HCImage Live
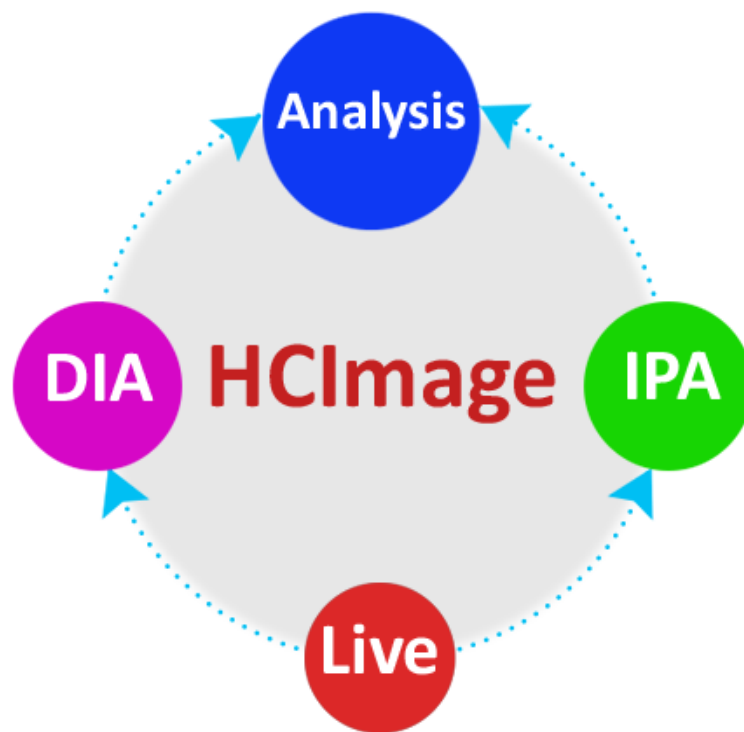
Getting Started Guide

Release 4.4

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INSTALLATION

## HCImage Live

1. Insert the HCImage Live installation DVD into the DVD-ROM drive. If autoplay is enabled, the HCImage Live setup will run automatically. If autoplay fails to start, locate your DVD-ROM drive and double-click **setup.exe**.
2. Click **Yes**, if prompted by the User Account Controls.
3. Review the Software License information and click **Yes**.
4. Review the README section for up-to-date information on software compatibility and support. When you are ready, click **Yes**.
5. On the Personalize screen, enter your registration information and click **Next**.
6. Choose the Destination Folder and click **Next**. It is recommended to install the software in the default path.
7. If you are ready to proceed with the installation, click **Install**.
8. Follow the instructions on each installation page.
9. Click **Finish**, when the installation is complete.
10. Install the appropriate DCAM-API drivers, see the instructions below, then turn the camera on before launching HCImage Live. If the drivers have not been installed, or the camera is not turned on before launching HCImage Live, the camera will not be available in the software.
11. Click the **HCImage Live** icon on the Desktop to launch HCImage Live.

Install DCAM-API Drivers

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

1. After installing HCImage Live from the DVD, you will be prompted to install DCAM-API, click **Yes**. If you downloaded HCImage Live, please go to http://www.dcam-api.com/ and download the DCAM-API drivers for Windows.
2. Click **Yes**, if prompted by the User Account Controls.

3. 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**.

4. Click **Next** to begin the installation.
5. Follow the instructions on each installation page.
6. Click **Finish** when the installation is complete.
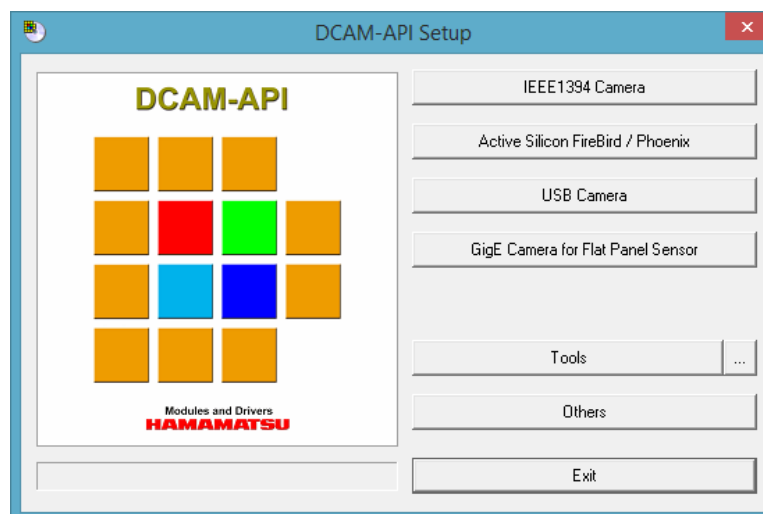
FILTER SETUP

