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HCImage Live

ORCA®-Flash4.0 V3 Guide

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INSTALLATION

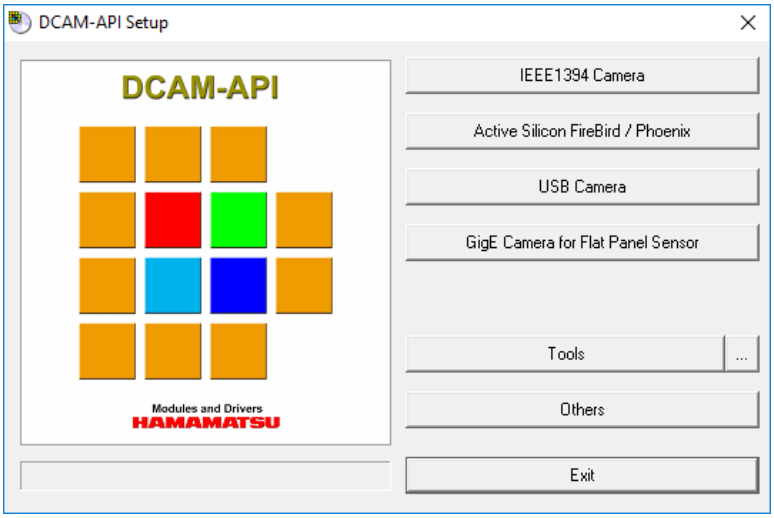
HCImage Live

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. Follow the instructions on installation wizard.
4. Click **Finish**, when the installation is complete.
5. 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.
6. Click the **HCImage Live** icon on the Desktop to launch HCImage Live.

DCAM-API® Drivers

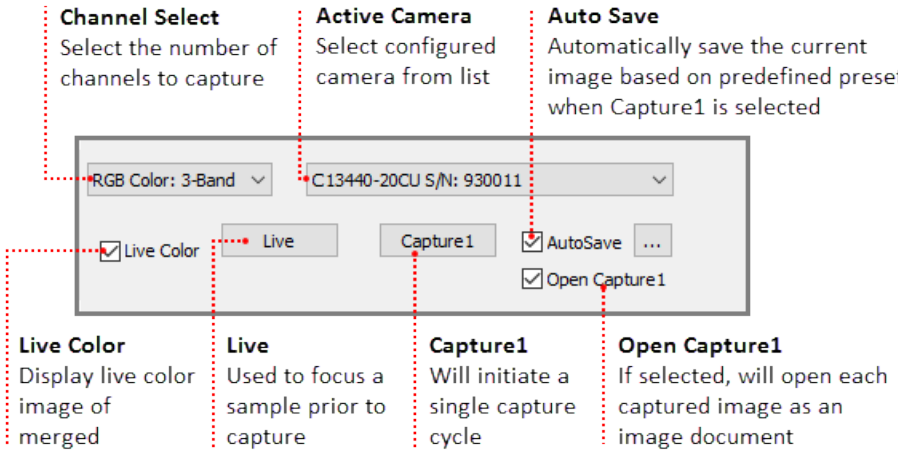
Before installing the camera driver, make sure that the camera is turned off.

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 https://www.dcam-api.com/ and download the DCAM-API® drivers for Windows.
2. Click **Yes**, if prompted by the User Account Controls.
3. [Camera Link] Select the **Active Silicon FireBird** module. [USB 3.0] Select the **USB Camera** module.
4. Click **Next** to begin the installation.
5. Follow the instructions on each installation page.
6. Click **Finish** when the installation is complete.



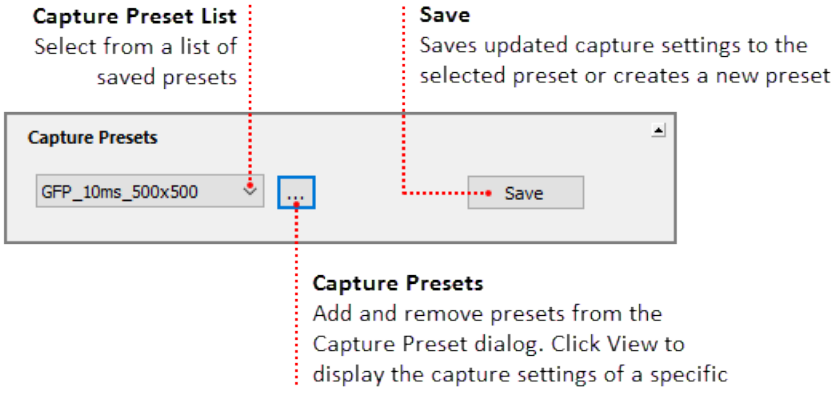
THE CAPTURE PANE

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. Each of the panels are described in detail below. 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

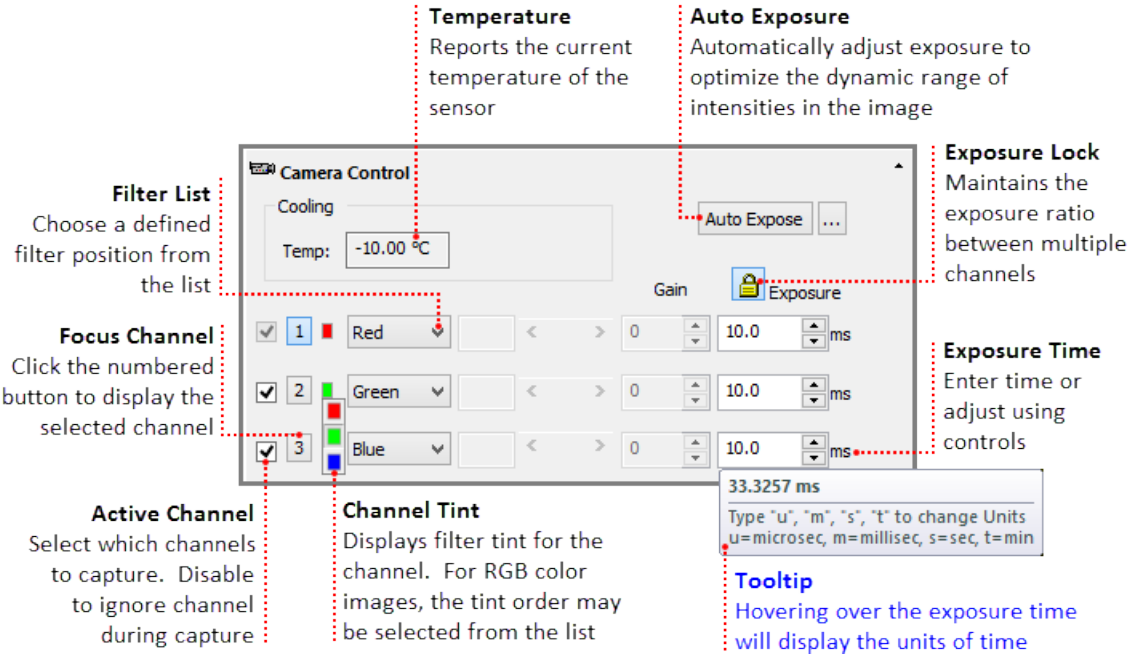
Capture settings can be saved as presets and then loaded when needed. Create multiple capture presets to easy change between frequently used capture settings. Capture presets may be selected from a list of saved presets available in the Capture Presets panel, located at the top of the Capture pane. To add, remove or view the settings of a preset, click the ellipsis to the right of the list, to open the Capture Presets dialog. 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.

Camera Control

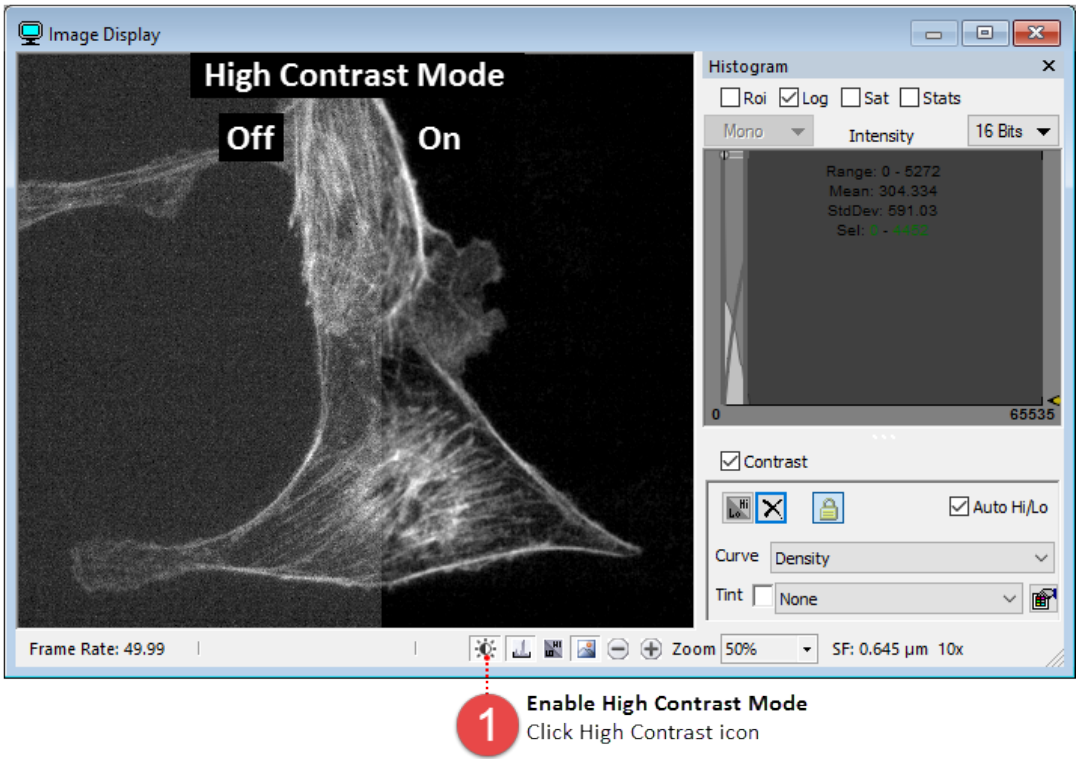
Manage capture settings using the individual channel and exposure controls.



Enhanced Visualization Mode

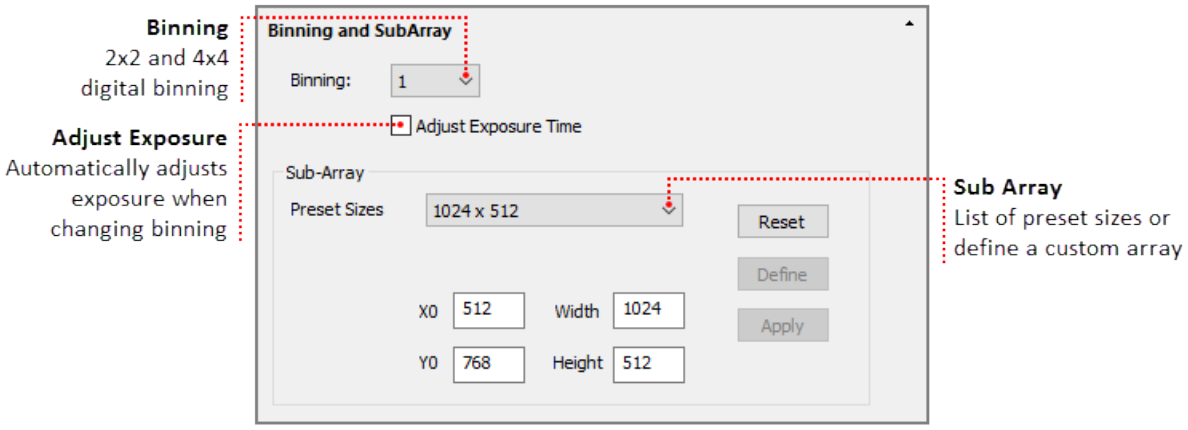
In low light observation, images from an sCMOS cameras may appear to have poor contrast. The High Contrast Mode can enhance the image to make it visually more appealing. Strictly for visual purposes, the processed image is displayed while the raw image is saved.

