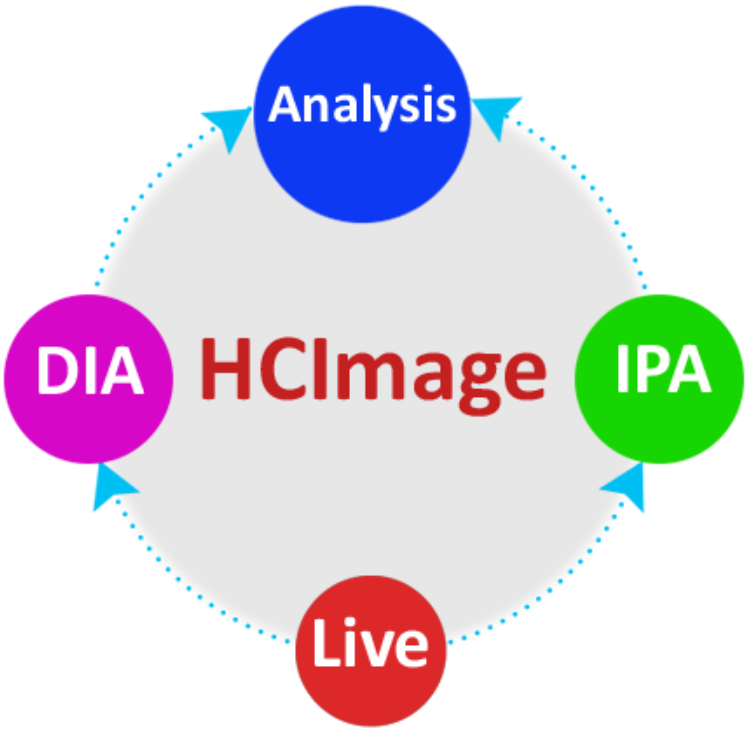
HAMAMATSU
PHOTON IS OUR BUSINESS

HCImage Live

Getting Started Guide



Release 4.5

November 2018

Hamamatsu Corporation
360 Foothill Road, Box 6910
Bridgewater, NJ 08807-0910
USA
+1 908.231.0960
sales@hamamatsu.com
http://sales.hamamatsu.com/

Software Support
hcsupport@hamamatsu.com
www.hcimage.com

Table of Contents

INSTALLATION

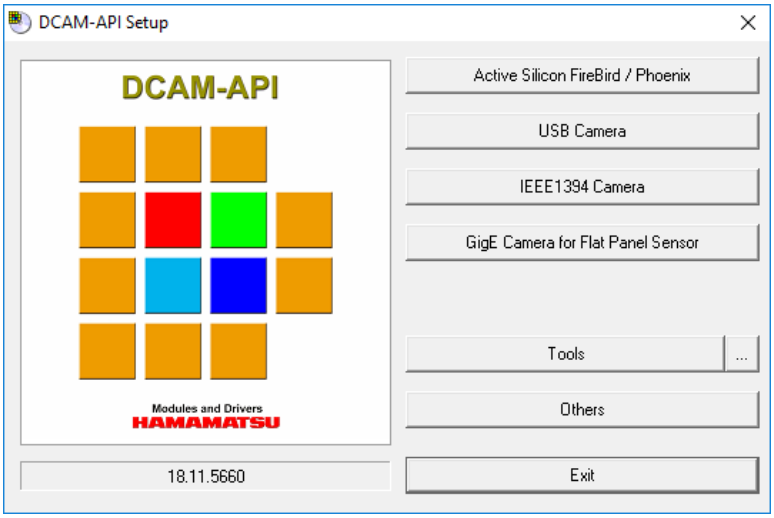
HCImage Live

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**.

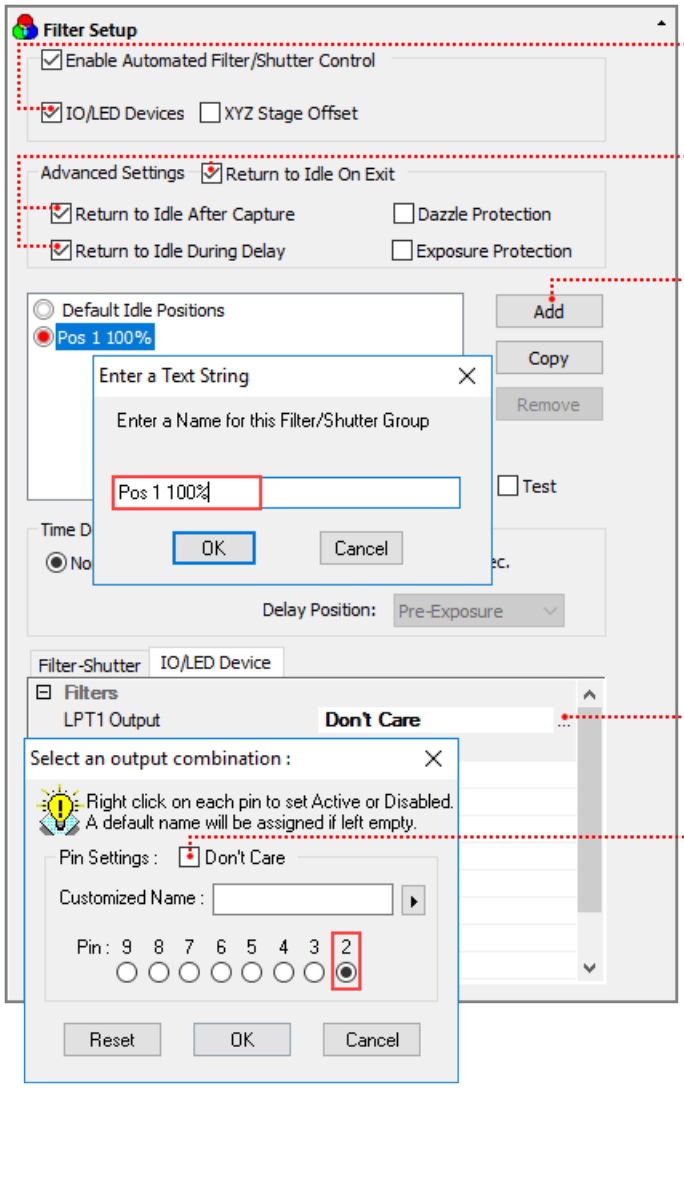


4. Click **Next** to begin the installation.
5. Follow the instructions on each installation page.
6. Click **Finish** when the installation is complete.

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

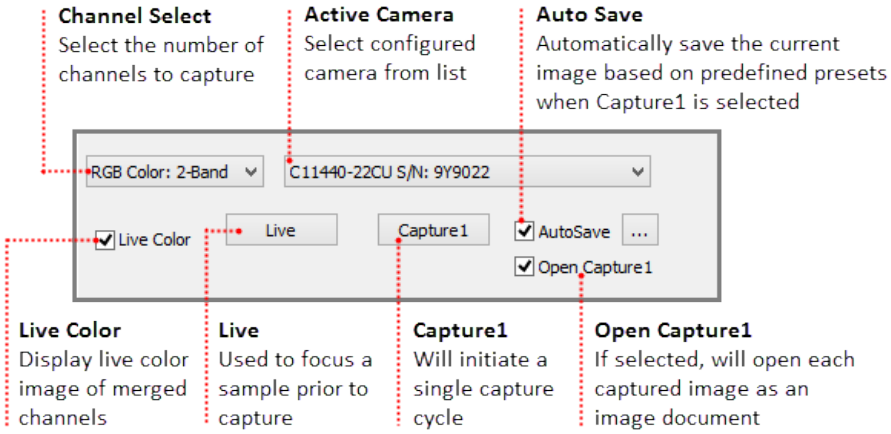
7 Add Remaining Filters
Repeat the steps to add the remaining filters and attenuations using the pin settings in the table below