## 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

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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.
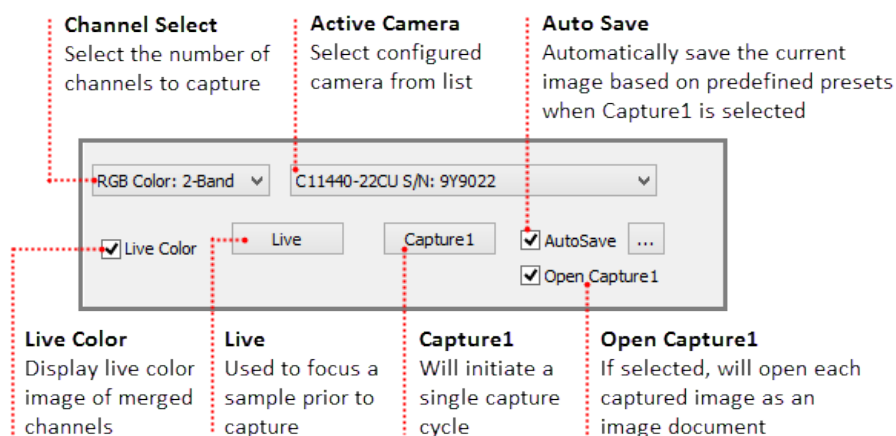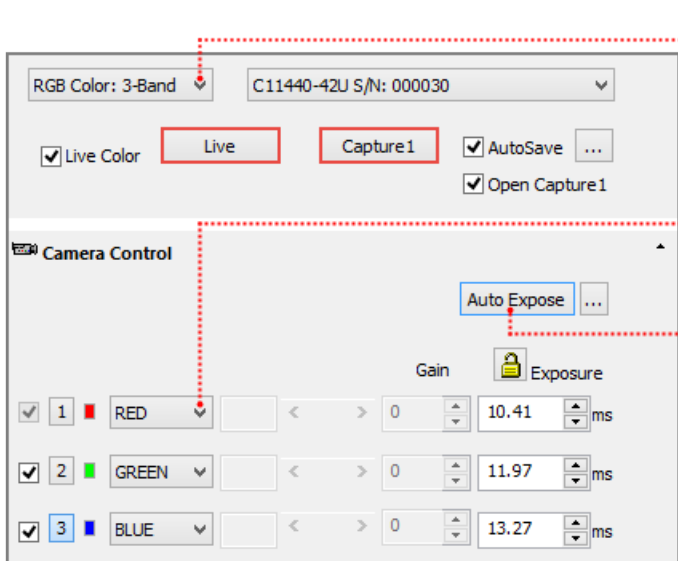

Capture Presets

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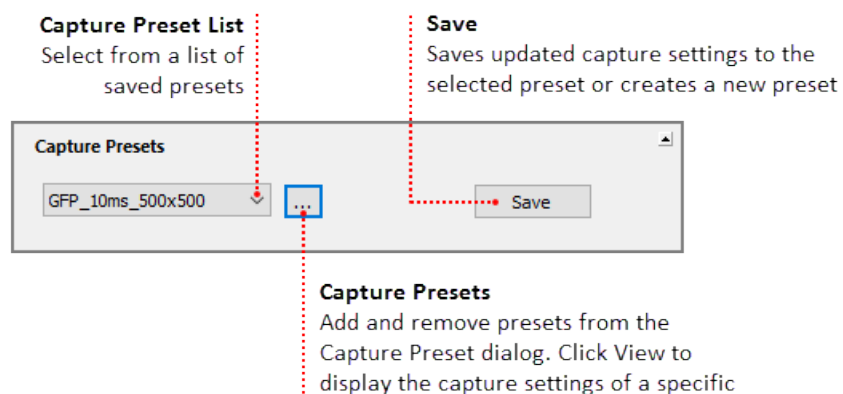
Note: Capture presets are not automatically saved before changing presets or exiting the software. To make changes to a saved capture preset, select the capture preset from the list, adjust the capture settings and click Save.

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Capture a Color Image

Capturing a color image requires filter setup, for instructions on configuring filters, please see "**Filter Setup**" on page 4.
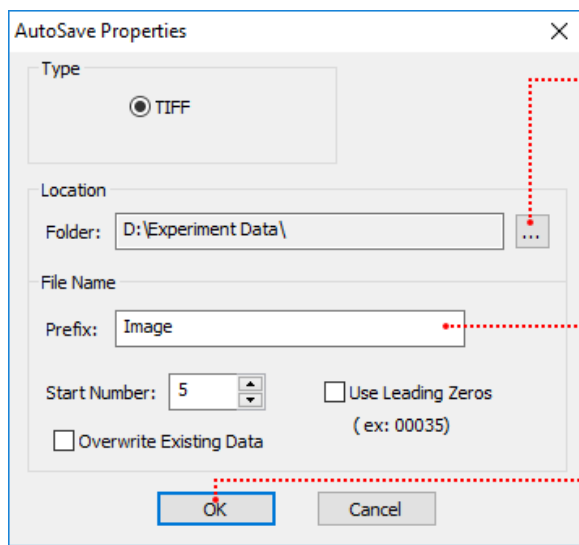

