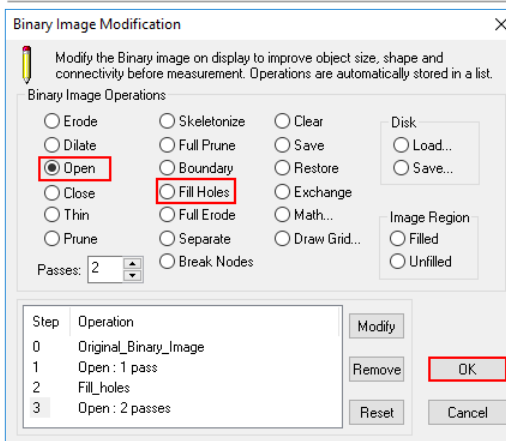
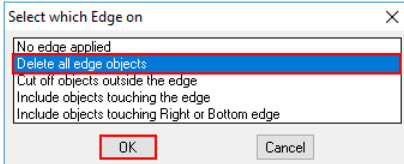
Output Format
Select whether to save the data to a data document or a spreadsheet

Sequence Intensity Analysis

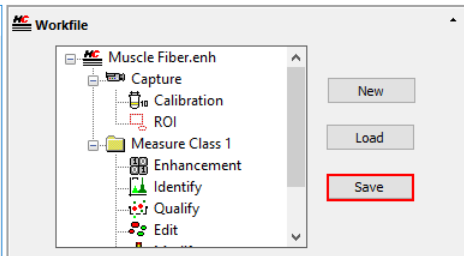
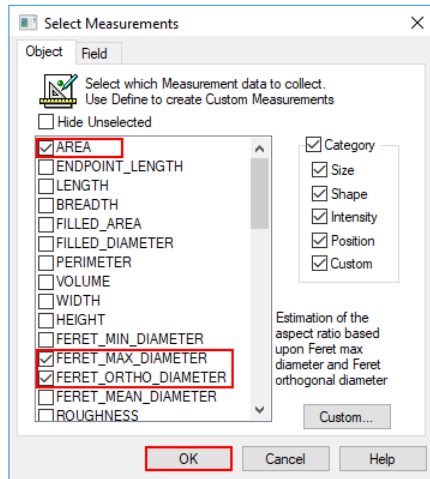
For Sequence Intensity Analysis, measure the intensity of a single object over time in an image sequence. The object of interest can be identified using the Advanced Analysis mode or drawn using the Simple Analysis mode (both shown above). The Simple mode is active by default but can be changed by clicking 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.



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.

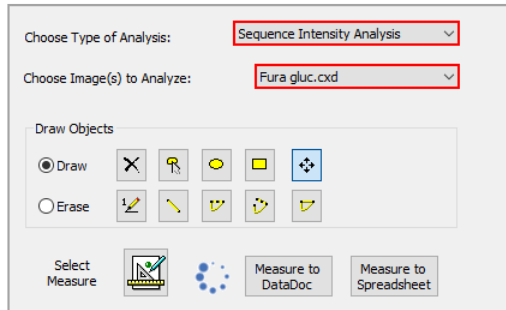


7 Select Measurements
Click the Measure icon, select Area, Ferret Max Diameter, Ferret Ortho Diameter, Ferret Elongation and Ferret Aspect Ratio. Click OK.

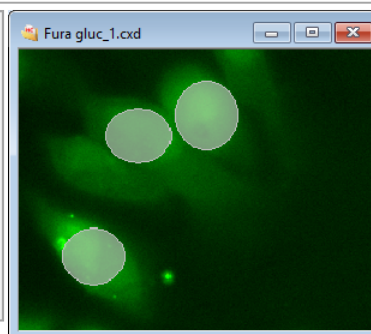
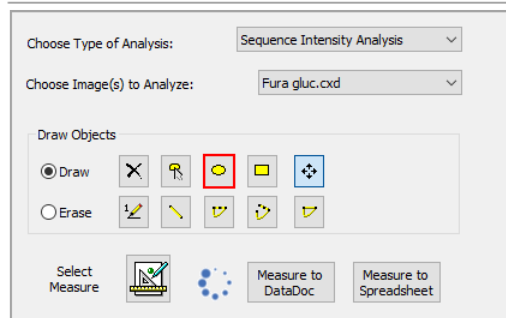
8 Save and Run the Workfile
Click the Save button and click Start.