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

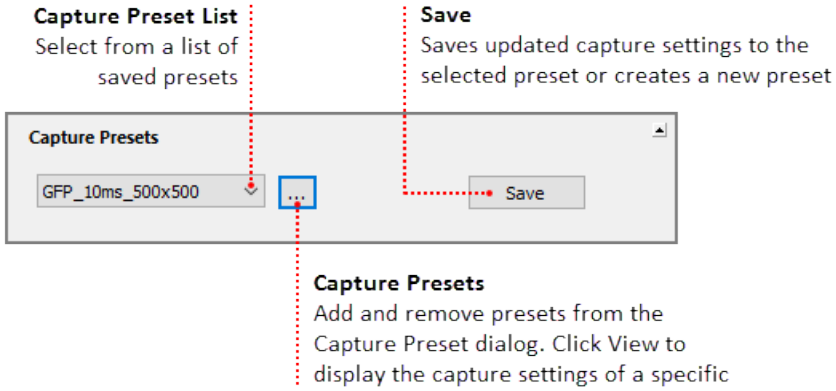
CAPTURE

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



Capture Presets

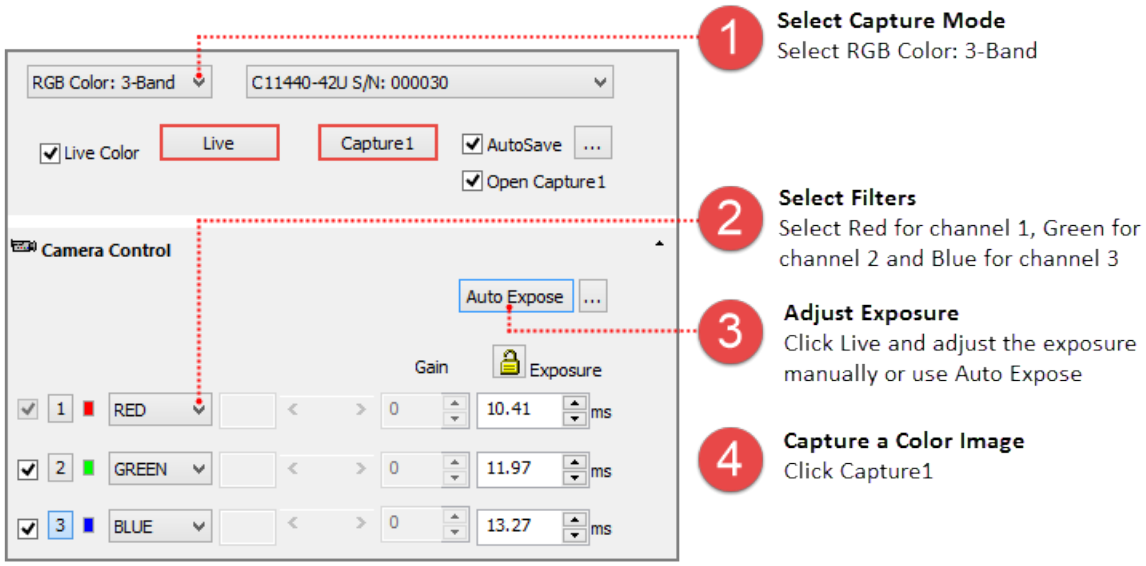
Capture presets save basic settings such as the capture mode, channels, filters, exposure times, as well as output trigger settings and advanced camera properties. For a list of the camera settings that are saved, select a capture preset from the Capture Presets dialog and click View. HCImage will load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



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

Capture a Color Image

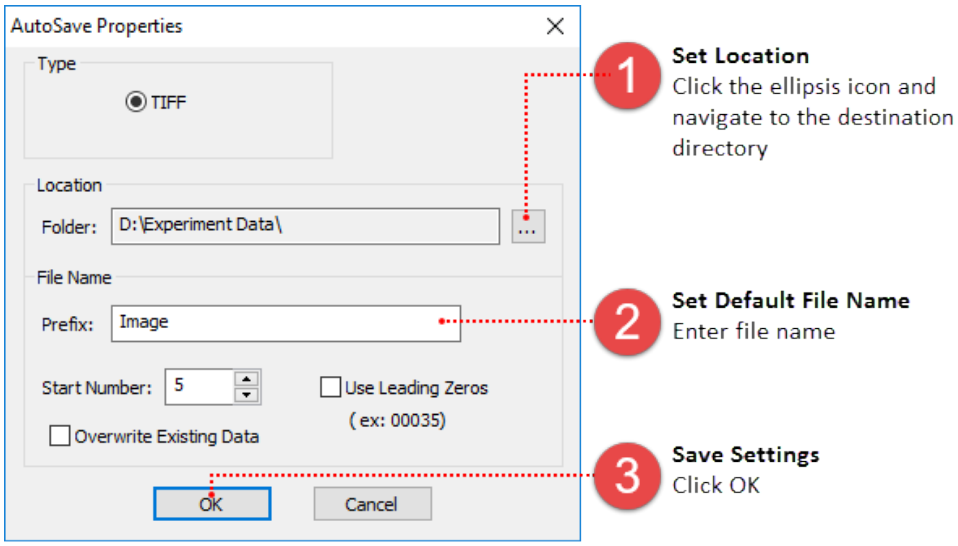
Capturing a color image requires filter setup, for instructions on configuring filters, please see "**Filter Setup**" on page 4.