Select Capture Mode
Select RGB Color: 3-Band

Select Filters
Select Red for channel 1, Green for channel 2 and Blue for channel 3

Adjust Exposure
Click Live and adjust the exposure manually or use Auto Expose

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.
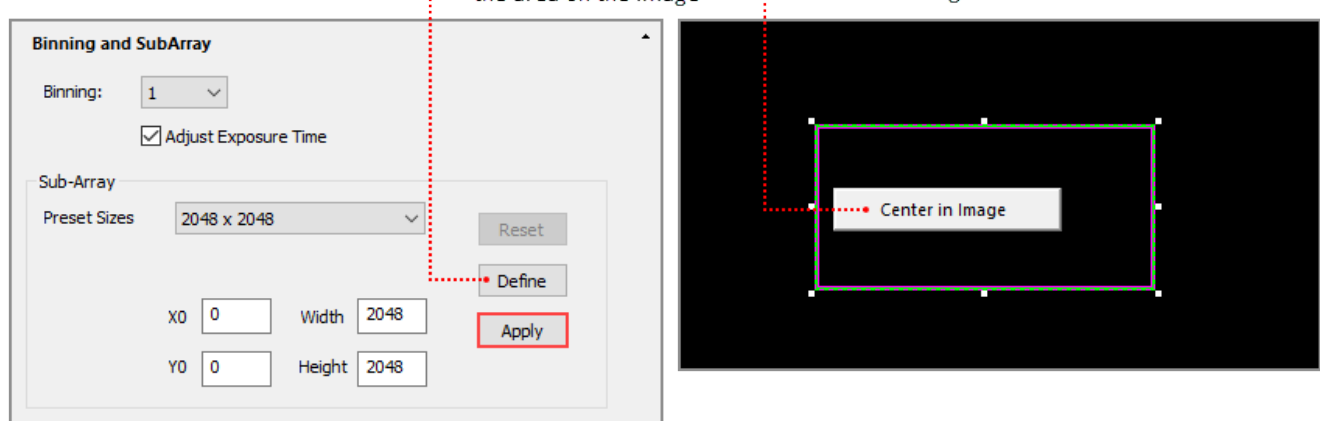

Set Location
Click the ellipsis icon and navigate to the destination directory

Set Default File Name
Enter file name

Save Settings
Click OK

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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.
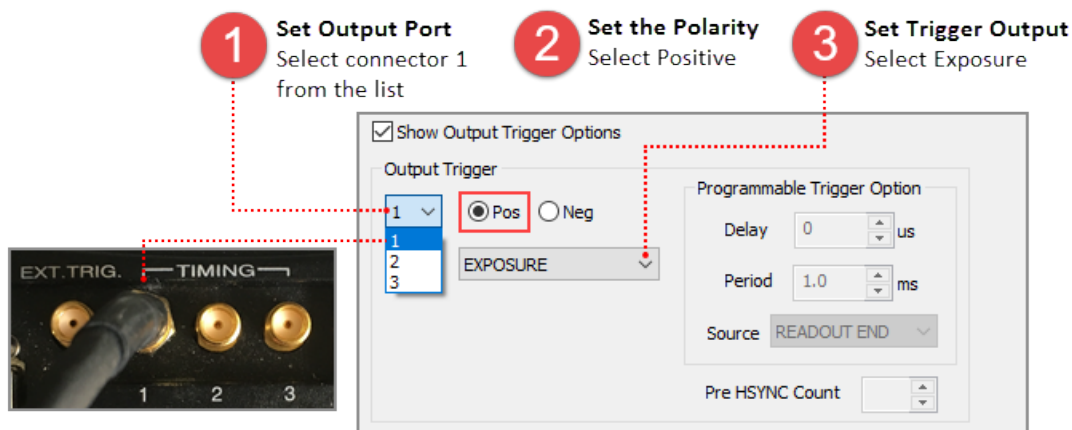

1 Define SubArray
Click Define and draw the area on the image

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

3 Apply SubArray
Click Apply

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.