How to Enable High Contrast Mode



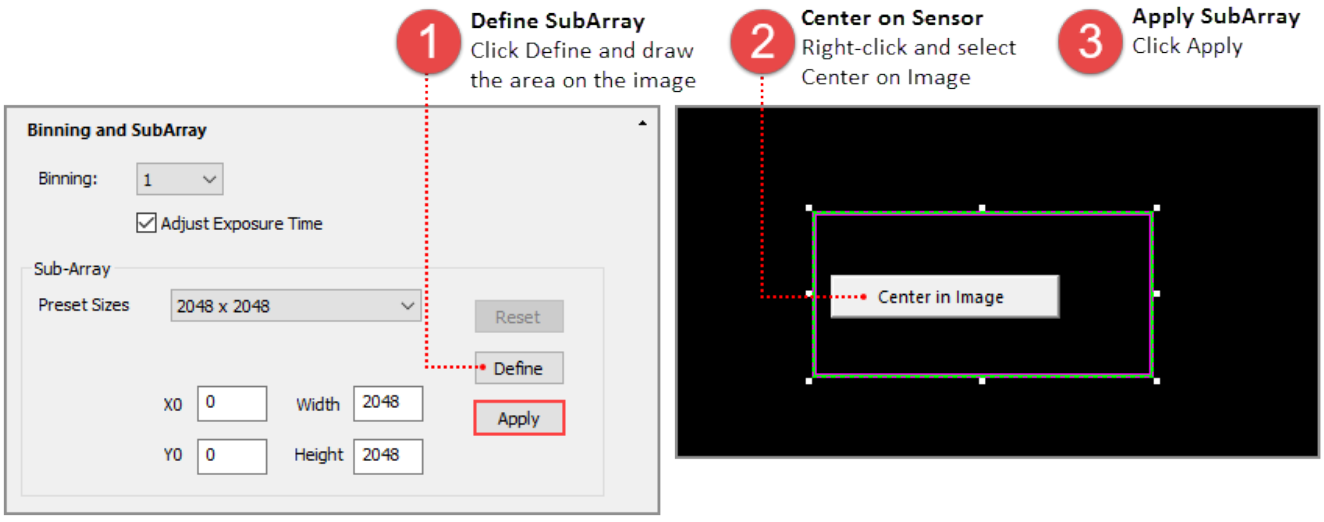
Binning and SubArray

With a CCD camera, 2x2 binning increases the signal to noise ratio by a factor of four and increases the speed of image acquisition by a factor of about two. With an sCMOS camera binning is purely digital, 2x2 binning increases the signal to noise ratio by a factor of two. Digital binning does not increase the speed of image acquisition. Adjust the spatial resolution using a subarray preset for increased speed and less data throughput. For sCMOS cameras a subarray must be centered on the camera sensor in order to achieve maximum speed. The subarray preset sizes for in the list are automatically centered (for sCMOS) but custom arrays are not. To center a custom array, see the example below.



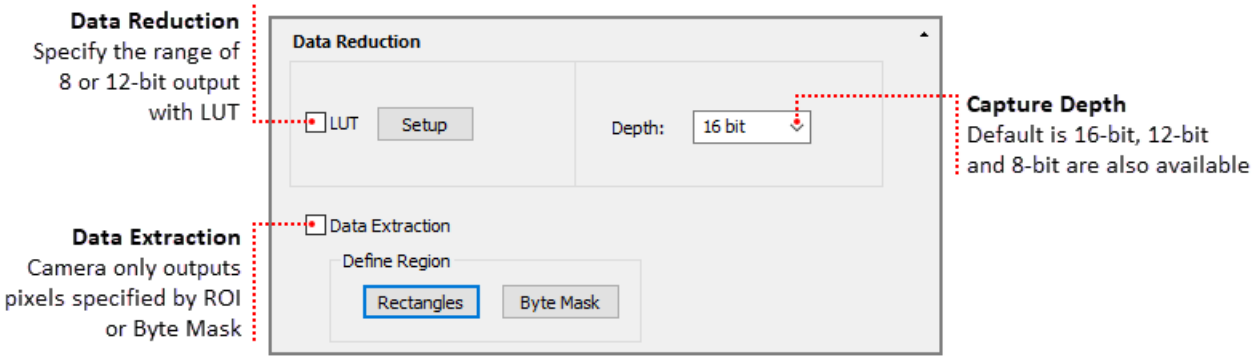
Define a Custom SubArray for Maximum Speed

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.



Data Reduction

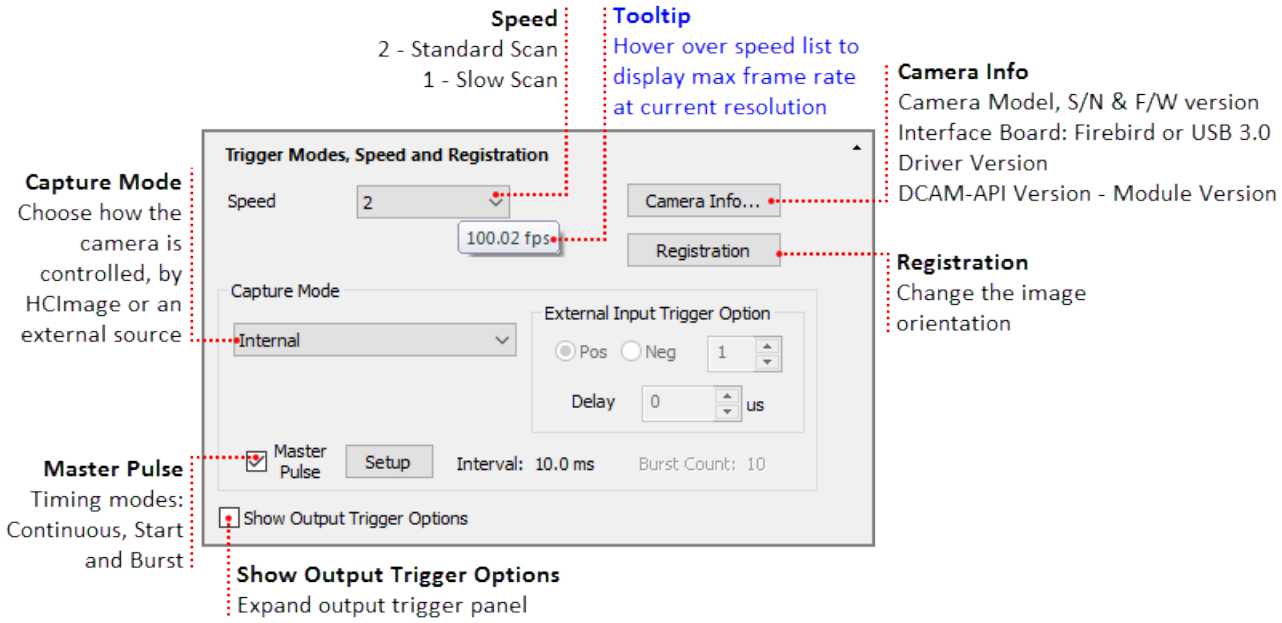
Reduce the amount of image data by using the LUT or Data Extraction. LUT is a customizable Look Up Table that allows the user to specify the range for 12 or 8-bit output. Data Extraction is used to define which pixels that the camera will output using ROI or byte masks. Data Reduction not only reduces the amount image data that will be acquired, it allows for higher frame rates through USB 3.0 with 12 or 8-bit output. For more information and examples on using data reduction, see "**Data Reduction**" on page 19.



Note: In order to achieve a reduced file size using Data Extraction or Data Reduction, the images must be acquired using High Speed Streaming to Disk and saved as DCIMG file type. Saving as or exporting to another file type (e.g., cxd or tiff) will result in 16-bit file size.

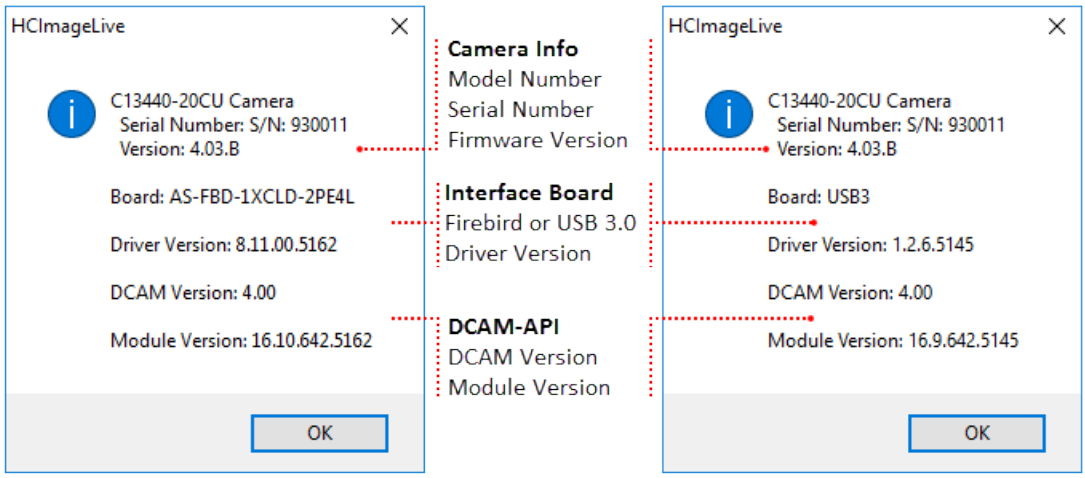
Trigger Modes, Speed and Registration

By default the camera is controlled through software but advanced triggering features available in certain cameras allow the camera to control external devices or be controlled by them. The speed, capture mode, master pulse and output trigger settings for these cameras can be adjusted based on the needs of the application. The example below describes the panel for the ORCA®-Flash4.0 V3.



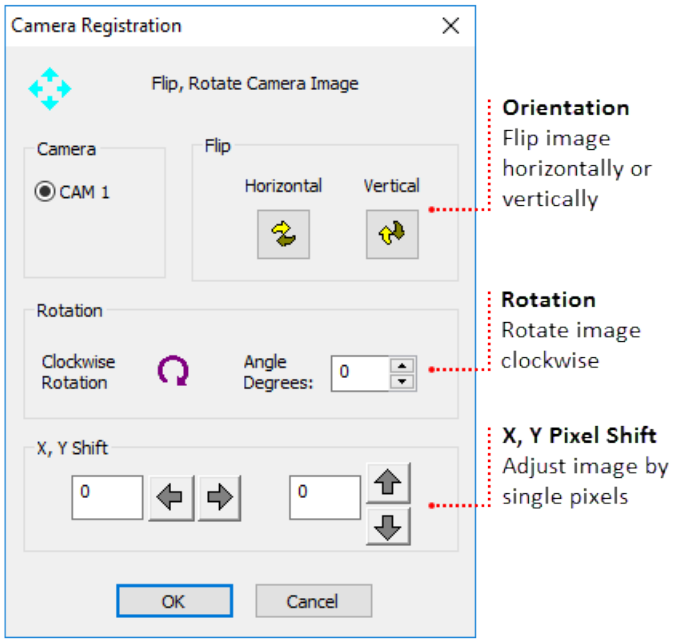
Camera Info

Provides information about the camera, interface board and DCAM-API® drivers.



Registration

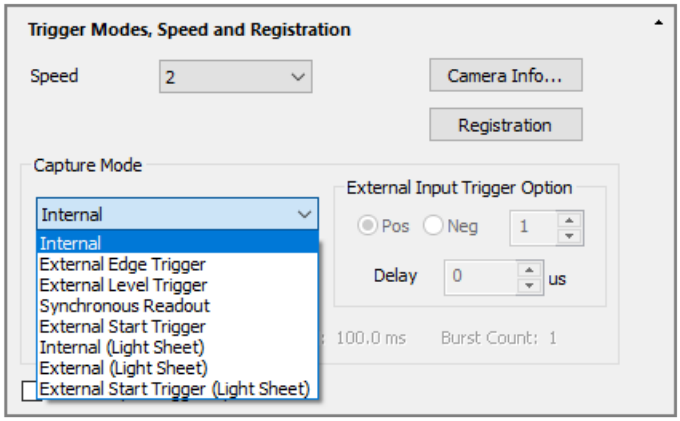
Adjust the orientation, rotation or pixel shift of the camera image.



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

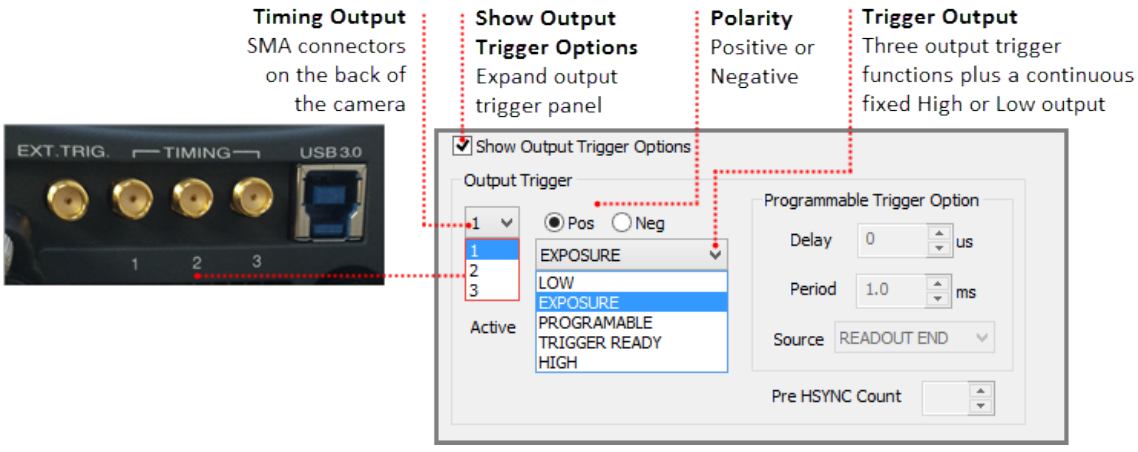
Capture Mode

By default, cameras are set to internal "free running" mode, where the software controls the exposure and readout timing. Most cameras also have external input trigger modes to synchronize with an external instrument where the external instrument becomes the master and the camera becomes the slave. The example below shows the capture modes available for the ORCA®-Flash4.0 V3 with the Camera Link and USB 3.0 interfaces. For more information about Capture Modes, please see "**External Input Trigger Modes**" on page 23.



Output Trigger Options

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. For this particular camera, there are three different trigger output functions, as well as a continuous High output (High output fixed) or continuous Low output (Low output fixed). For more information, please see "**Camera Trigger Output**" on page 31.