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

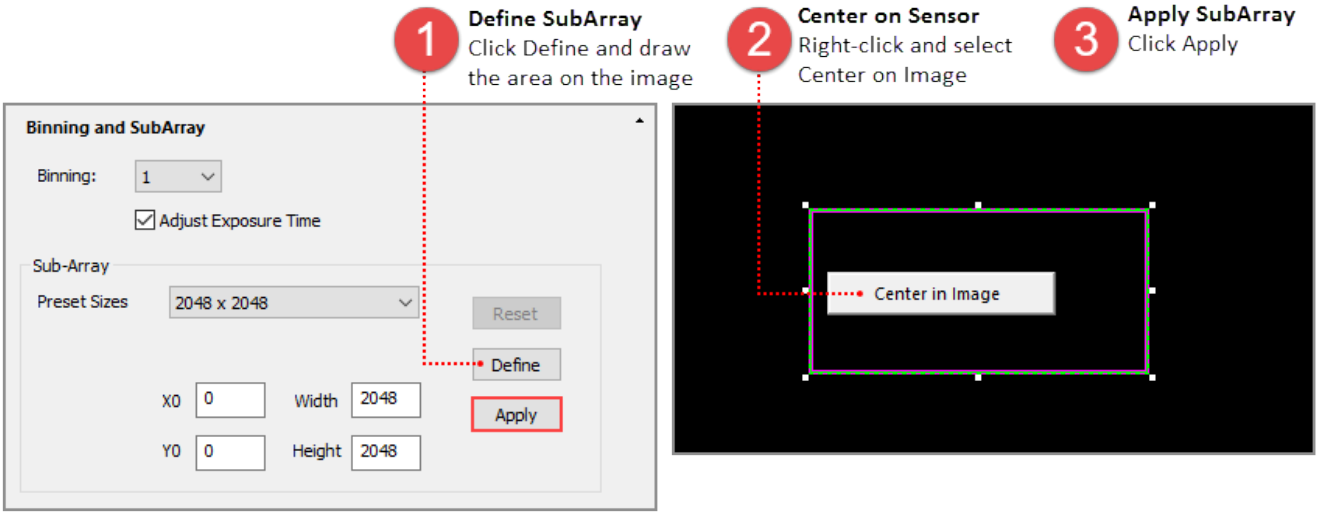
How to use AutoSave

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



Define a Custom SubArray for Maximum Speed

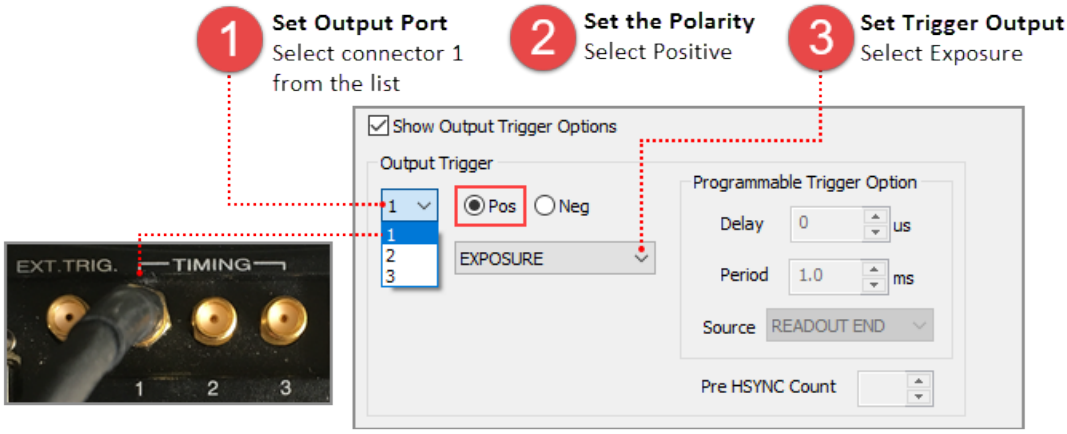
Click Live, focus on the sample and move the area of interest into the center of the image. Follow the steps below to define a custom subarray.



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

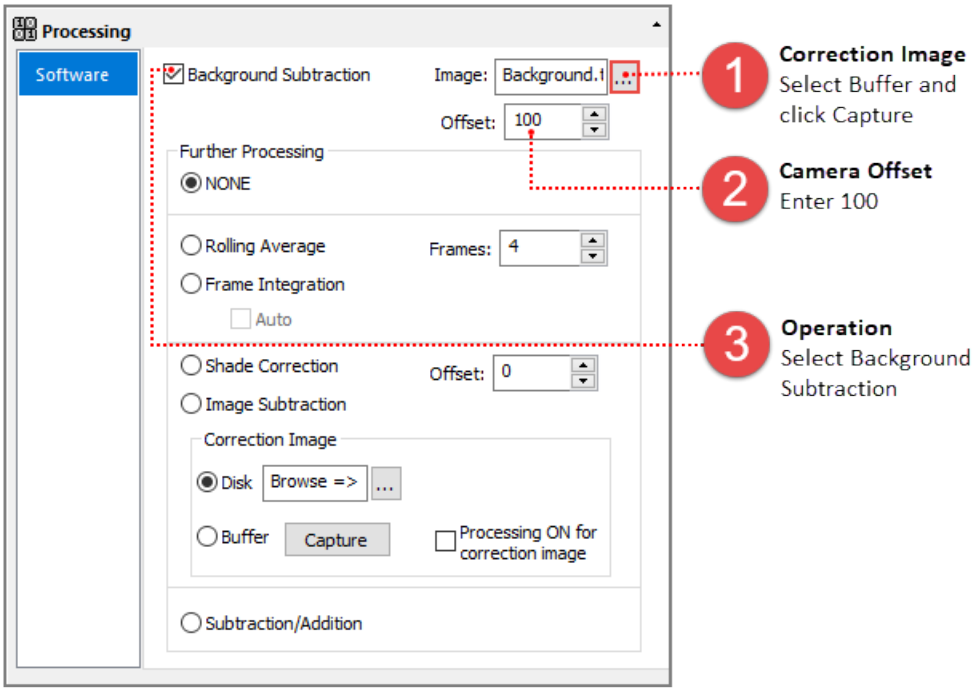
Control an LED using Output Trigger from the Camera

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.

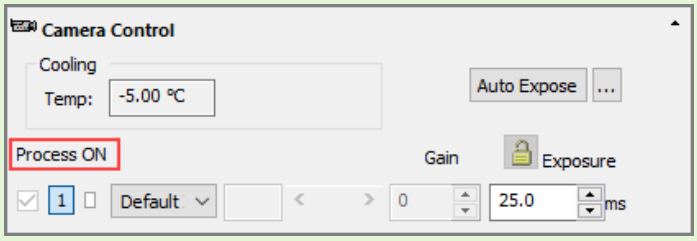


How to Setup a Background Subtraction

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.



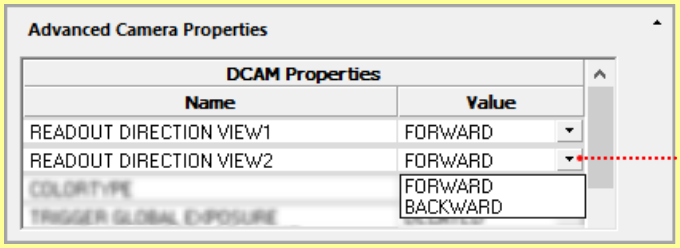
Hint: HCImage remembers the capture settings from the previous session, if background subtraction was left enabled, Process ON will be displayed in the Camera Control panel. The display image may appear distorted or black.



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 and the ORCA-Flash4.0 cameras, 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.

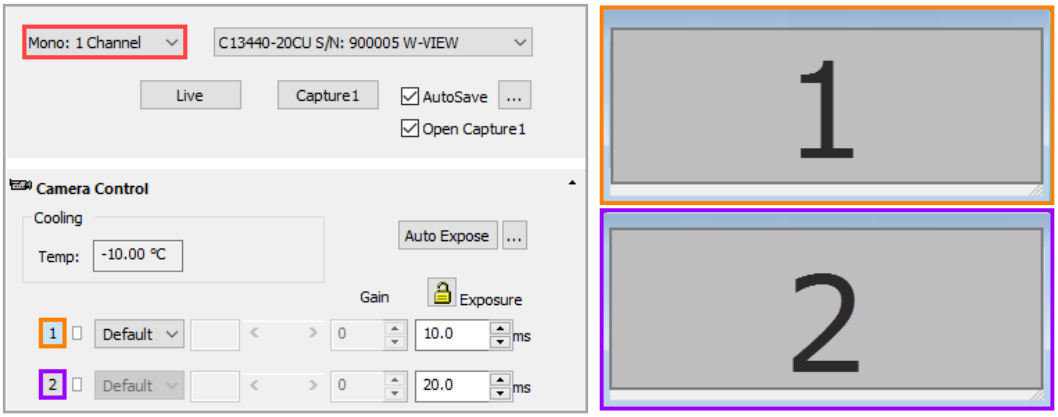


W-VIEW Capture Modes

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.

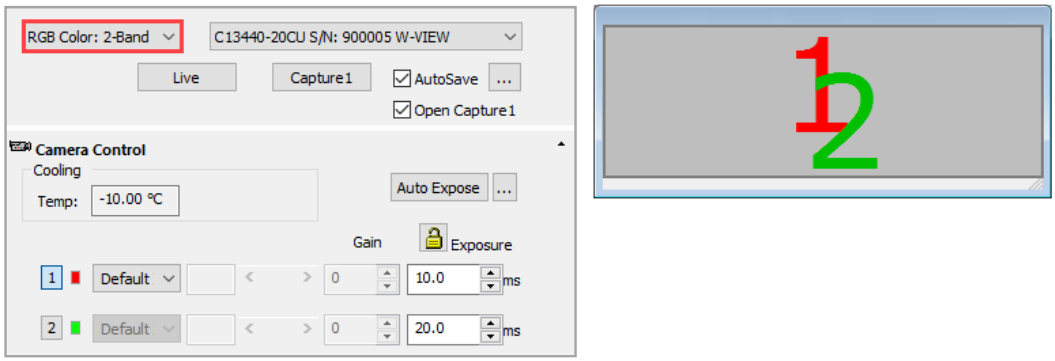
Mono 1 Channel

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.