1 Set Output Port
Select connector 1 from the list

2 Set the Polarity
Select Positive

3 Set Trigger Output
Select Exposure

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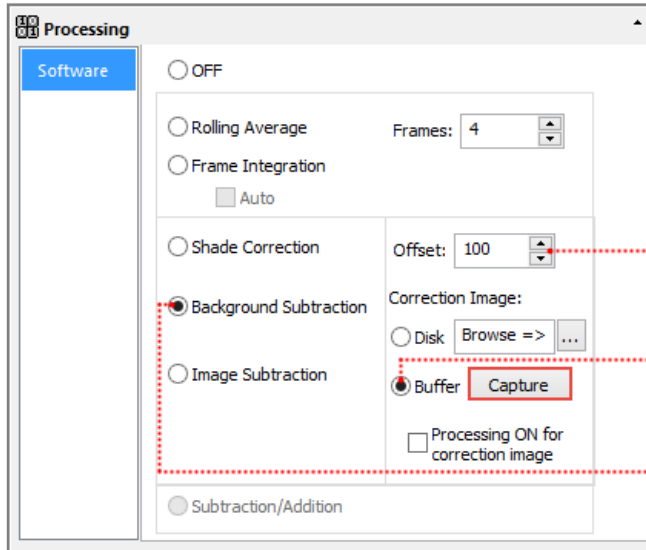
Hint: HCImage remembers the capture settings from the previous session, if background subtraction was left enabled, the following message will appear the next time HCImage is launched.
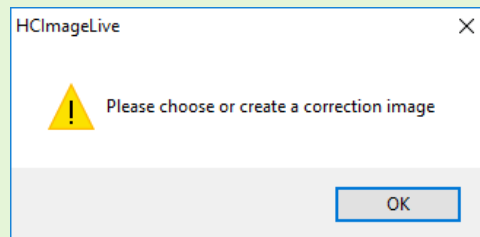
W-VIEW Mode

The W-VIEW mode allows for independent exposure time settings, independent readout directions and separate position offset for subarray.

Note: With W-VIEW mode, the readout direction for View 1 (top half) and for View 2 (bottom half) can be set to Forward or Backward under DCAM Properties in the Advanced Camera Properties panel.

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W-VIEW Capture Modes

HCImage Live will automatically detect the ORCA-Flash4.0 V3 and LT as two cameras, a normal camera and as a camera in W-VIEW mode. Select C13440-20CU S/N:#### W-VIEW for W-VIEW mode from the Capture Device list. The capture modes are explained below.
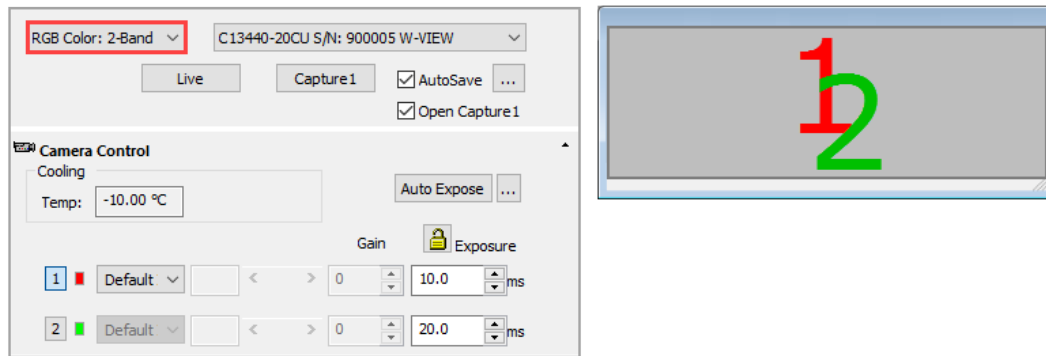
Mono 1 Channel

In the single channel monochrome mode, the user can select which image to display, only one image will be displayed at a time. Click on the 1 or 2 button to select which image will be displayed.
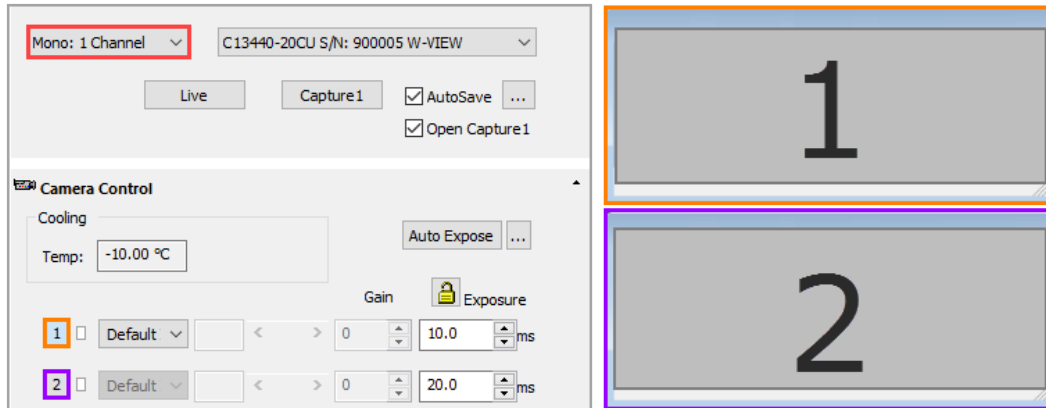
RGB Color 2-Band

The RGB Color 2-Band mode displays a merged red-green image from image 1 and 2.