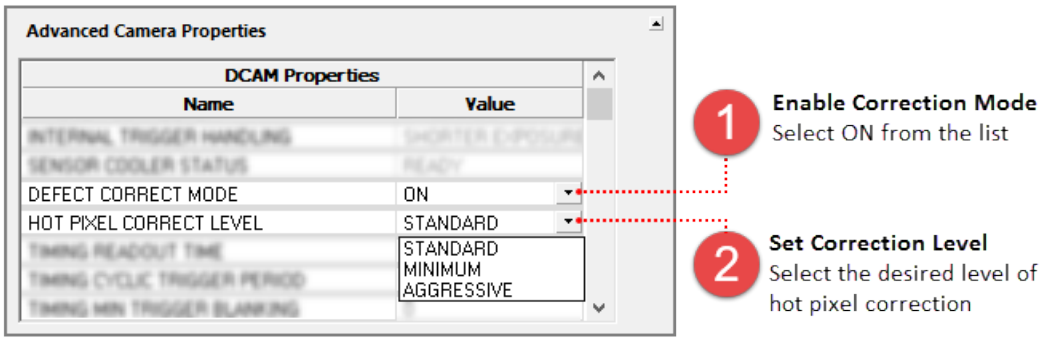
Advanced Camera Properties

DCAM Properties are a list of camera parameters reported by DCAM. The camera properties and reported values are specific to the connected camera and in some cases provide access to additional functionality based on the capture mode. For example, the ORCA®-Flash4.0 V3 supports multiple levels of pixel correction for quantitative and qualitative control of images.

Correction Level for White Spots	When Exposure Time Is	Ratio of the number of pixels to be corrected to the total number of pixels
Aggressive	1 second or longer	Approximately 0.1 %
Standard (default)	less than 1 second	Approximately 0.05 %
Minimum	10 milliseconds or less	Less than 0.001 %
Off	Zero	0 %

How to Select Pixel Correction Level

In the Capture pane, expand the Advance Camera Properties panel and locate Hot Pixel Correct Level in the list of DCAM Properties. When the Correction Mode is ON, select the level of hot pixel correction from the list.



Processing

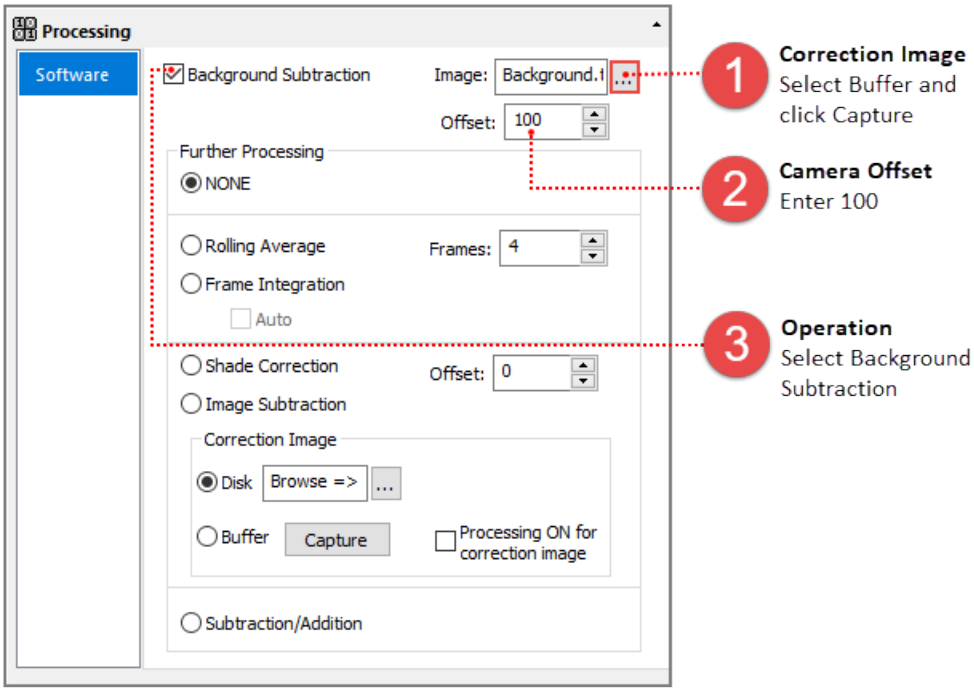
The Processing Panel provides the opportunity to enhance images during focus and acquisition by incorporating image-processing operations during or immediately after image Capture. To select an Image Processing operation, first expand the Process Pane and then select the Operation Type. Rolling Average and Frame Integration are used for noise reduction. Use the image arithmetic functions like Shade Correction, Background Subtraction or Image Subtraction to remove artifacts from the incoming image. Clicking Capture1 will initiate image capture with the selected image processing operations applied.

Note: For Image Correction or Arithmetic, the user must first choose a source or background image. The image may be the current image saved in a buffer or one previously saved to disk. To use the current image, make sure Processing is OFF, select Buffer, click Capture and then select Shade Correction, Background Subtraction or Image Subtraction. Use the same method when using an image from Disk.

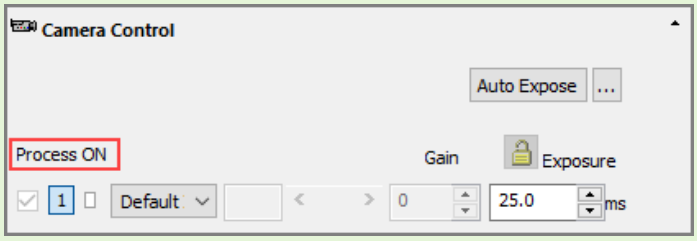
Hint: Enable Processing ON for correction image when you would like to capture a correction image using Rolling Average or Frame Integration. When you are ready to capture the correction image, select Rolling Average and enter the number of frames, enable Processing ON for correction image and then click the Capture button to the right of Buffer. The captured averaged image is stored in the buffer and ready to use a correction image.

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.

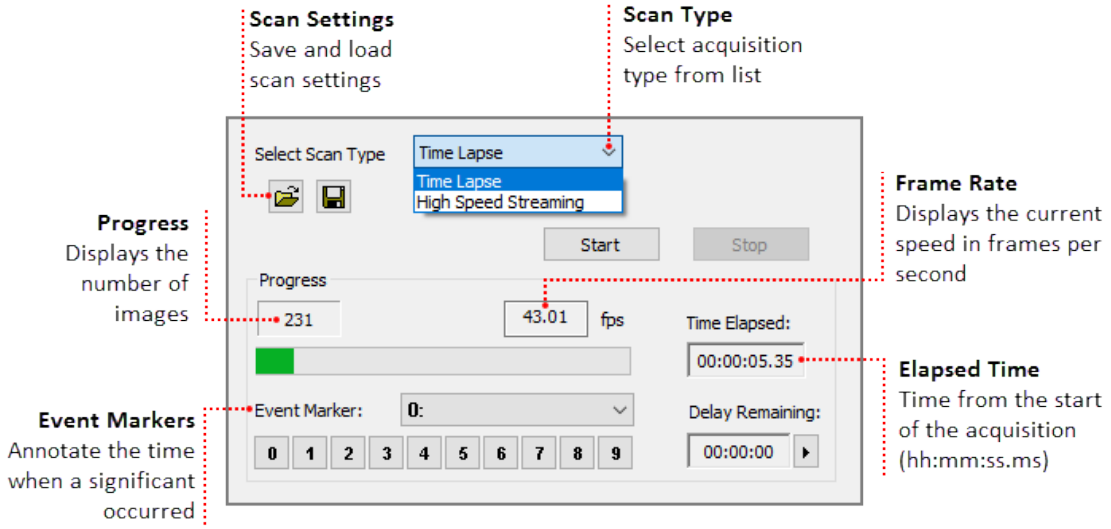


Hint: HCImage remembers the capture settings from the previous session, if background subtraction was left enabled, Process ON will be displayed in the Camera Control panel. The display image may appear distorted or black.



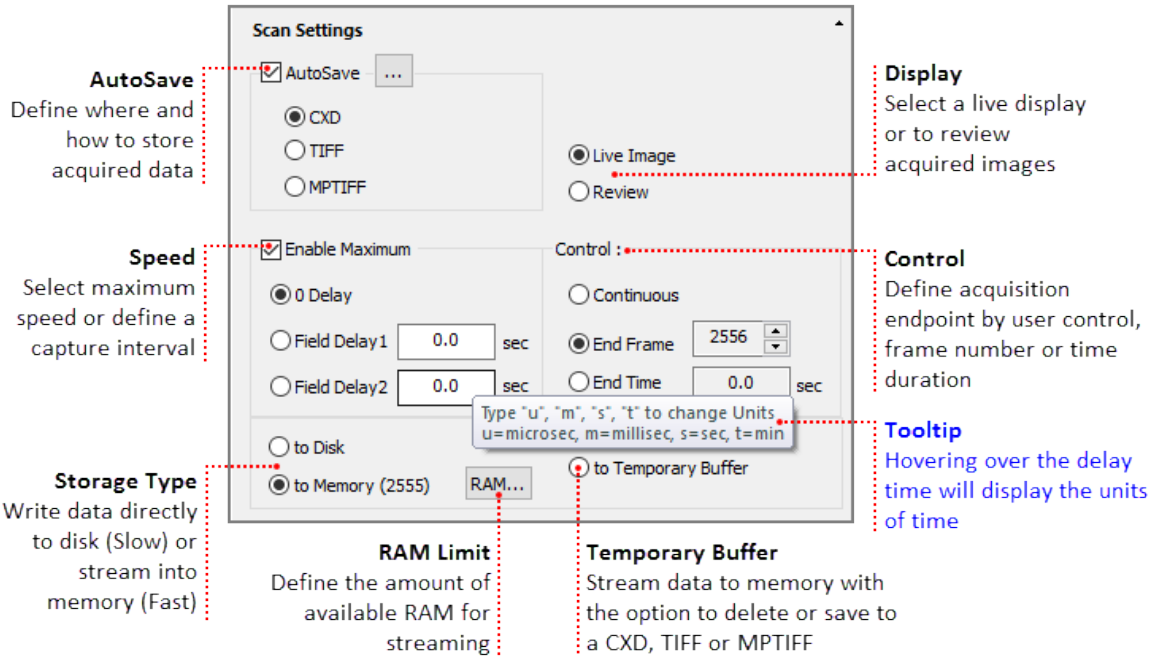
THE SEQUENCE PANE

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.



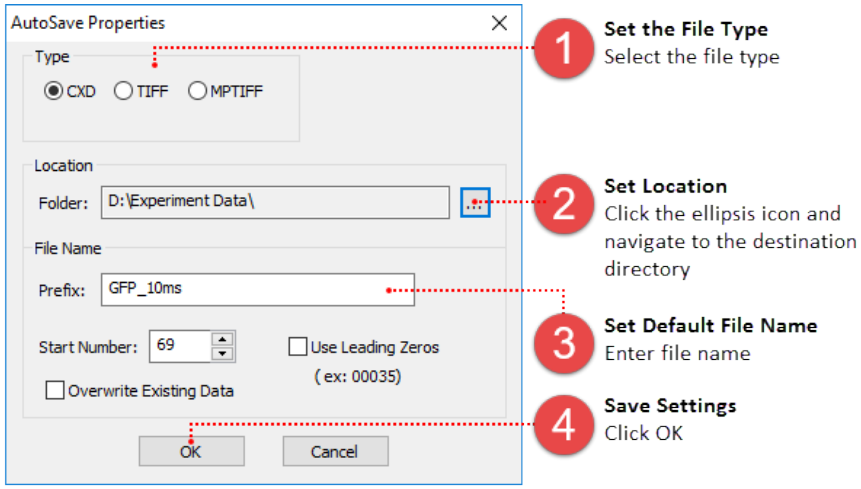
Time Lapse

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

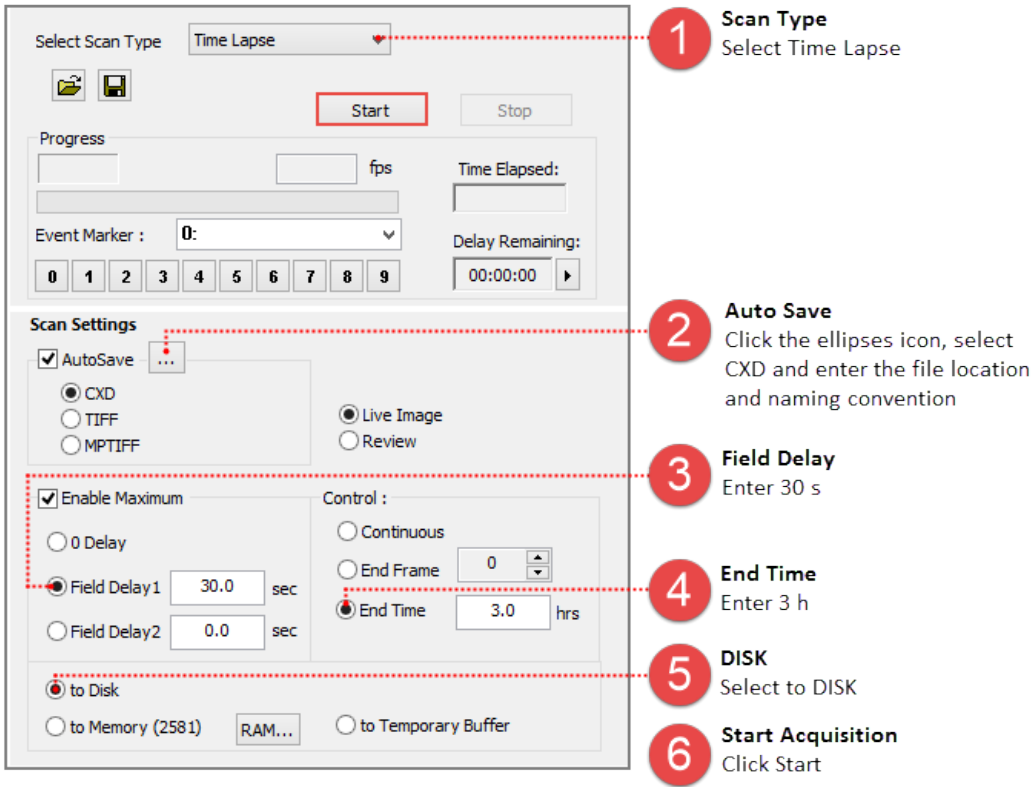
In the AutoSave Properties dialog, the user can determine how and where to store the acquired data. Image data can be saved as a CXD, TIFF or MPTIFF. The example below provides a description of the Auto Save Properties dialog.