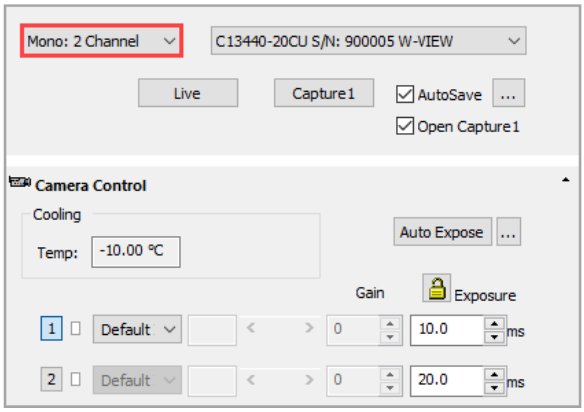
RGB Color 2-Band

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).



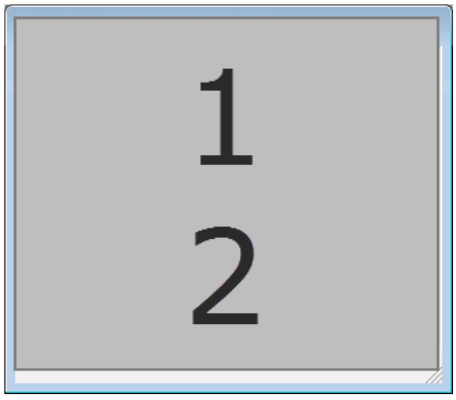
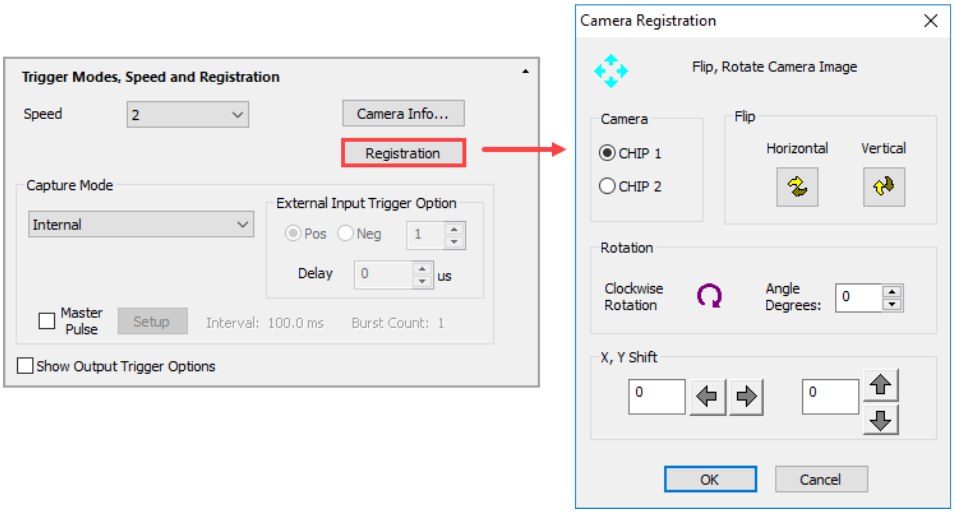


Image Alignment

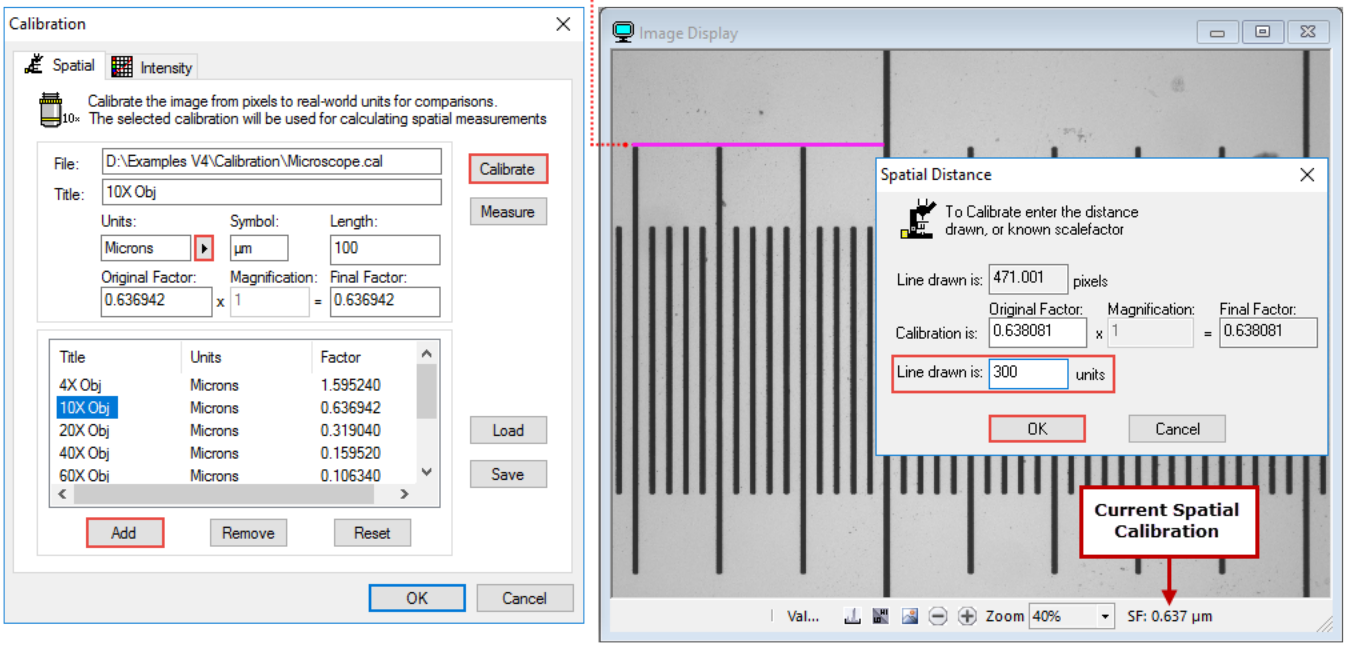
The Camera Registration feature allows the users while Live to flip and rotate the image. Click on the Registration button in the Trigger Modes, Speed and Registration pane to open the Camera Registration dialog.



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.

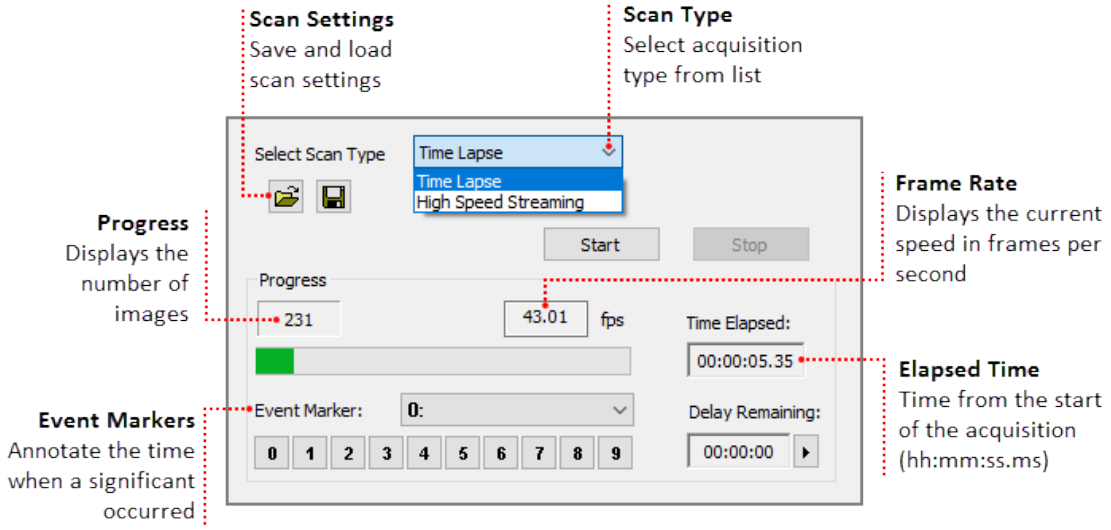
1. **Add Calibration** Click Add and enter a title (e.g., 10x)
2. **Select Units** Select Microns from the Units list
3. **Calibrate the Image** Click Calibrate. Draw a line to span the distance to measure
4. **Enter the Distance** Enter the known distance and click OK