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. HImage 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.

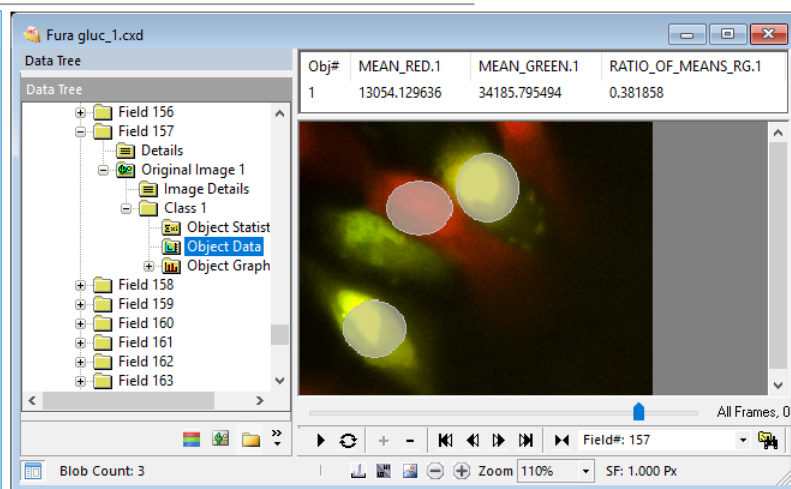
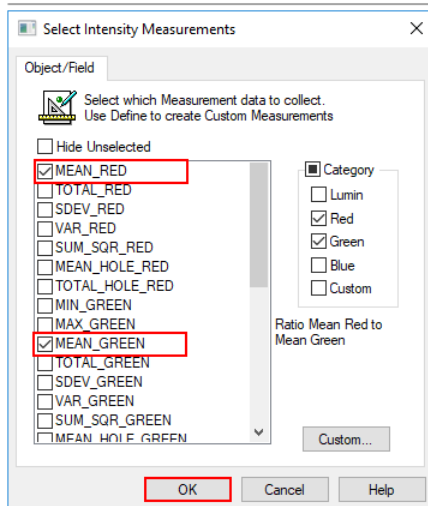


- 1 Enable Sequence Intensity Analysis**
Open the dataset and select Sequence Intensity Analysis from the drop-menu.

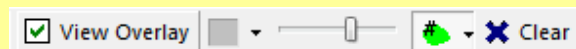


- 2 Draw Object**
Click the Ellipse icon and manually identify the object of interest.
Tooltip
Press SHIFT to draw a circle.

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

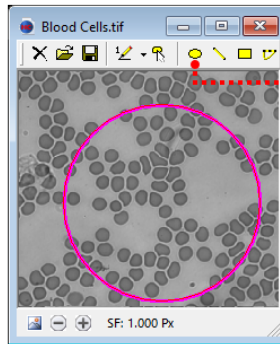
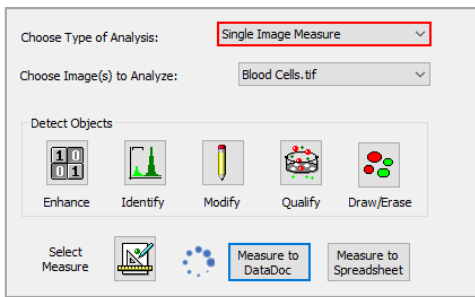



Note: Change the overlay color to silver instead of green in the **Change Overlay Color** icon. Use the translucency slider to adjust the overlay transparency or hide it by selecting **View Overlay**. Click **Clear** to delete the overlay.