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.

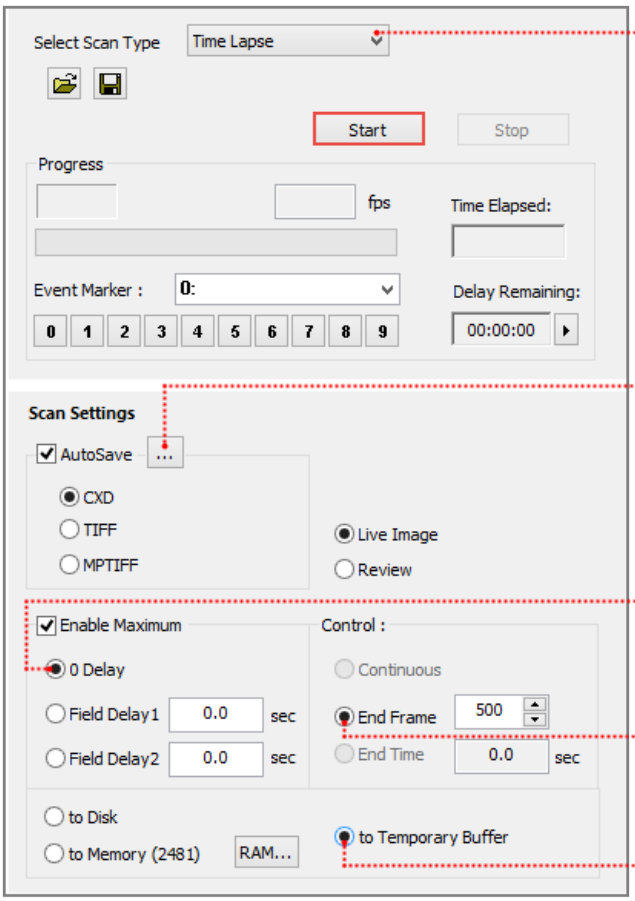
Setup a Time Lapse - Save to Disk

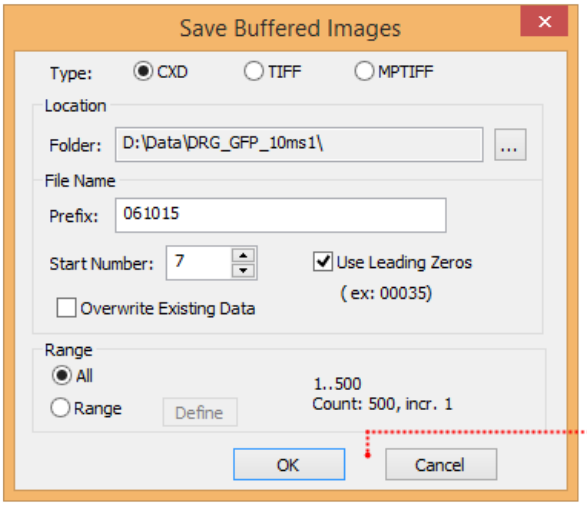
The time lapse in this example will acquire an image every 30 seconds for 3 hours and the data will be saved as a cxd. Once your are satisfied with capture settings and the sample is in focus, go to the Sequence pane and follow the steps below.



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.



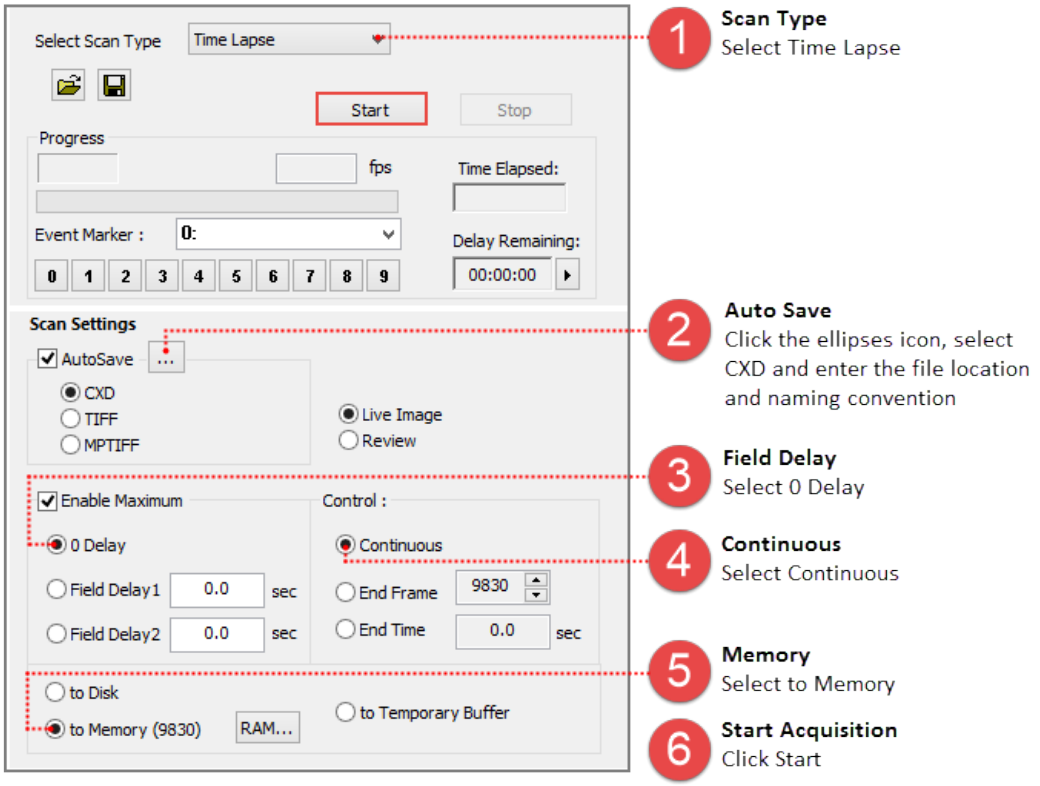


1. **Scan Type**
   Select Time Lapse
2. **Auto Save**
   Click the ellipses icon, select CXD and enter the file location and naming convention
3. **Field Delay**
   Select 0 Delay
4. **End Frame**
   Enter 500
5. **Temporary Buffer**
   Select to Temporary Buffer
6. **Start Acquisition**
   Click Start
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

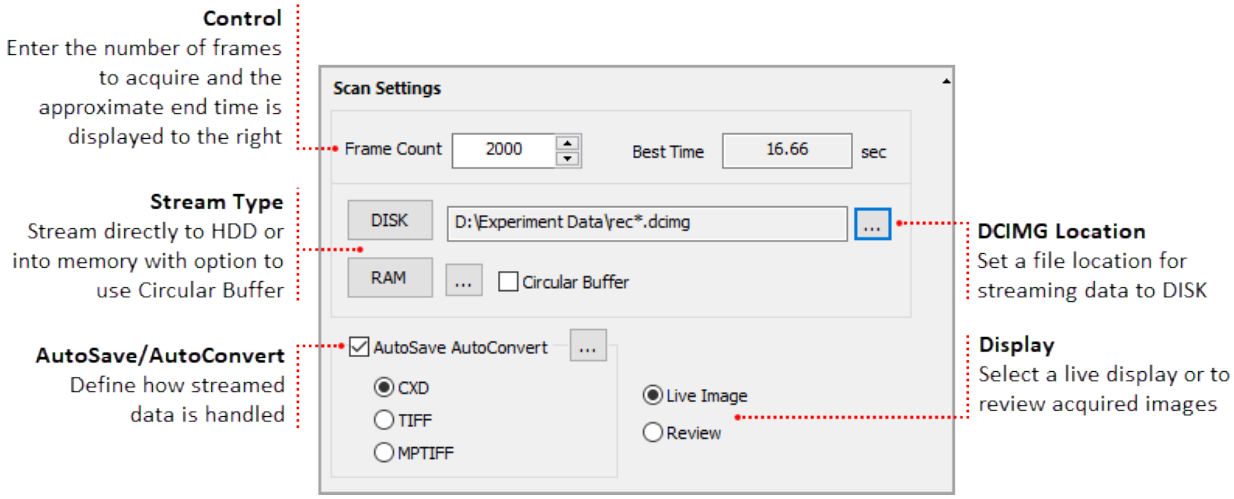
The time lapse in this example will store images in memory until the acquisition is stopped or runs out of memory at which point the acquired images are saved to disk for the remainder of the time lapse. Once your are satisfied with capture settings and the sample is in focus, go to the Sequence pane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

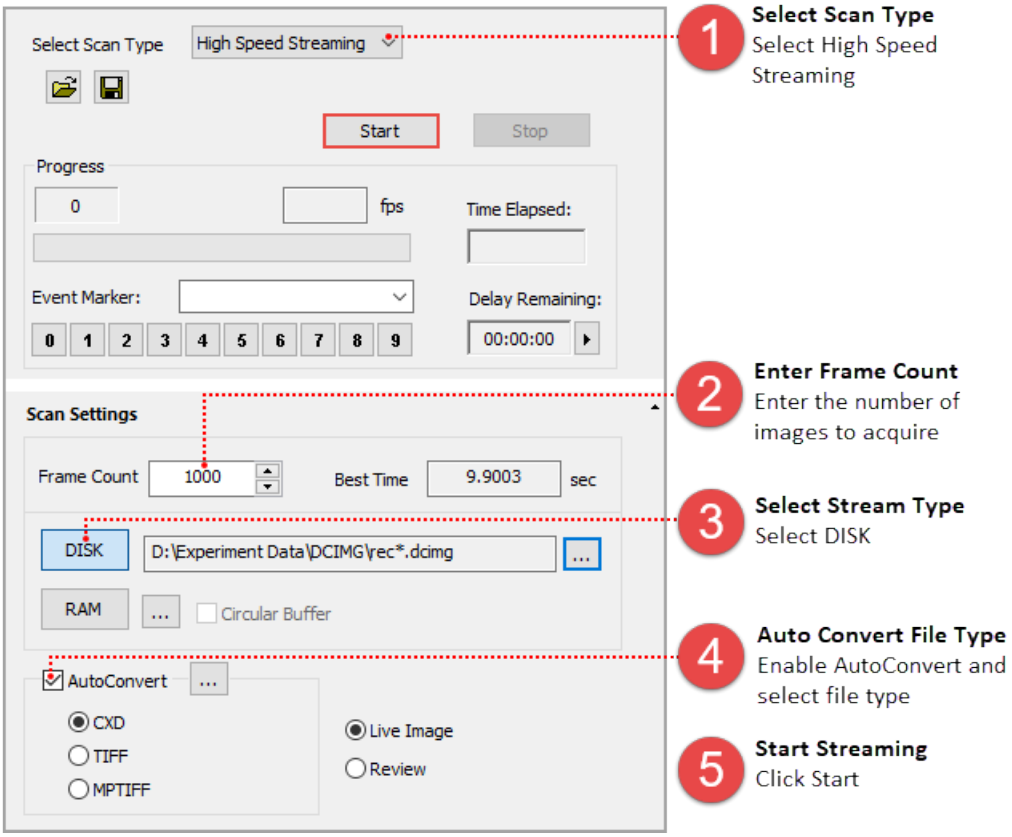
Note: Acquisition rates will vary based on the PC configuration, for information about the computer requirements, please see the PC Recommendations for ORCA®-Flash4.0 V3.