Mono 2 Channel

In the two channel monochrome mode, both images 1 and 2 are displayed (i.e., the whole camera sensor is displayed).

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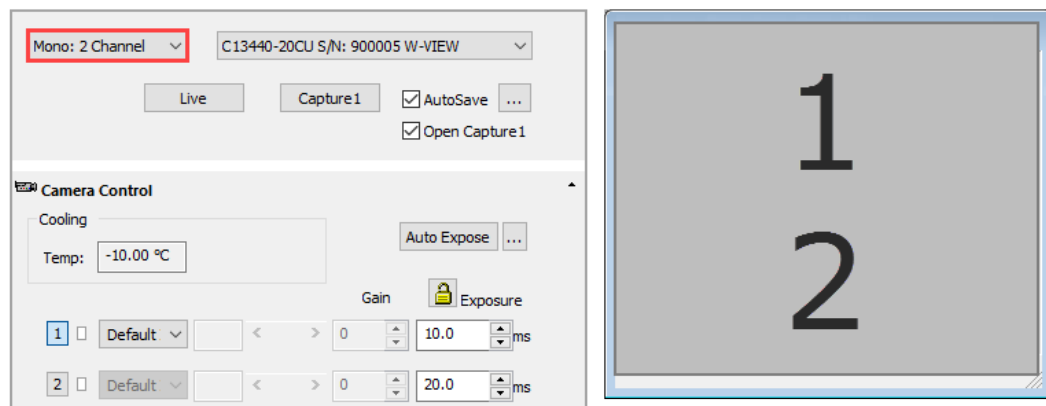
Image Alignment

The Camera Registration feature allows the users while Live to flip and rotate the image. Click on the Registration button in the Trigger Modes, Speed and Registration pane to open the Camera Registration dialog.
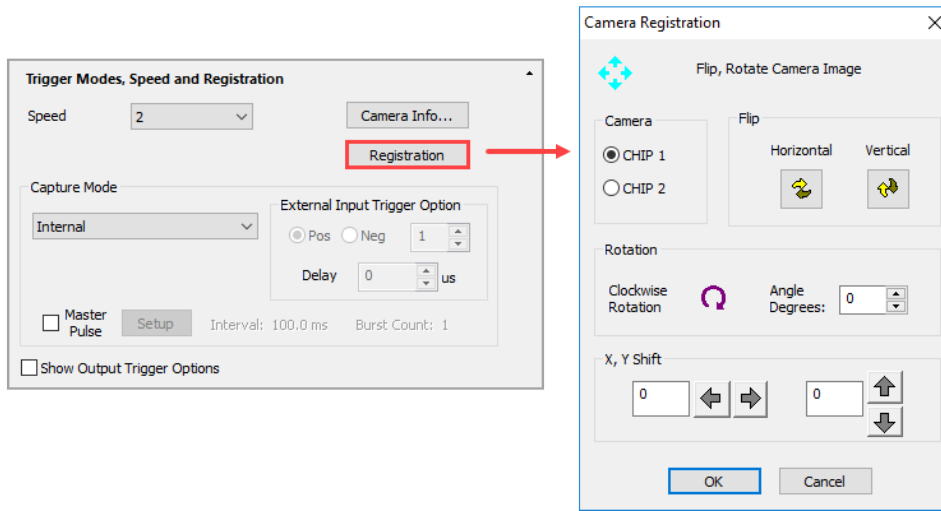
Calibrate an Image from Pixels to Microns

Capture an image with some known distance, for example a micrometer. Cick on the **Calibration Properties** icon (Calibration) on the Analysis toolbar and follow the steps 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