HAMAMATSU
PHOTON IS OUR BUSINESS

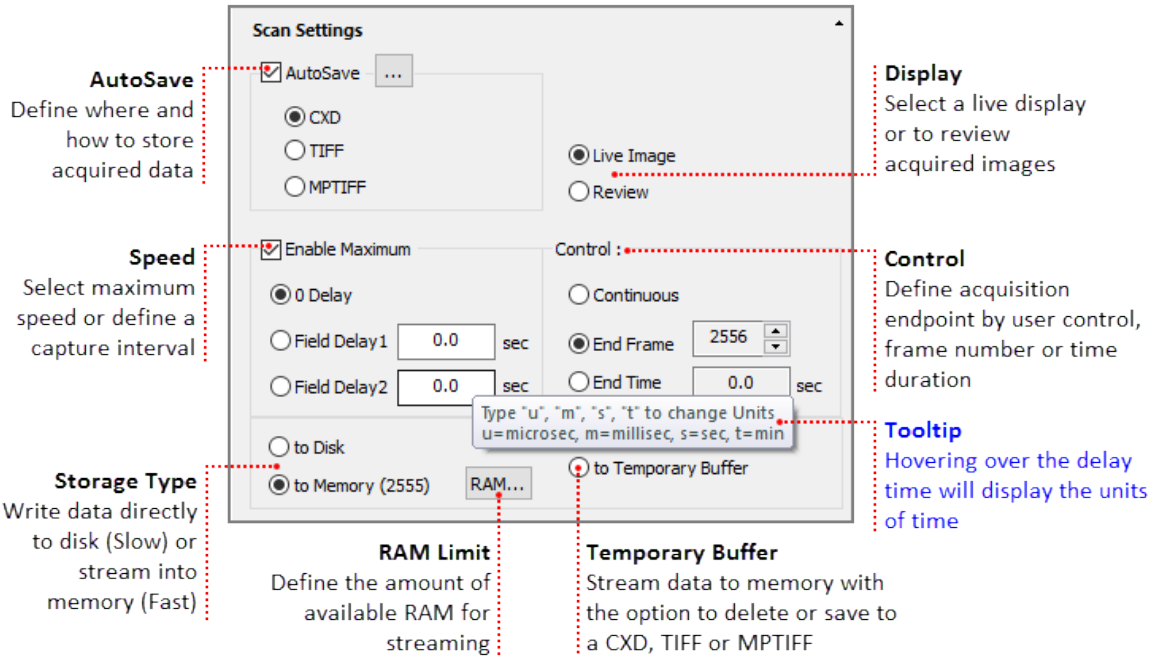
SEQUENCE

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



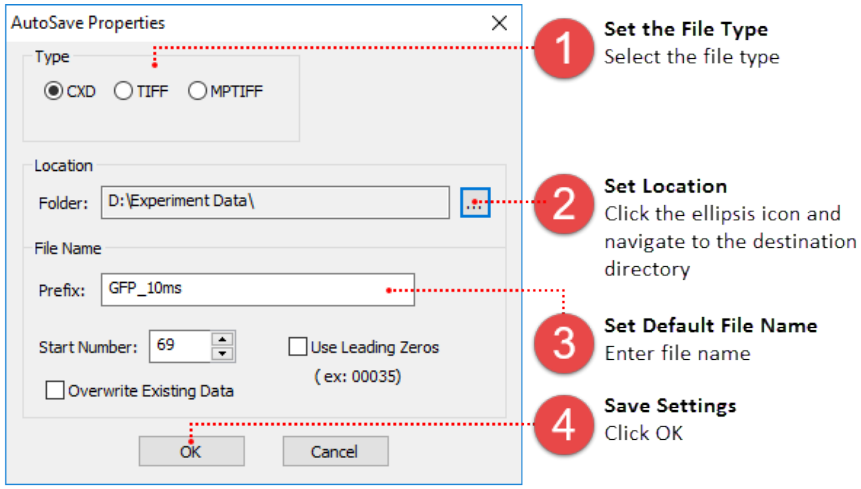
Setting up a Time Lapse

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



How to Use AutoSave

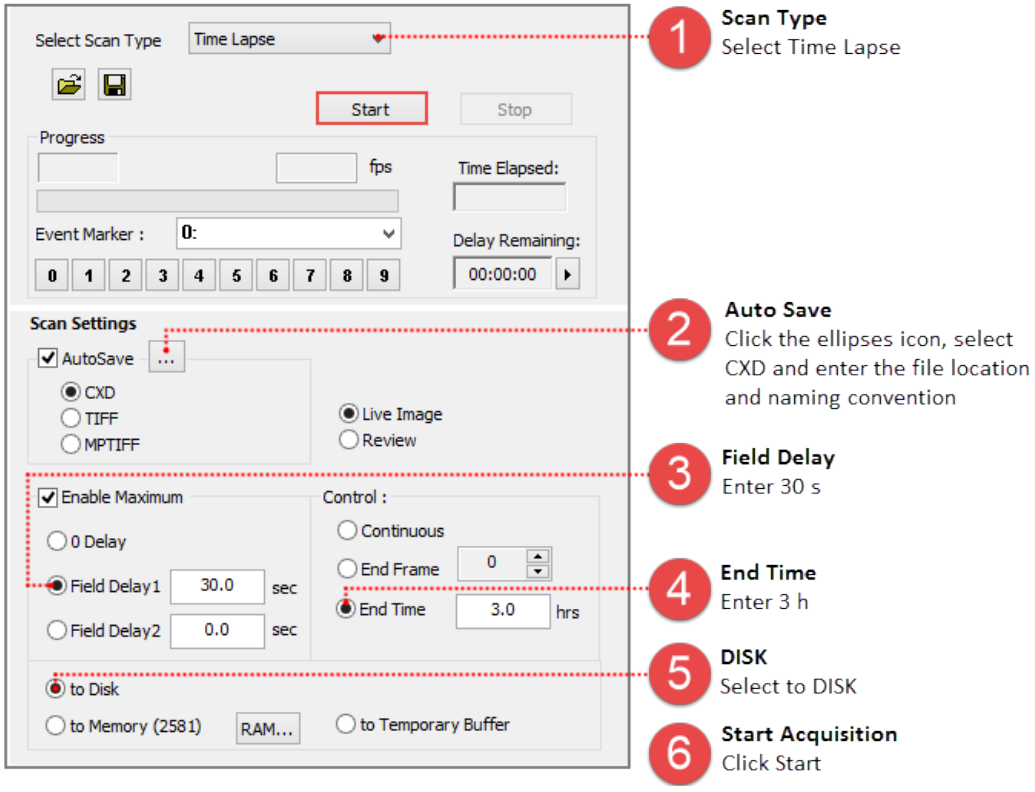
In the AutoSave Properties dialog, the user can determine how and where to store the acquired data. Image data can be saved as a CXD, TIFF or MPTIFF. The example below provides a description of the Auto Save Properties dialog.