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

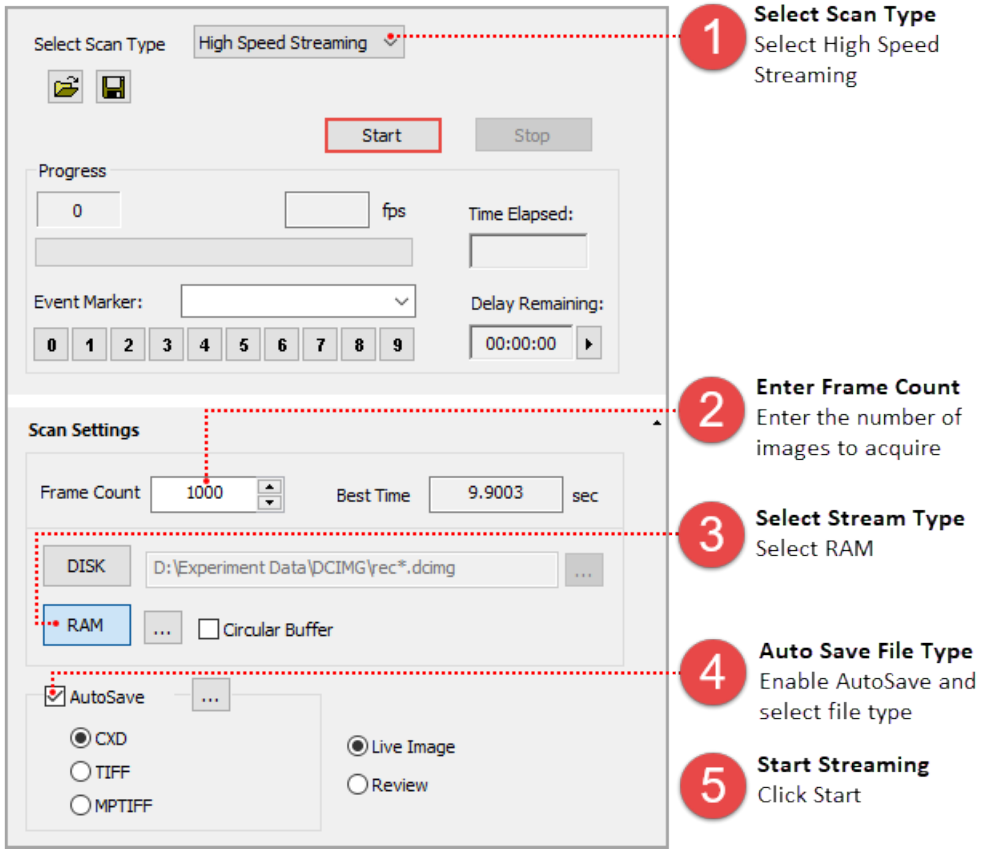
5 **Start Streaming**
Click Start

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

Steps for Streaming to RAM

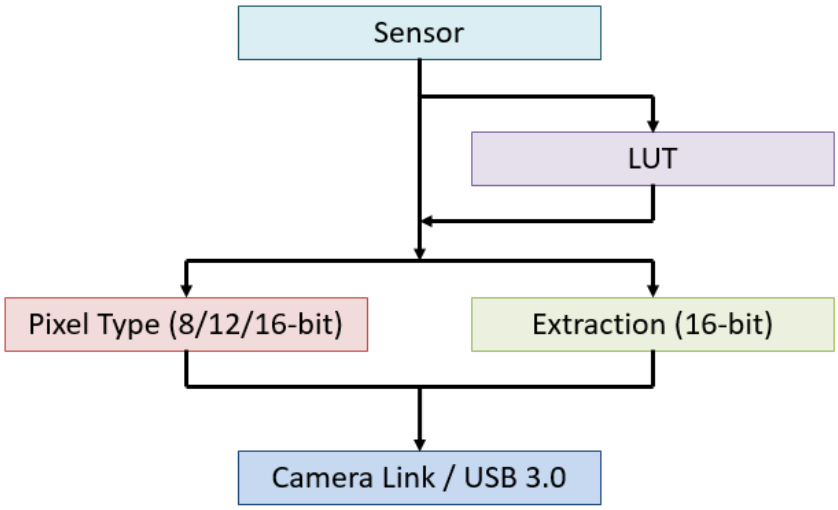
Acquired data is stored in memory with the option to review the image sequence before saving or deleting it. In the AutoSave Properties dialog, the user can determine how and where to store the acquired data. Once your are satisfied with capture settings and the sample is in focus, go to the Sequence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



DATA REDUCTION

Reduce the amount of image data by using the LUT or Data Extraction. LUT is a customizable Look Up Table that allows the user to specify the range for 12 or 8-bit output. Data Extraction is used to define which pixels that the camera will output using ROI or byte masks.



Data Reduction not only reduces the amount image data that will be acquired, it allows for higher frame rates through USB 3.0 with 12 or 8-bit output.

Resolution*1	Output Bit Depth	Camera Link frames per second*2	USB 3.0 frames per second*2
2048 x 2048	16	100	40
	12	100	53
	8	100	80
2048 x 1024	16	200	80
	12	200	106
	8	200	160
2048 x 512	16, 12 or 8	400	200
2048 x 8	16, 12 or 8	25655	20524

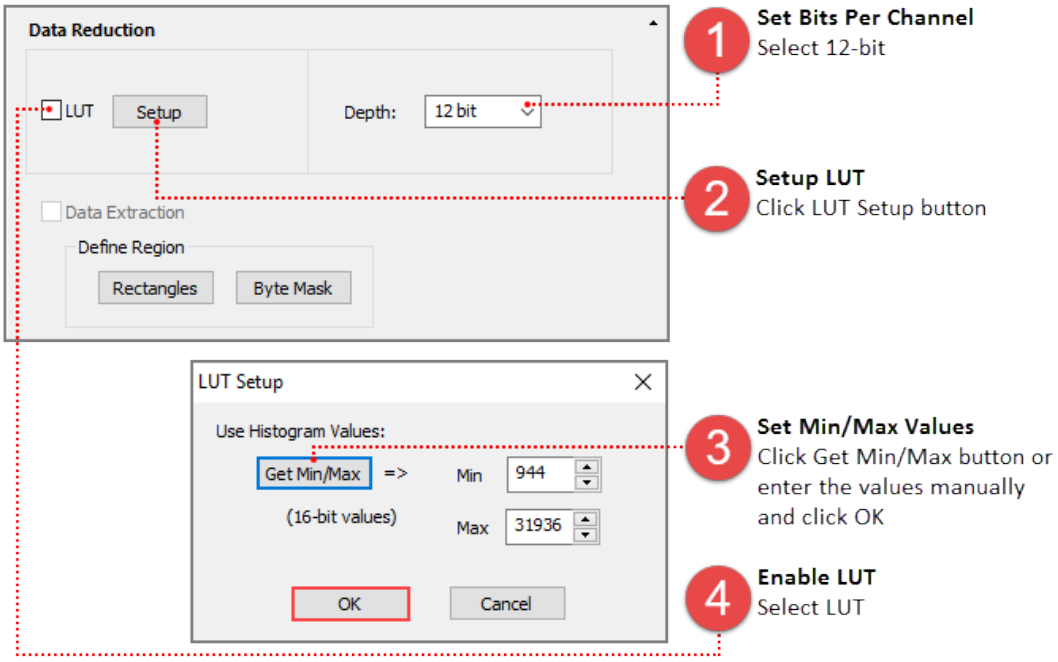
*1 Pixels centered on sensor, horizontal x vertical *2 In standard scan mode

Note: In order to achieve a reduced file size using Data Extraction or Data Reduction, the images must be acquired using High Speed Streaming to Disk and saved as DCIMG file type. Saving as or exporting to another file type (e.g., cxd or tiff) will result in 16-bit file size.

LUT

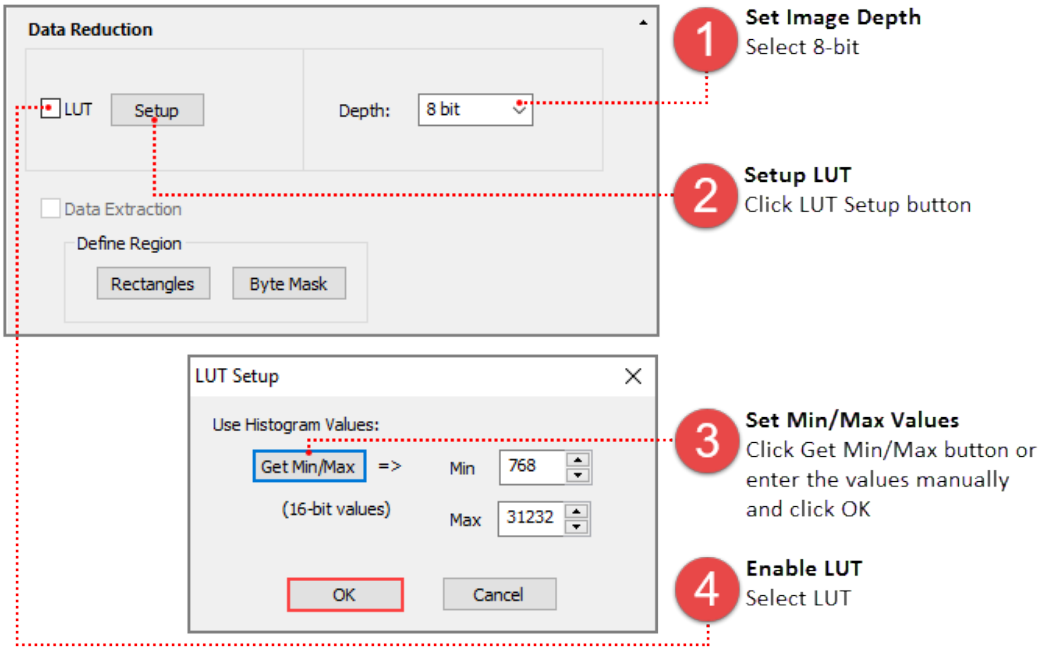
How to Setup LUT Range 12-bit

Configure the capture settings and follow the instructions below.



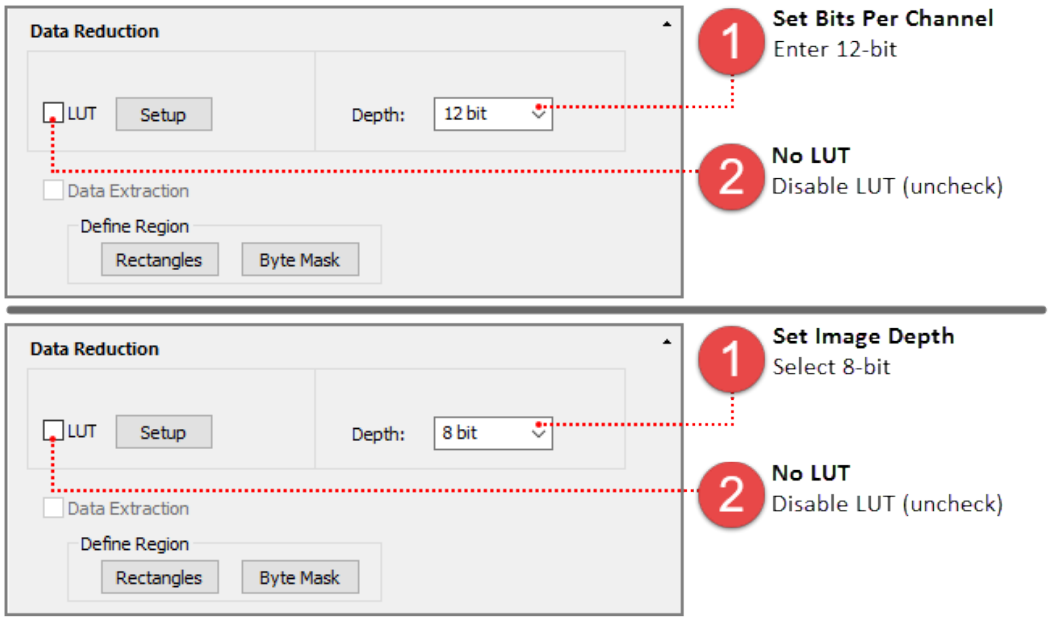
How to Setup LUT Range 8-bit

Configure the capture settings and follow the instructions below.



How to Setup 12-bit / 8-bit without LUT

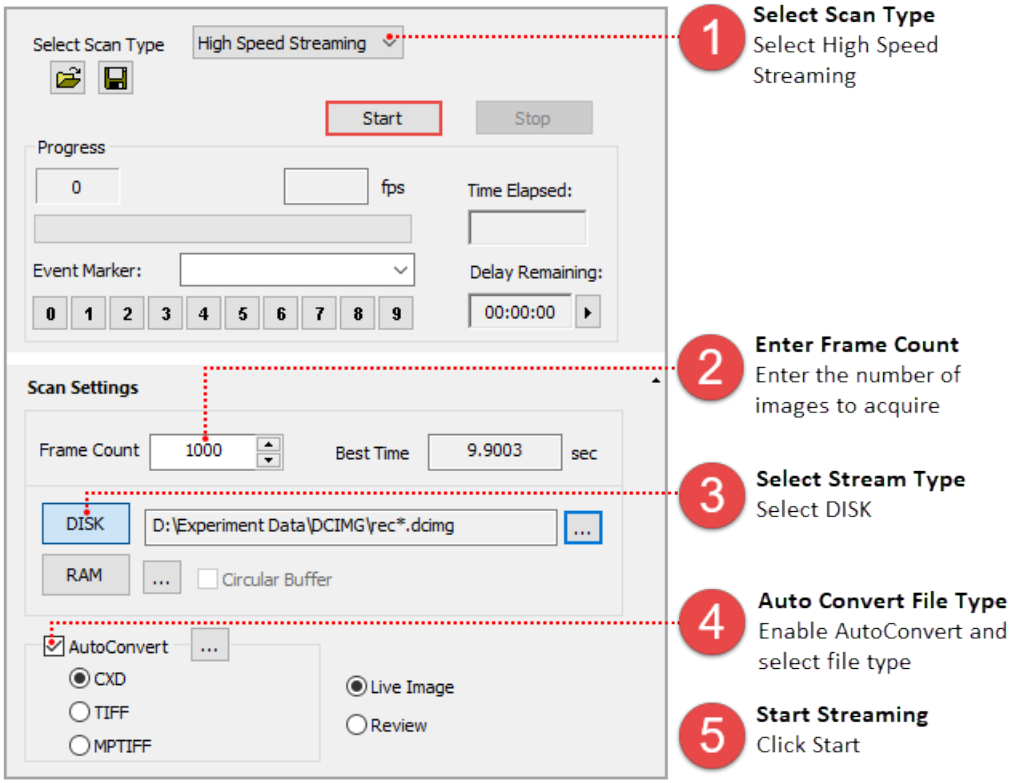
Configure the capture settings and follow the instructions below.