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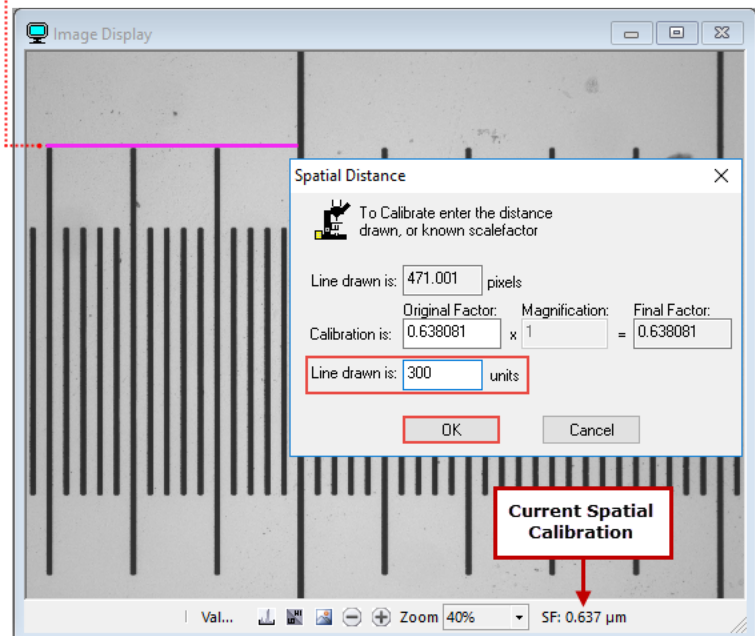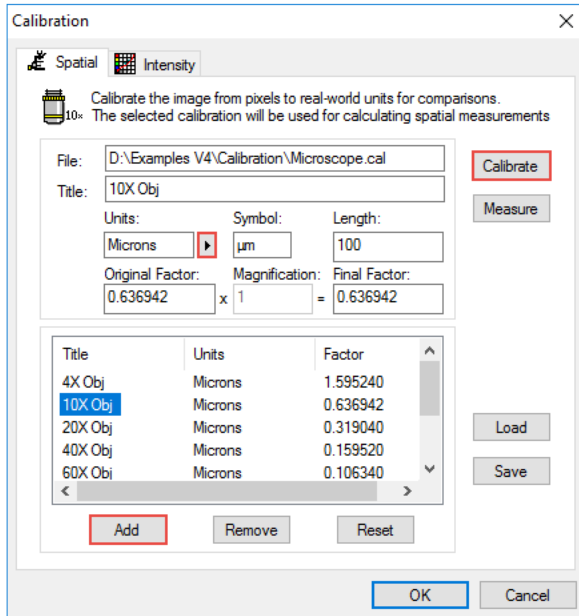
SEQUENCE

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.

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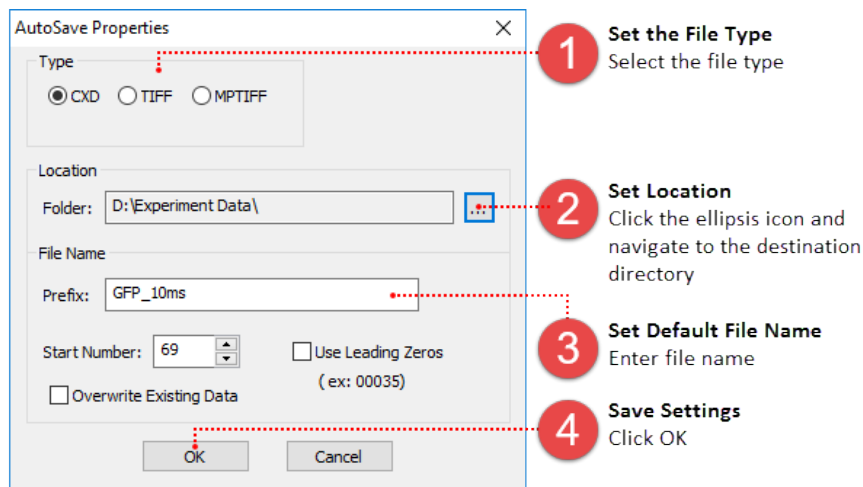
Note: MPTIFF files have a 65,000 image limit or 4 GB size limit. For image sequences exceeding these limits, multiple MPTIFF files will be saved and numbered sequentially.