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

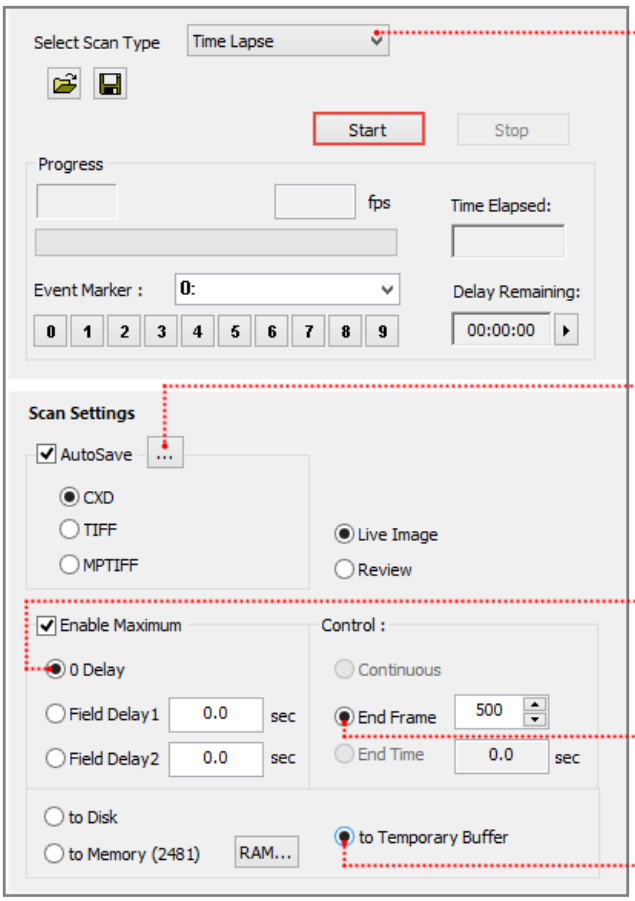
Setup a Time Lapse - Save to Disk

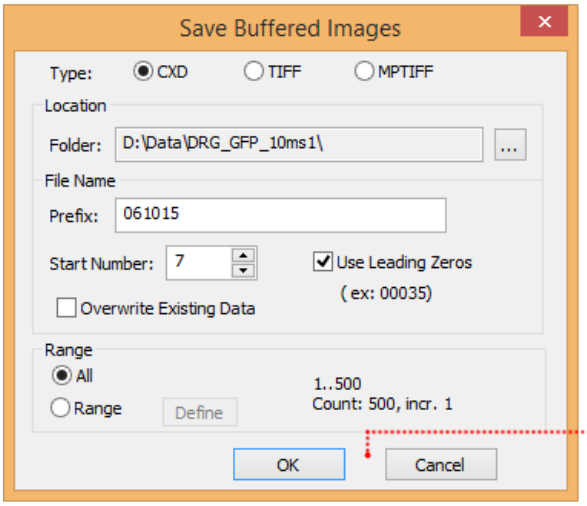
The time lapse in this example will acquire an image every 30 seconds for 3 hours and the data will be saved as a cxd. Once your are satisfied with capture settings and the sample is in focus, go to the Sequence pane and follow the steps below.



Setup a Time Lapse - Save to the Temporary Buffer

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



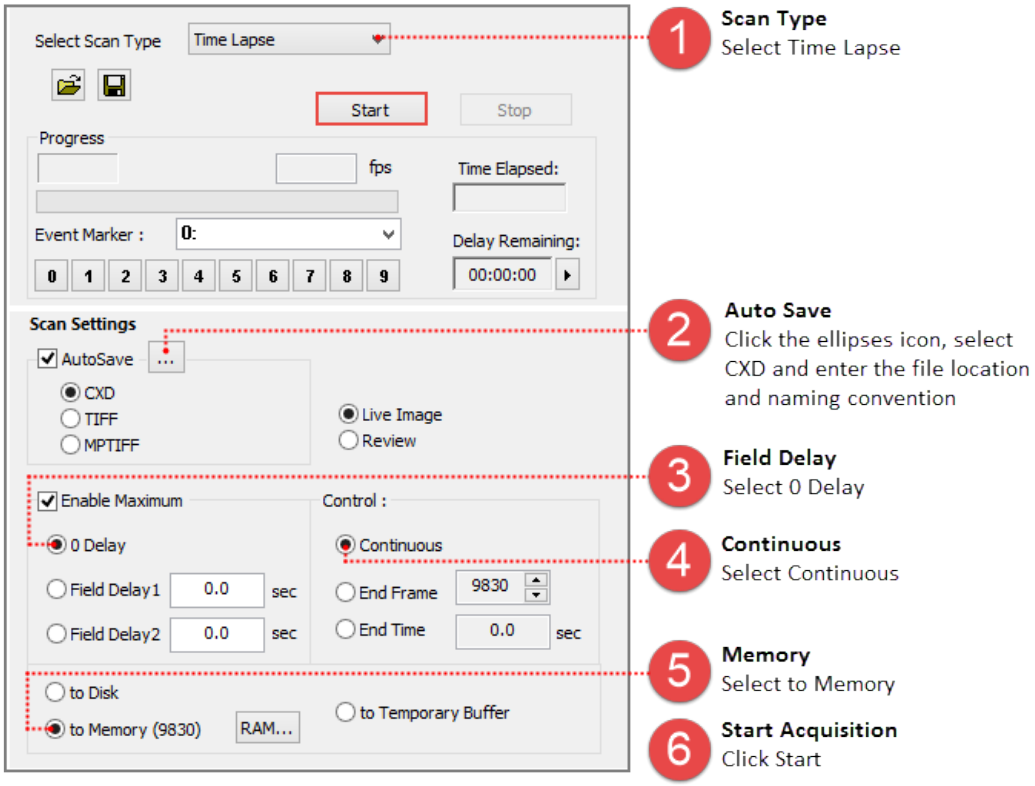


1. **Scan Type**
   Select Time Lapse
2. **Auto Save**
   Click the ellipses icon, select CXD and enter the file location and naming convention
3. **Field Delay**
   Select 0 Delay
4. **End Frame**
   Enter 500
5. **Temporary Buffer**
   Select to Temporary Buffer
6. **Start Acquisition**
   Click Start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

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

Setup a Time Lapse - Save to Memory

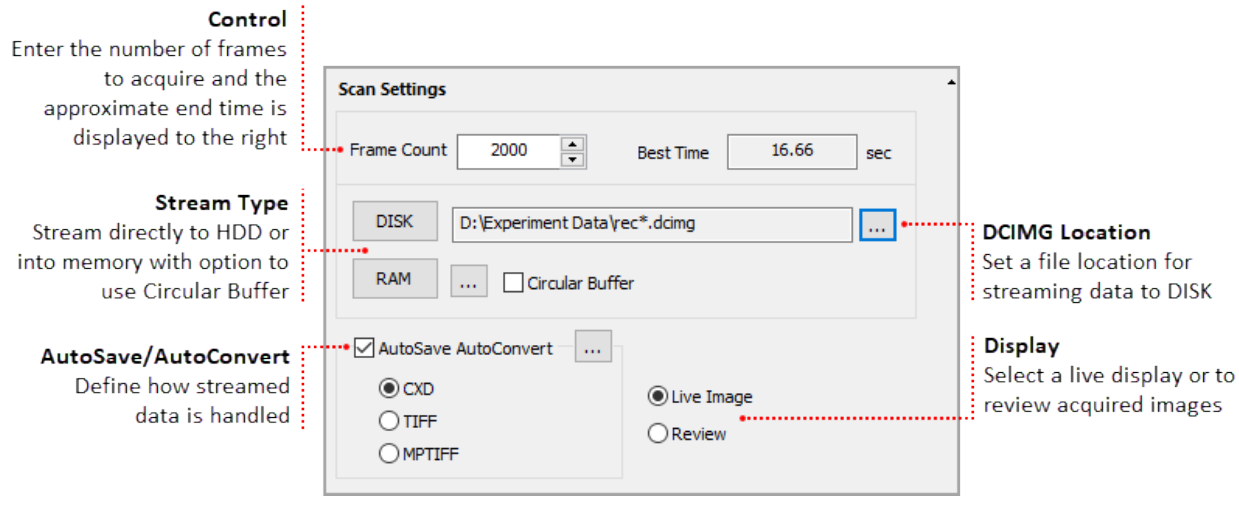
The time lapse in this example will store images in memory until the acquisition is stopped or runs out of memory at which point the acquired images are saved to disk for the remainder of the time lapse. Once your are satisfied with capture settings and the sample is in focus, go to the Sequence pane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