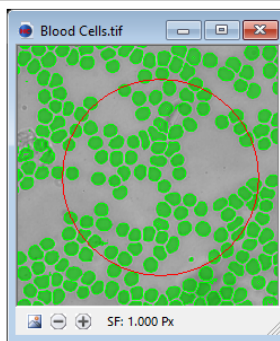
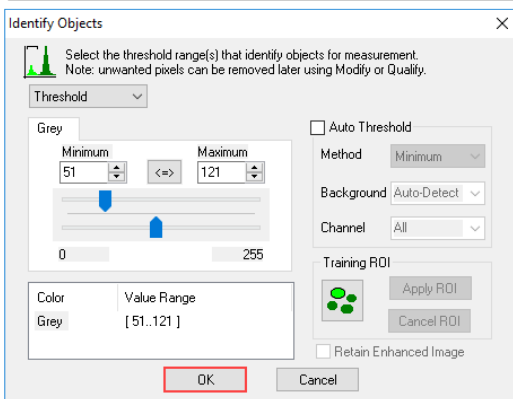



Single Image - Measure Analyze Objects Inside of a ROI

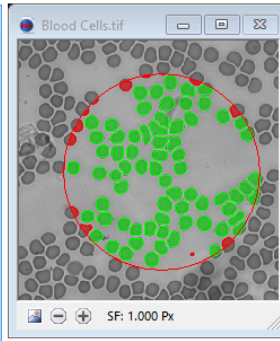
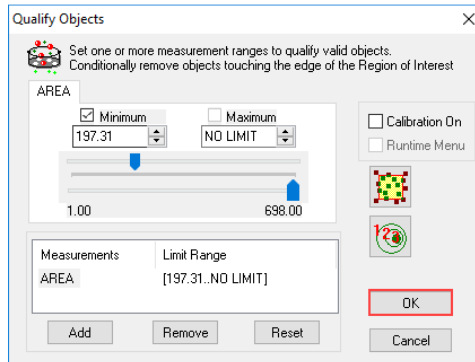
Enable the Advanced Analysis mode by clicking View on the Menu bar, then highlighting Analysis Mode and selecting Advanced. Open an image, go to the Analysis pane and select Single Image Measure from the drop-down list. Click on the image and follow the instructions below.




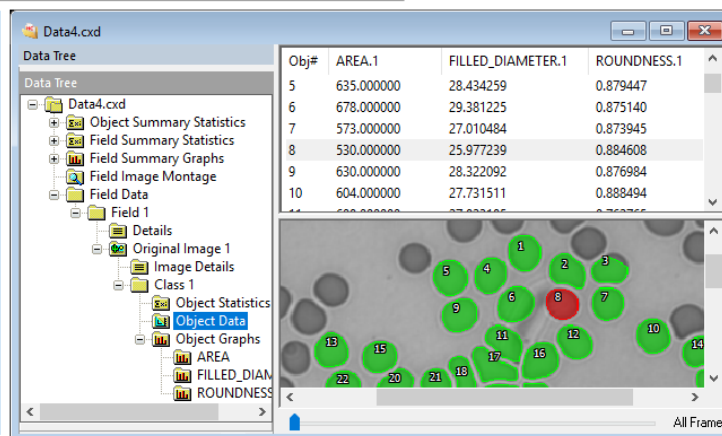
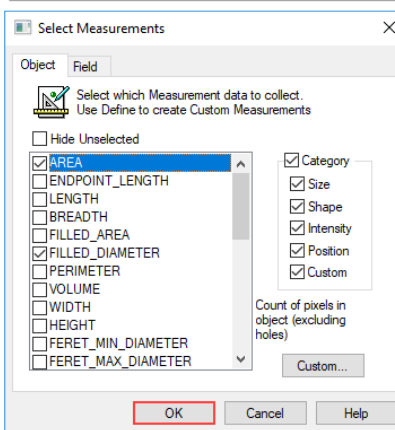
1 Define Region of Interest
 Activate the Image region layer , click the ellipse icon and draw region of interest