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.
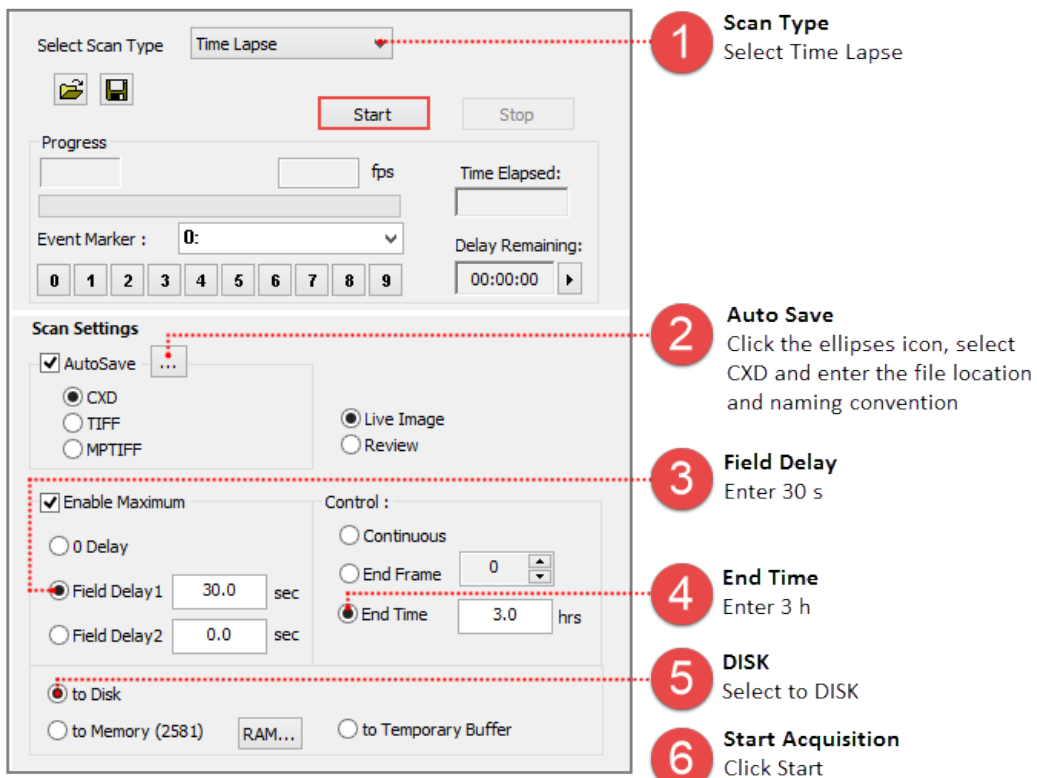
Setup a Time Lapse - Save to the Temporary Buffer

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.

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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.

Scan Type
Select Time Lapse

Auto Save
Click the ellipses icon, select CXD and enter the file location and naming convention

Field Delay
Select 0 Delay

Continuous
Select Continuous

Memory
Select to Memory

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

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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.

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

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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.
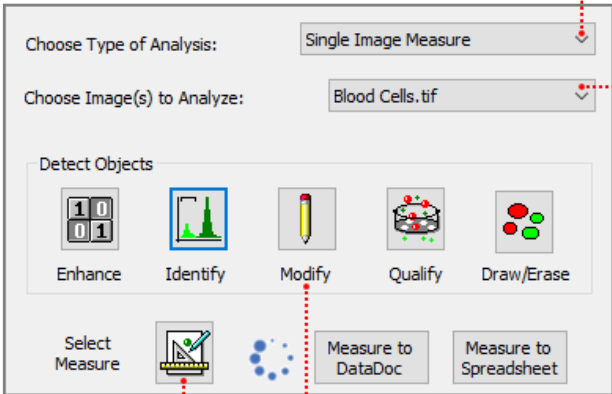

1 Select Scan Type
Select High Speed Streaming

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

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ANALYSIS

The Analysis panel provides all of the necessary tools for collecting data from images and image sequences. HCImage provides a Simple and an Advanced analysis mode for defining objects of interest, please see below.

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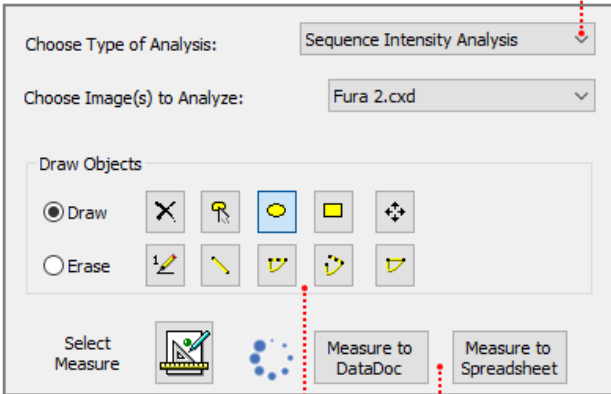Measure the area, length and intensity of multiple objects in a single image

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

Choose Type of Analysis: Single Image Measure

Choose Image(s) to Analyze: Blood Cells.tif

Detect Objects

Enhance   Identify   Modify   Qualify   Draw/Erase

Select Measure   Measure to DataDoc   Measure to Spreadsheet

Choose Type of Analysis: Sequence Intensity Analysis

Choose Image(s) to Analyze: Fura 2.cxd

Draw Objects

◉ Draw   ○ Erase

Select Measure   Measure to DataDoc   Measure to Spreadsheet

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

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
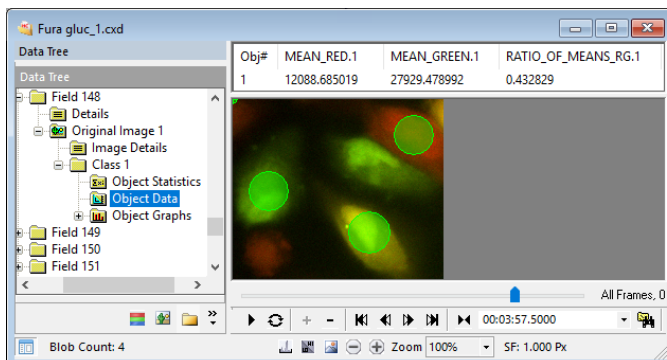
Sequence Intensity Analysis - Simple Mode