High Speed Streaming

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

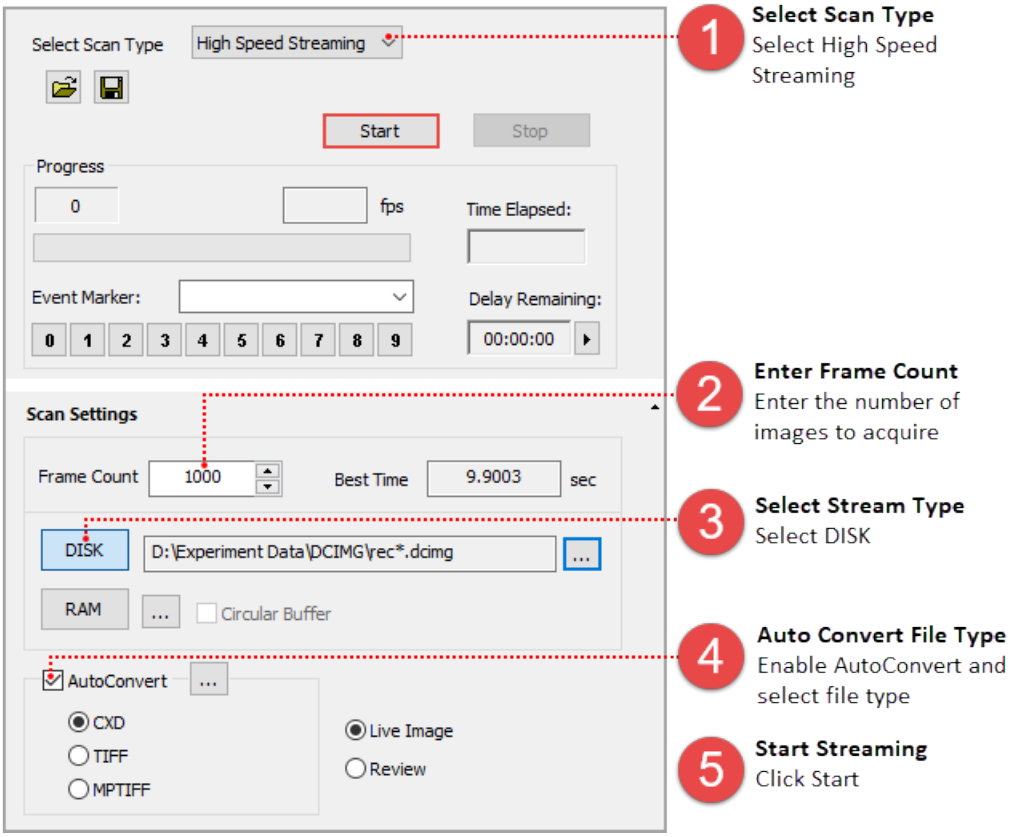
Note: Acquisition rates will vary based on the PC configuration, for information about the computer requirements, please see the PC Recommendations for ORCA-Flash4.0 V3 / LT+.



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

Steps for Streaming to Disk

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



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

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

3 **Select Stream Type**
Select DISK

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

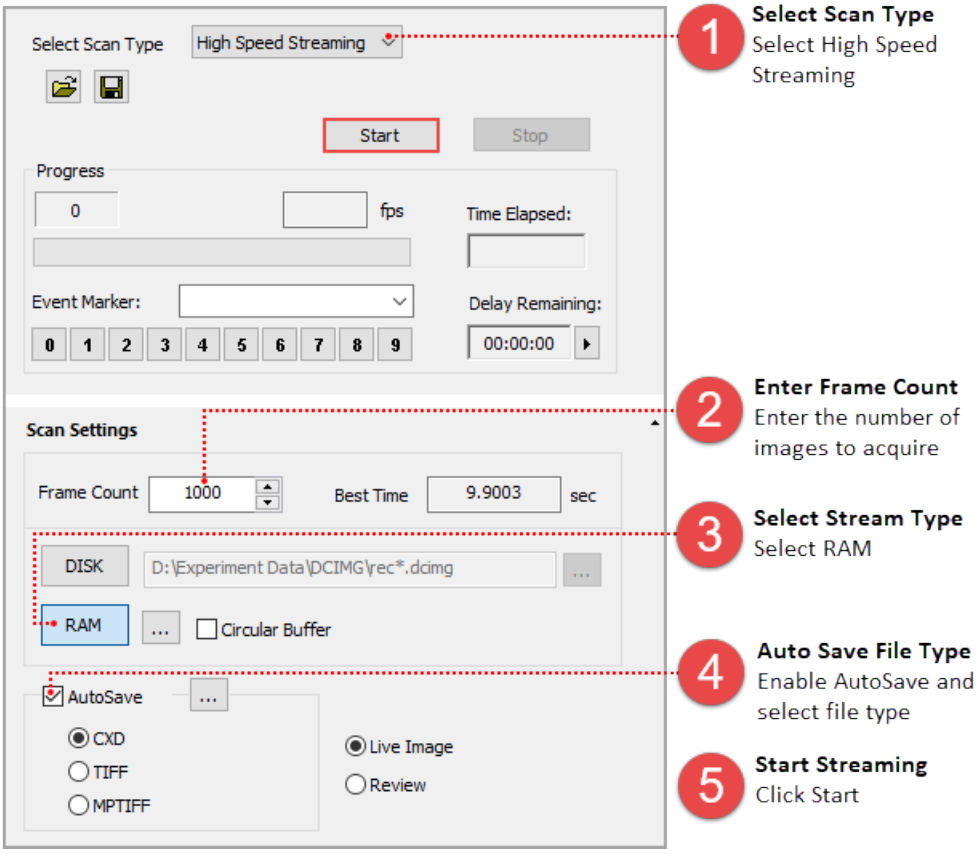
5 **Start Streaming**
Click Start

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

Steps for Streaming to RAM

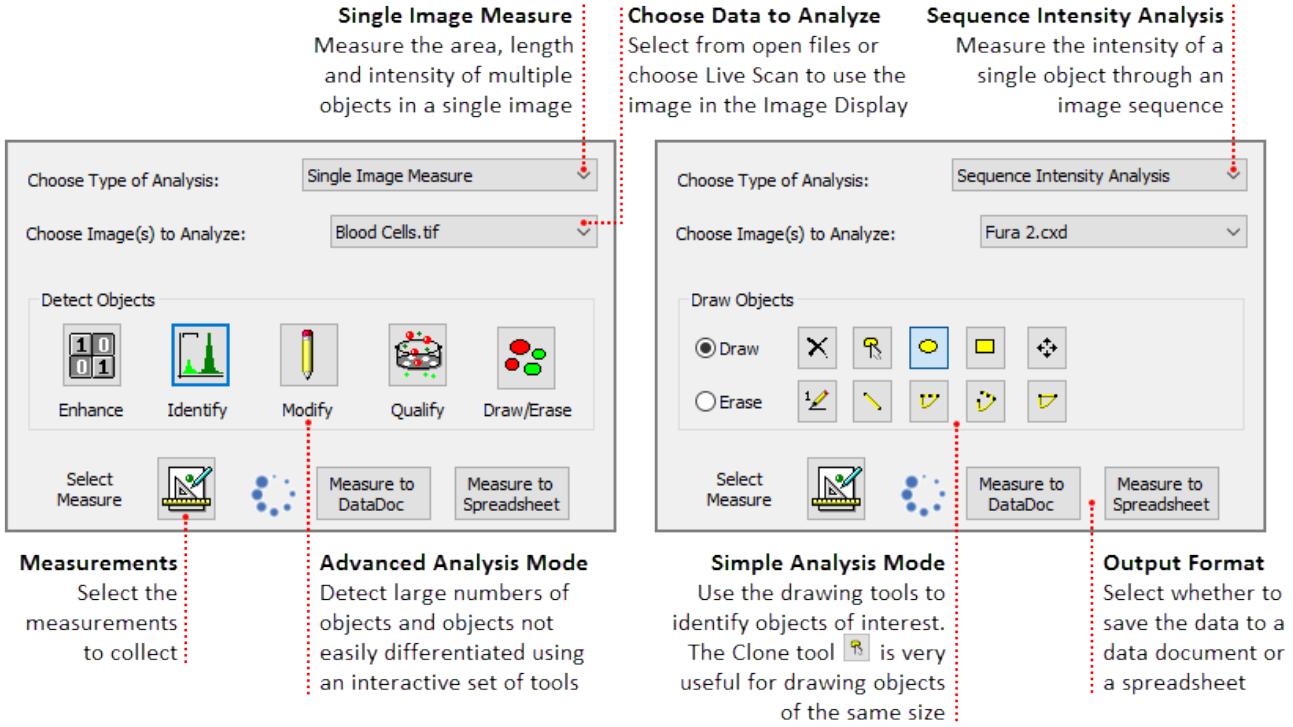
Acquired data is stored in memory with the option to review the image sequence before saving or deleting it. In the AutoSave Properties dialog, the user can determine how and where to store the acquired data. Once your are satisfied with capture settings and the sample is in focus, go to the Sequence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