Note: The camera outputs MSB (most significant bit) 12-BIT / 8-bit images. This is not suitable for darker images as the lower 4-bits or 8-bits are lost. It works well for brighter images when detail intensity is not important.

How to Save Data with LUT

Once the capture and LUT settings have been configured, go to the Sequence pane and select High Speed Streaming from the Select Scan Type list.

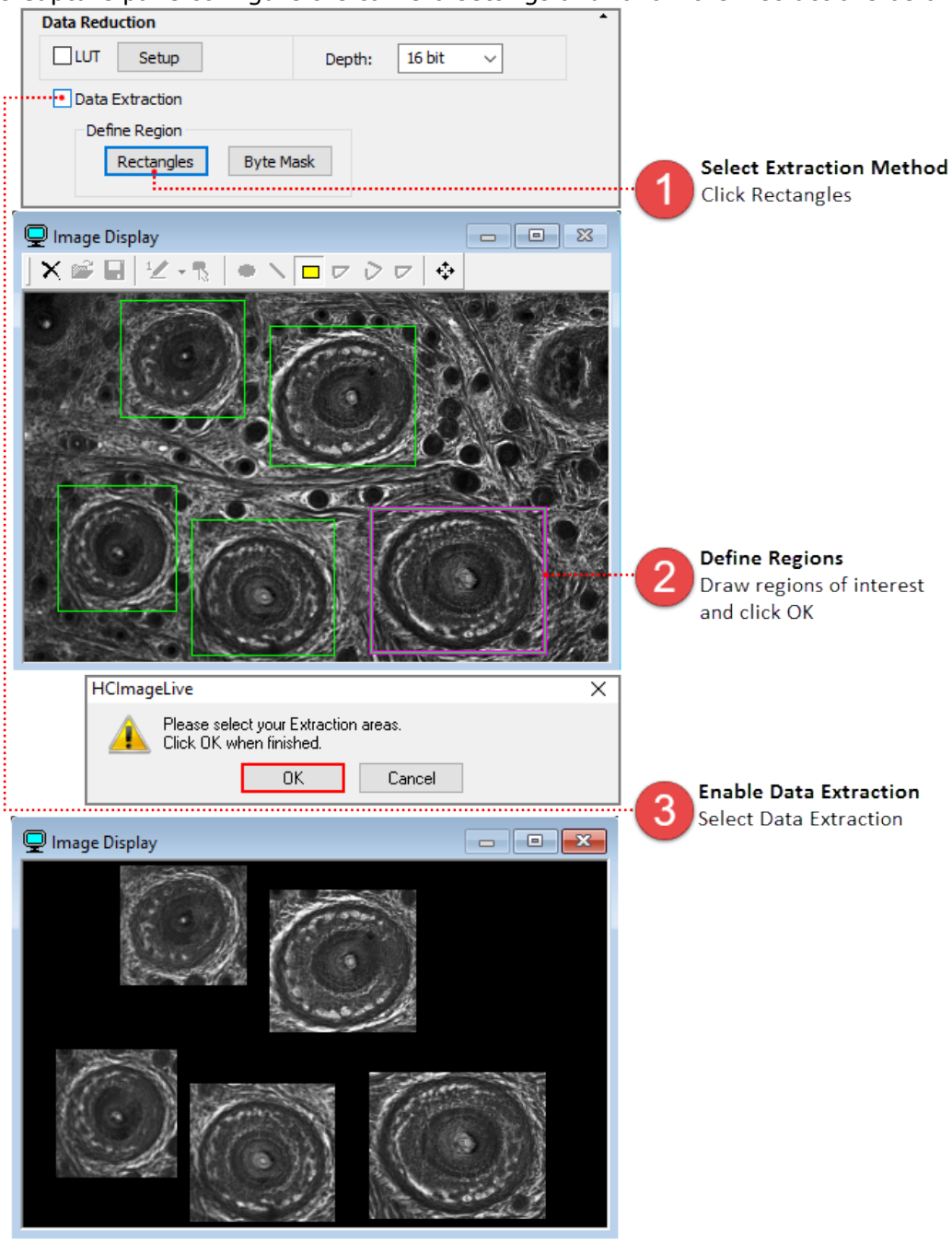


Data Extraction

This can reduce the amount of image data by defining which pixels that the camera will output using ROI or bitmap masks. Data Extraction can reduce the amount of data when streaming to DCIMG.

How to Define ROI for Data Extraction

In the Capture pane configure the camera settings and follow the instructions below.

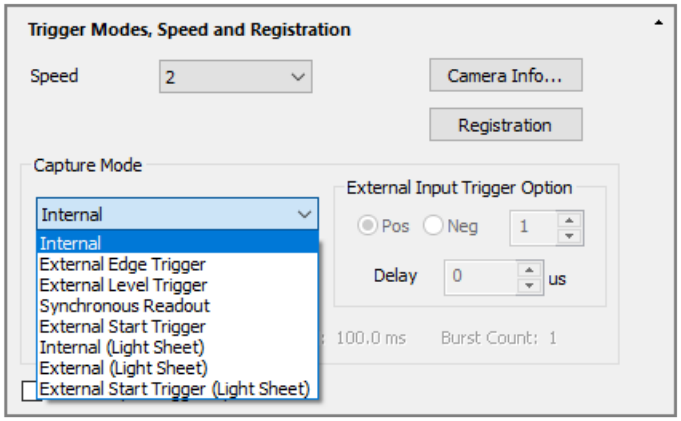


How to Save Extracted Data

In order to achieve a reduced file size using Data Extraction, the images must be acquired using High Speed Streaming to Disk and saved as DCIMG file type. Saving as or exporting to another file type (e.g., cxd or tiff) will result in 16-bit file size. For instructions on saving as DCIMG, please see "**LUT**" on page 20.

EXTERNAL INPUT TRIGGER MODES

The camera has various external input trigger functions to synchronize the camera with the external equipment. In the external trigger mode, the external equipment becomes a master and the camera becomes a slave. For information on Light Sheet capture modes, please see "**Light Sheet Mode**" on page 37.



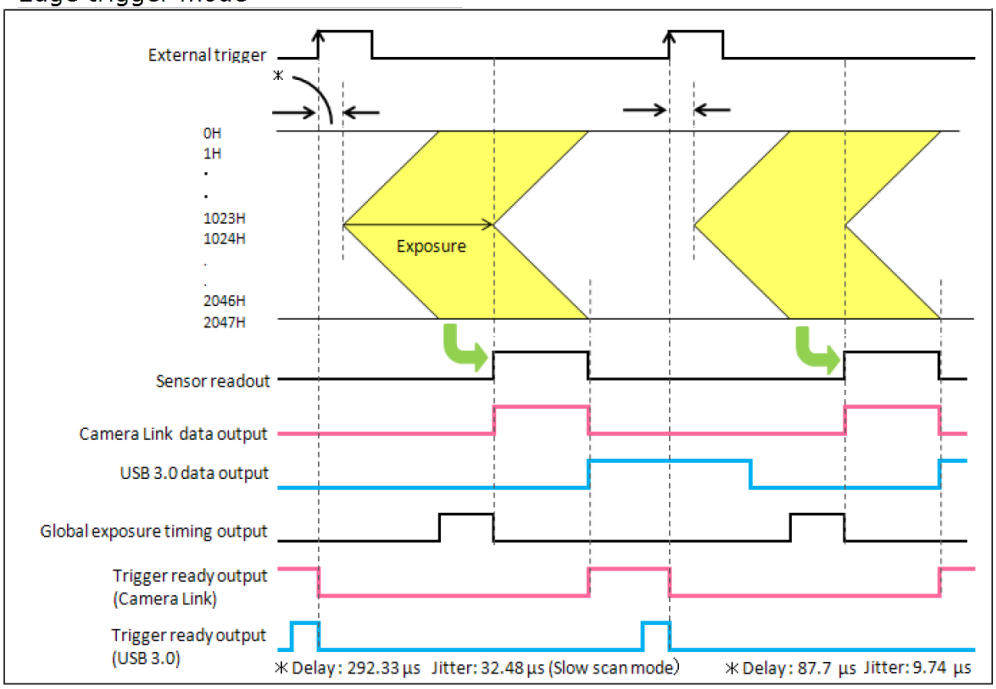
External Trigger Delay Function

For each external input trigger mode of the camera, a delay can be set between the input trigger signal and the start of capture of the camera. A delay from 0 µs to 10 s (10 µs steps) can be entered in the Delay box under External Input Trigger Option.

External Edge Trigger

An external signal triggers the start of exposure timing for each frame (i.e., the rising/falling edge of the external pulse triggers the capture of a frame). The exposure time is set by HCImage.

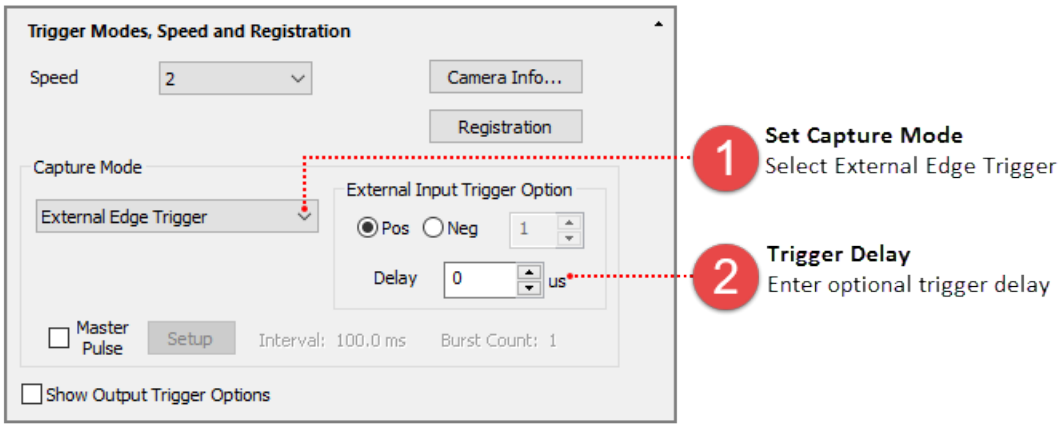
Edge trigger mode



External Edge and Level Trigger: Maximum Speed = Exposure Time + Readout Time

Setup Capture Mode for External Edge Trigger

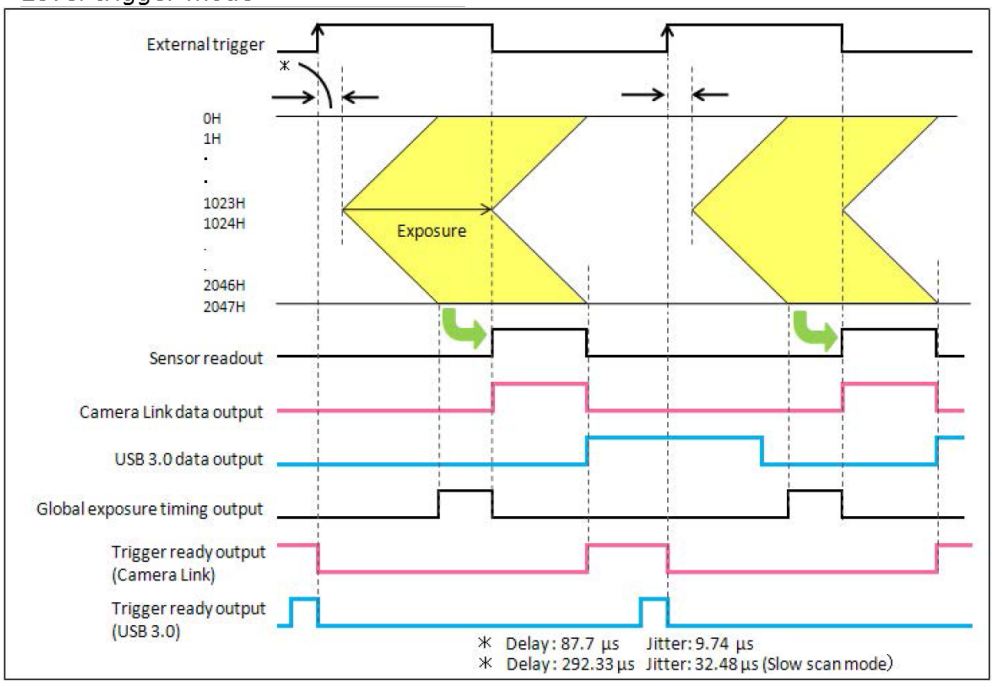
Follow the steps below to enable external edge trigger mode in HCImage. Enter the exposure time in the Camera Control panel. Click Live and the software will wait until the camera receives the external signal before displaying an image. A stream of triggers are required for continuously updated images.