Sequence Intensity Analysis will measure the intensity of a single object in an image sequence. If multiple areas are drawn or identified, they are treated as a single object. Go to View in the menu bar, then highlight Analysis Mode and select Simple. Make sure that the image sequence to analyze is open, then select Sequence Intensity Analysis from the Choose Type of Analysis drop-menu and follow the instructions below.
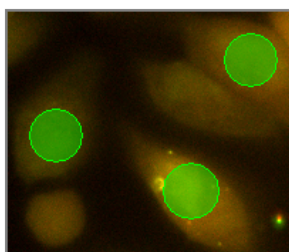
1 **Draw Object**
Click the Ellipse icon and manually identify the object of interest
**Tooltip**
Press SHIFT to draw a circle.
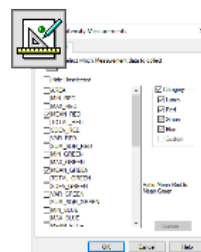
2 **Measure the Image**
Click the Measure icon, select Mean Red, Mean Green, Ratio of Means RG and click OK. Click the Measure to DataDoc button or the Measure to Spreadsheet button (both are shown below)

Fura gluc_1.cxd

Obj#	MEAN_RED.1	MEAN_GREEN.1	RATIO_OF_MEANS_RG.1
1	12088.685019	27929.478992	0.432829

Data Tree
- Field 148
  - Details
  - Original Image 1
    - Image Details
    - Class 1
      - Object Statistics
      - Object Data
      - Object Graphs
- Field 149
- Field 150
- Field 151

All Frames, 0

00:03:57.5000

Blob Count: 4   Zoom 100%   SF: 1.000 Px

Fura gluc_1.csv

	A	B	C	D	E	F
1	Field Data					
2		Field#	MEAN_RED.1	MEAN_GREEN.1	RATIO_OF_MEANS_RG.1	
3		1	8661.491742	27626.192988	0.313525	
4		2	8662.284555	27637.028108	0.313430	
5		3	8676.067227	27667.617502	0.313582	
6		4	8671.206027	27599.880614	0.314175	
7		5	8704.006955	27593.263402	0.315440	
8		6	8678.729644	27683.110982	0.313503	
9		7	8693.033903	27660.238771	0.314279	
10		8	8693.869603	27654.514054	0.314374	
11		9	8706.153579	27791.834251	0.313263	
12		10	8703.217618	27681.066358	0.314410	
13		11	8732.595769	27730.422486	0.314910	

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# Analyze a Single Image - Advanced Mode

Enable the Advanced Analysis mode by clicking View in the menu bar, then highlighting Analysis Mode and selecting Advanced. Make sure that the image to analyze is open, then select Single Image Measure from the Choose Type of Analysis drop-menu and follow the instructions below.
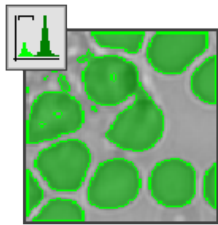

1 Threshold the Image
Click the Identify icon and adjust the min/max sliders until the objects of interest are highlighted by the green binary overlay. Click OK
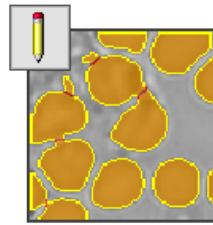
2 Modify the Binary Image
Click the Modify icon and select binary operations such as Open, Close and Separate to edit the binary overlay. Click OK
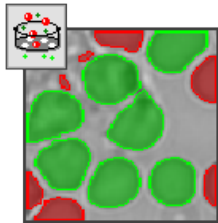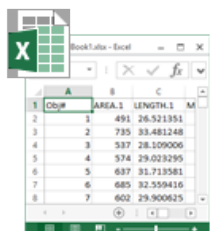

3 Remove Unwanted Objects
Click the Qualify icon and adjust the min/max sliders to reject unwanted objects by area. Rejected objects will appear red. Click OK
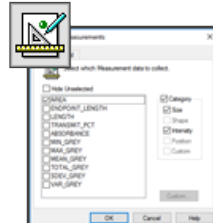
4 Measure the Image
Click the Measure icon, select Area and click OK. Click the Measure to DataDoc button

5 Export Data to Excel
While in the Object Data folder, go to Edit in the menu bar and select Copy to Excel. Please note, Excel must be installed on the system

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