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



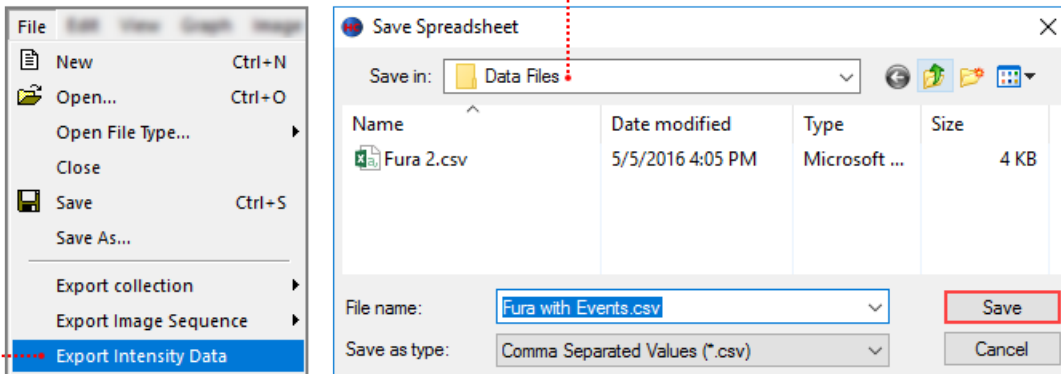
4 Analyze Objects of Interest
 Click the Measure icon , select measurements, click OK and select Measure to DataDoc 

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.

- 1 Export Intensity Data**
Go to File and select Export Intensity Data
- 2 Save to Spreadsheet**
Set the destination, enter file name and click Save

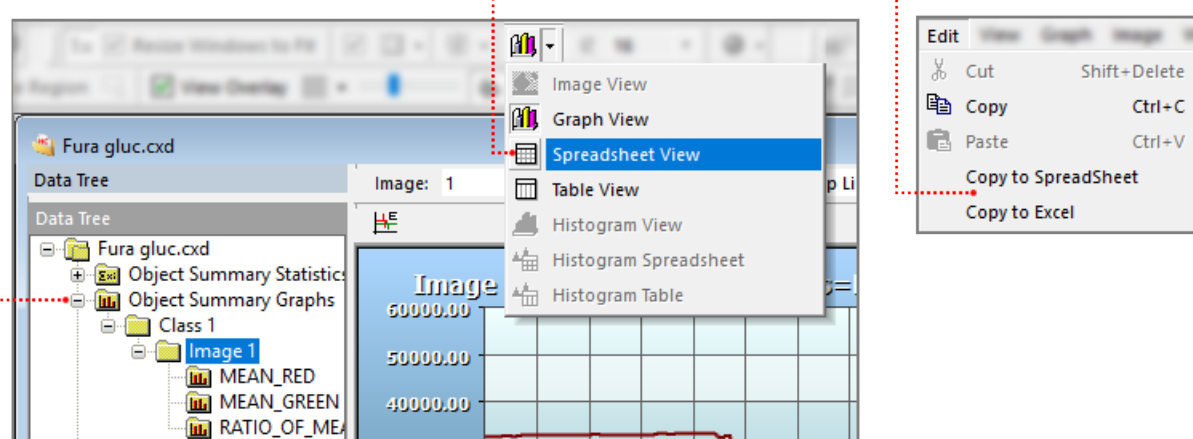


The 'File' menu is shown with 'Export Intensity Data' highlighted. The 'Save Spreadsheet' dialog box is open, showing the 'Save in' location as 'Data Files', the file name 'Fura with Events.csv', and the 'Save as type' as 'Comma Separated Values (*.csv)'. The 'Save' button is highlighted.

Copy to Spreadsheet or Excel

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

- 1 Display Object Data**
Go to Object Summary Graphs and select the data to display
- 2 Spreadsheet View**
Go to the Current View icon and select Spreadsheet View
- 3 Copy Object Data**
Go to Edit and select Copy to Spreadsheet or Copy to Excel



The software interface shows the 'Data Tree' with 'Image 1' selected. The 'View' menu is open, showing 'Spreadsheet View' selected. The 'Edit' menu is also open, showing 'Copy to Spreadsheet' selected.

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