External Level Trigger

In external level trigger mode, an external signal controls the start of exposure timing and the exposure time length. The exposure time is determine by the trigger pulse width.

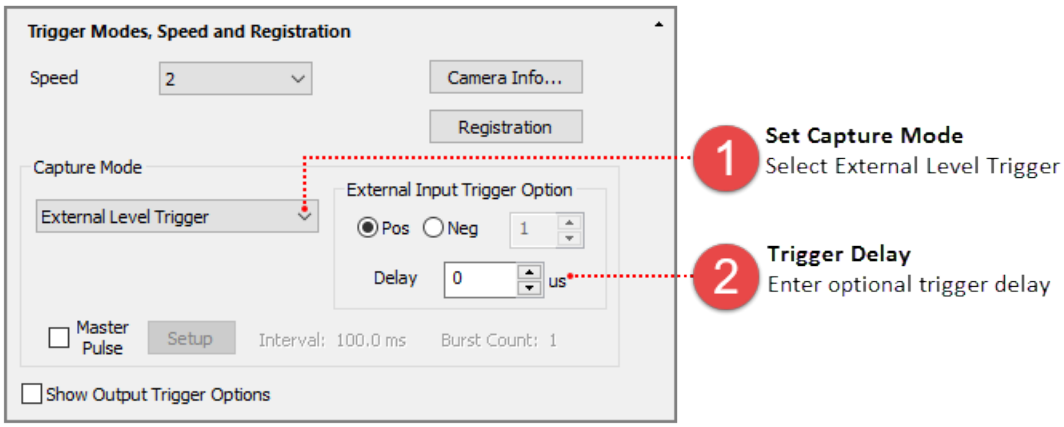
Level trigger mode



Note: In External Level Trigger mode, the exposure time in the Camera Control panel is grayed out because the exposure time is controlled by an external source.

Setup Capture Mode for External Level Trigger

Follow the steps below to enable external level trigger mode in HCImage. Click Live and the software will wait until the camera receives the external signal before displaying an image. A stream of triggers are required for continuously updated images.

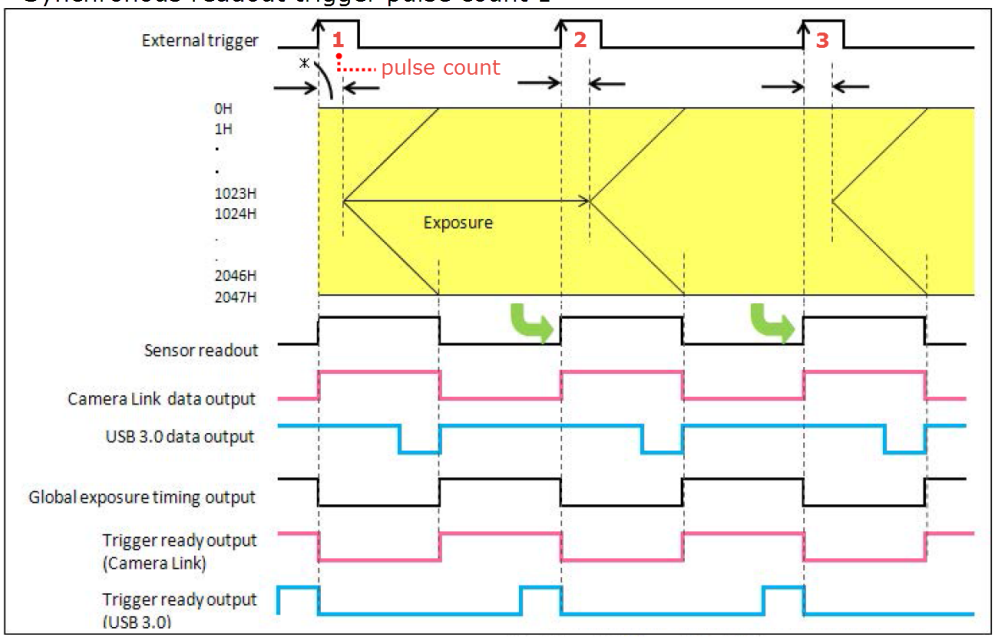


Synchronous Readout Trigger

(1) Normal operation (pulse count 1)

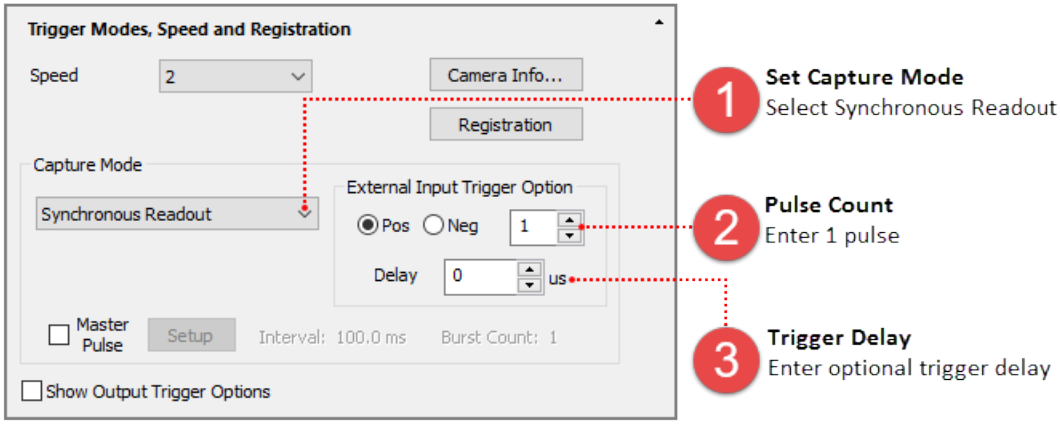
The synchronous readout trigger mode is used for continuous imaging when it is necessary to control the exposure start timing of each frame from an outside source and also when it is necessary to secure as long exposure time as possible. In the synchronous readout trigger mode, the camera ends each exposure, starts the readout and also, at the same time, starts the next exposure at the edge of the input trigger signal (rising /falling edge). That is, the interval between the same edges of the input trigger becomes the exposure time. So the first pulse starts exposing the first frame, the second pulse stops the exposure and starts the readout of the first frame and at the same time starts exposing the second frame. The third pulse stops the exposure and starts the readout of the second frame and at the same time, starts exposing the third frame. The minimum exposure time is equal to the readout time.

Synchronous readout trigger pulse count 1



Setup Synchronous Readout Trigger (pulse count 1)

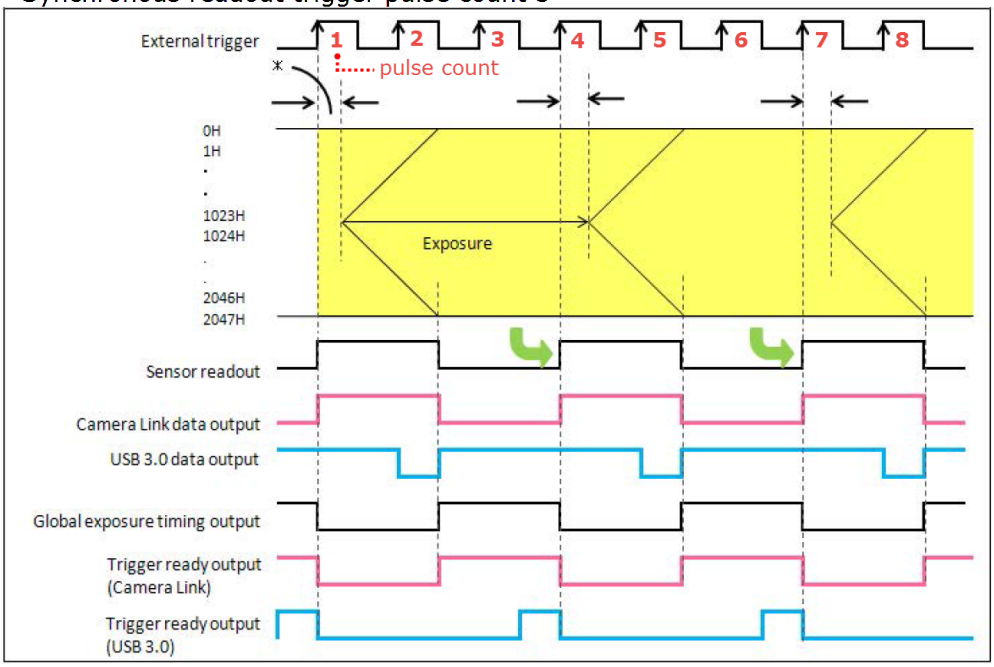
Follow the steps below to enable synchronous readout mode in HCImage. Click Live and the software will wait until the camera receives the external signal before displaying an image. A stream of pulses are required for continuously updated images or at least 2 pulses are required to capture a single image.



(2) Pulse count

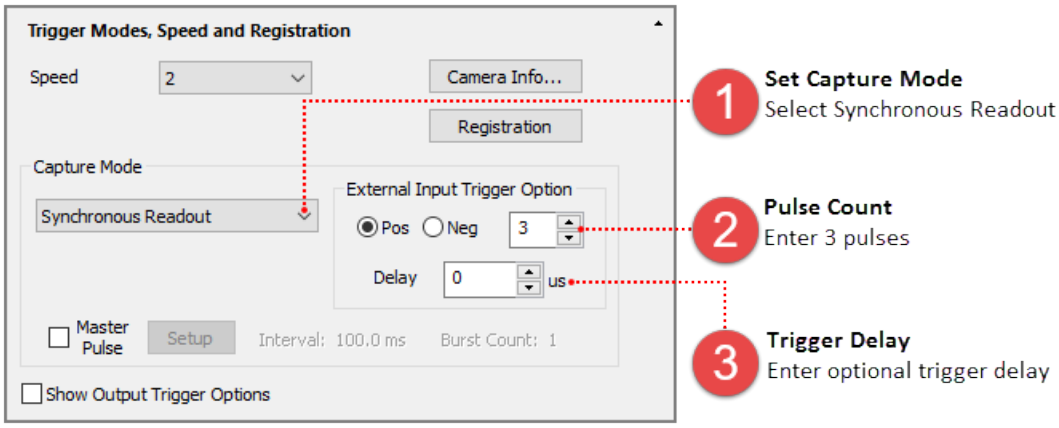
Useful for synchronizing the camera with a spinning disk confocal, the exposure time is determined by a specified number of timing pulses or pulse count. For example, the pulse count is set to 3. The first pulse starts exposing the first frame, the fourth pulse stops the exposure and starts the readout of the first frame and at the same time starts exposing the second frame. The seventh pulse stops the exposure and starts the readout of the second frame and at the same time, starts exposing the third frame.

Synchronous readout trigger pulse count 3



Setup Synchronous Readout Trigger (pulse count 3)

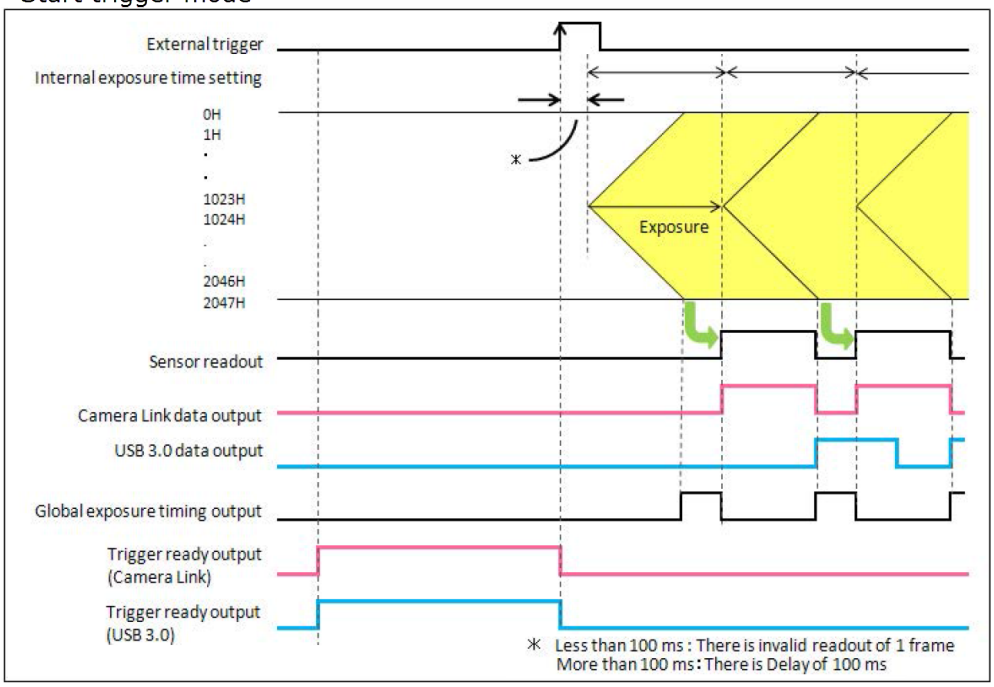
Follow the steps below to enable synchronous readout mode in HCImage. Click Live and the software will wait until the camera receives the required external signal pulses before displaying an image. A stream of pulses are required for continuously updated images or at least 4 pulses are required to capture a single image.