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



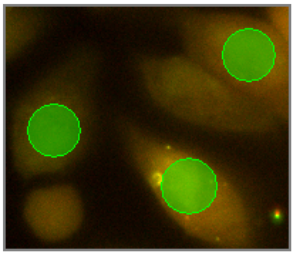
ANALYSIS

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.

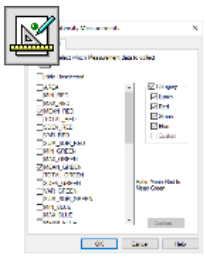


Sequence Intensity Analysis - Simple Mode

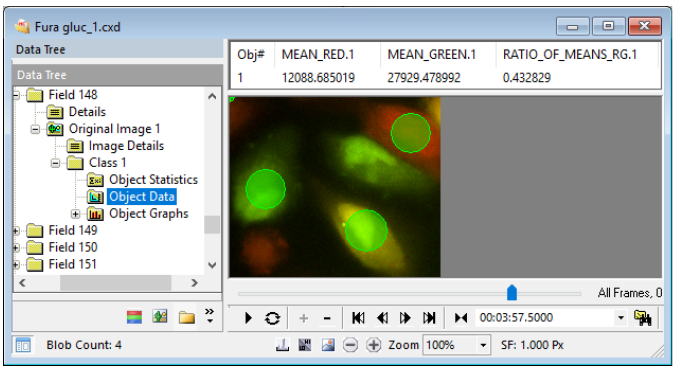
Sequence Intensity Analysis will measure the intensity of a single object in an image sequence. If multiple areas are drawn or identified, they are treated as a single object. Go to View in the menu bar, then highlight Analysis Mode and select Simple. Make sure that the image sequence to analyze is open, then select Sequence Intensity Analysis from the Choose Type of Analysis drop-menu and follow the instructions below.

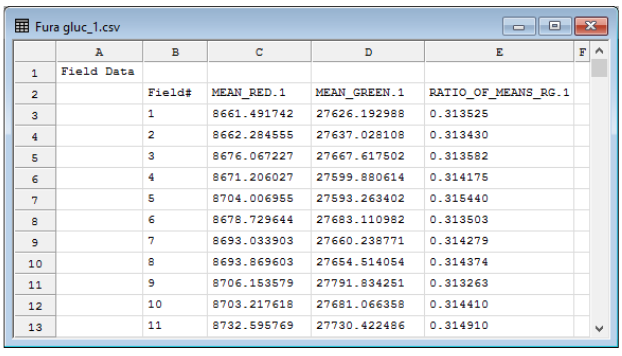


1. **Draw Object**
Click the Ellipse icon and manually identify the object of interest
Tooltip
Press SHIFT to draw a circle.



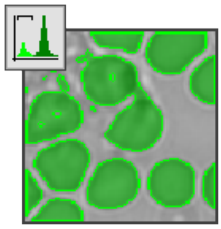
2. **Measure the Image**
Click the Measure icon, select Mean Red, Mean Green, Ratio of Means RG and click OK. Click the Measure to DataDoc button or the Measure to Spreadsheet button (both are shown below)



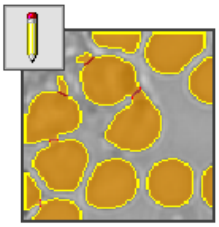


Analyze a Single Image - Advanced Mode

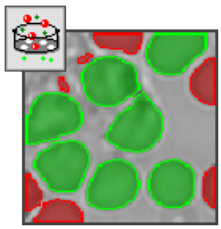
Enable the Advanced Analysis mode by clicking View in the menu bar, then highlighting Analysis Mode and selecting Advanced. Make sure that the image to analyze is open, then select Single Image Measure from the Choose Type of Analysis drop-menu and follow the instructions below.



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

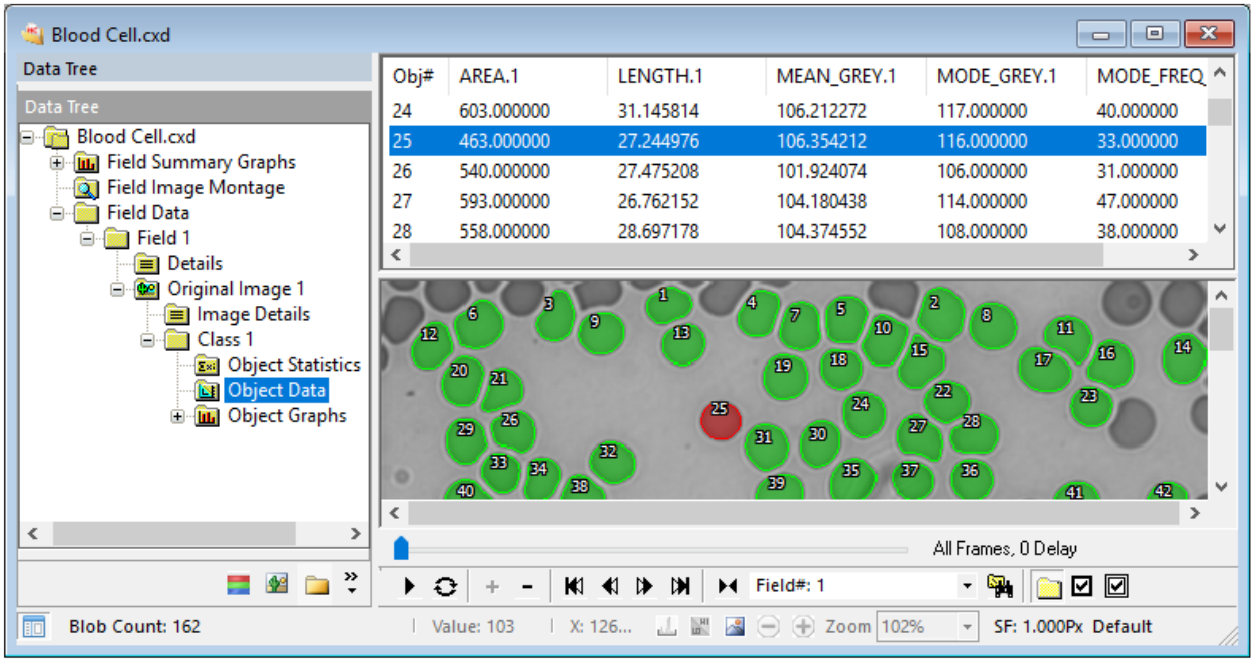


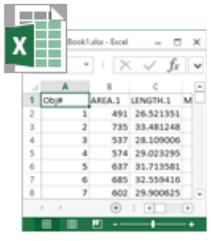
2 **Modify the Binary Image**
Click the Modify icon and select binary operations such as Open, Close and Separate to edit the binary overlay. Click OK



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