External Start Trigger

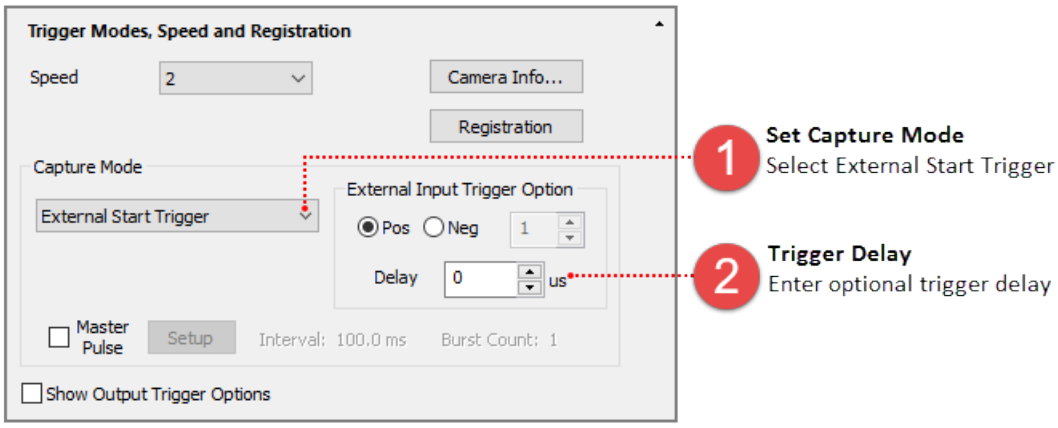
External start trigger mode utilizes a single trigger pulse to start acquiring images. An external signal triggers the start of exposure and then the camera acquires images at max frame rate in internal "free running" mode. This mode is only available when acquiring a single channel.

Start trigger mode



Setup Capture Mode for External Start Trigger

Follow the steps below to enable external start trigger mode in HCImage. Enter the exposure time in the Camera Control panel. Click Live and the software will wait until the camera receives the external signal before displaying an image.



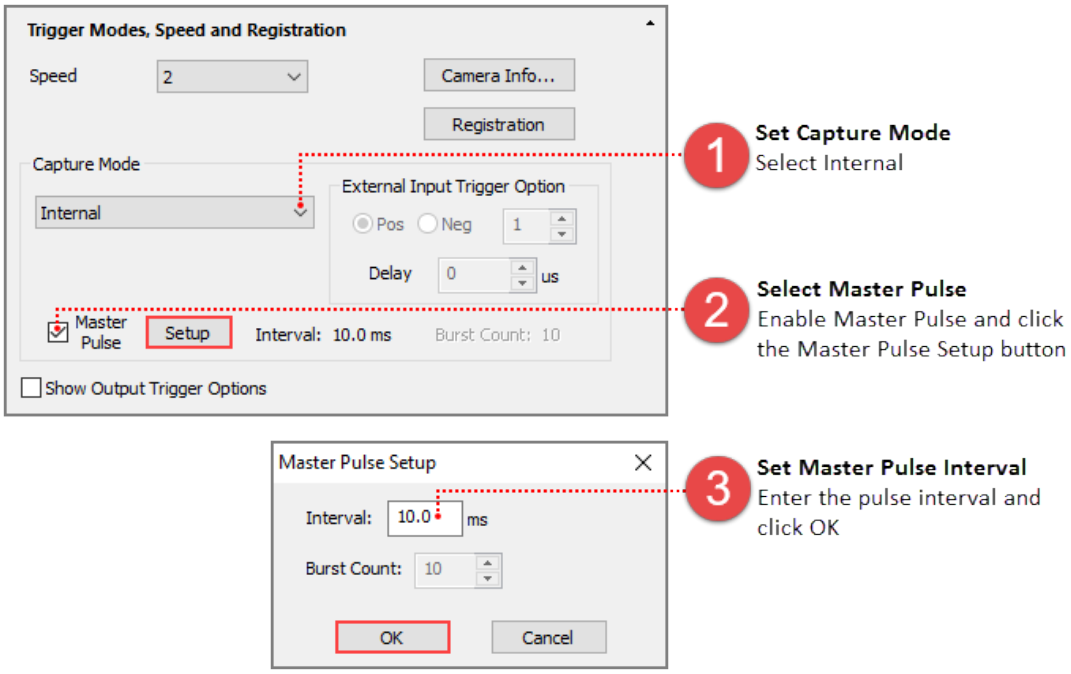
MASTER PULSE

The ORCA®-Flash4.0 V3 has a pulse generator built into the camera. Master Pulse can be used to control the timing of the camera as well as for synchronizing another camera and devices. Master Pulse timing modes include: Continuous, Start and Burst.

Master Pulse Mode	Required Capture Mode	Description
Continuous	Internal	Pulse is output at a specified interval which allows for control of the frame rate
Start	External Start Trigger	External source triggers the start of timing and then pulse output is at specified interval
Burst	External Edge Trigger	External source triggers a specified number of pulses

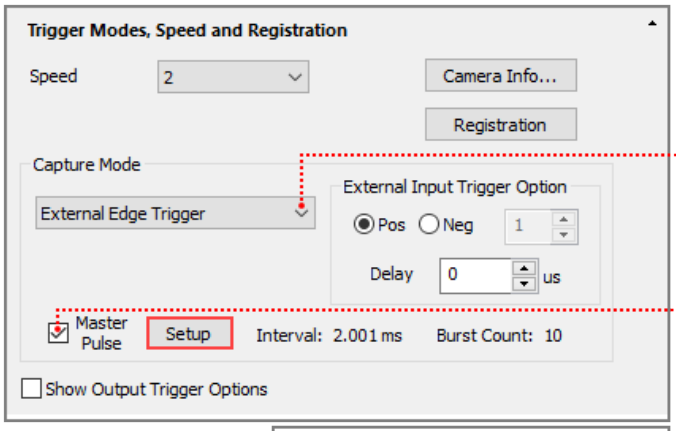
How to Set Master Pulse Continuous Mode

In the Capture pane enter the exposure time and then expand the Trigger Modes, Speed and Registration panel. Follow the steps below to configure the Master Pulse with Internal mode. Once configured, click Live and the camera will wait for the external trigger to begin acquiring images.



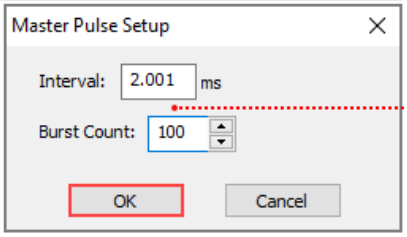
How to Set Master Pulse Burst Mode

Make sure that the trigger cable is connected to the External Trigger SMA port on the back of the camera. In the Capture pane enter the exposure time and then expand the Trigger Modes, Speed and Registration panel. Follow the steps below to configure the Master Pulse Burst Mode with the external edge trigger. Once configured, click Live and the camera will wait for the external trigger to begin acquiring images.



1 Set Capture Mode
Select External Edge Trigger

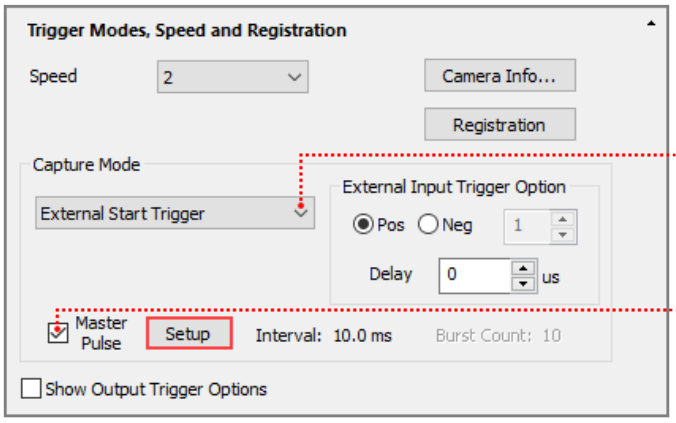
2 Select Master Pulse
Enable Master Pulse and click the Master Pulse Setup button



3 Configure Master Pulse
Enter the pulse interval, the number of pulses and click OK

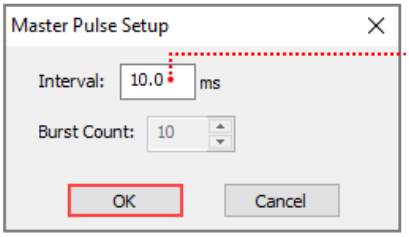
How to Set Master Pulse Start Mode

In the Capture pane enter the exposure time and then expand the Trigger Modes, Speed and Registration panel. Follow the steps below to configure the Master Pulse with the external start trigger. Once configured, click Live and the camera will wait for the external trigger to begin acquiring images.



1 Set Capture Mode
Select External Start Trigger

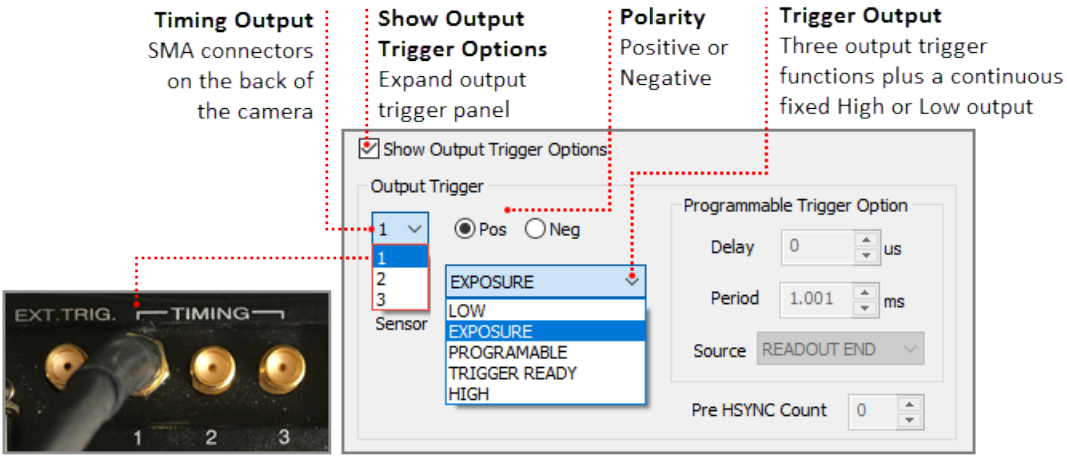
2 Select Master Pulse
Enable Master Pulse and click the Master Pulse Setup button



3 Set Master Pulse Interval
Enter the pulse interval and click OK

CAMERA TRIGGER OUTPUT

The camera provides a range of trigger output signals to synchronize with an external instrument where the camera becomes the master and the external instrument becomes the slave. There are three different trigger output functions, as well as a continuous High output (High output fixed) or continuous Low output (Low output fixed). These three different trigger output functions can be selected by software command, and they are output from any of the Timing out connectors.

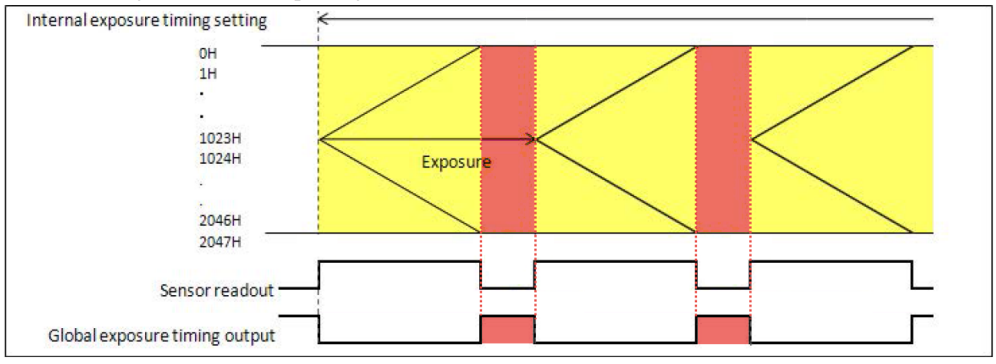


Global Exposure Output

Global exposure output is used to precisely control the on/off timing of an external illumination source in order to synchronize with the global exposure period, when all of the sensor lines expose at the same time. This mode is typically used for controlling a pulsed illumination source such as a laser or LEDs, as well as a fast shutter.

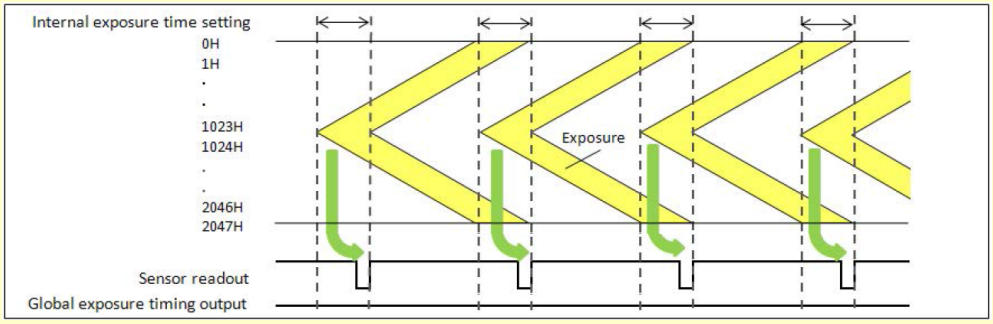
Global Exposure Time = Exposure Time - Readout Time

Global exposure timing output

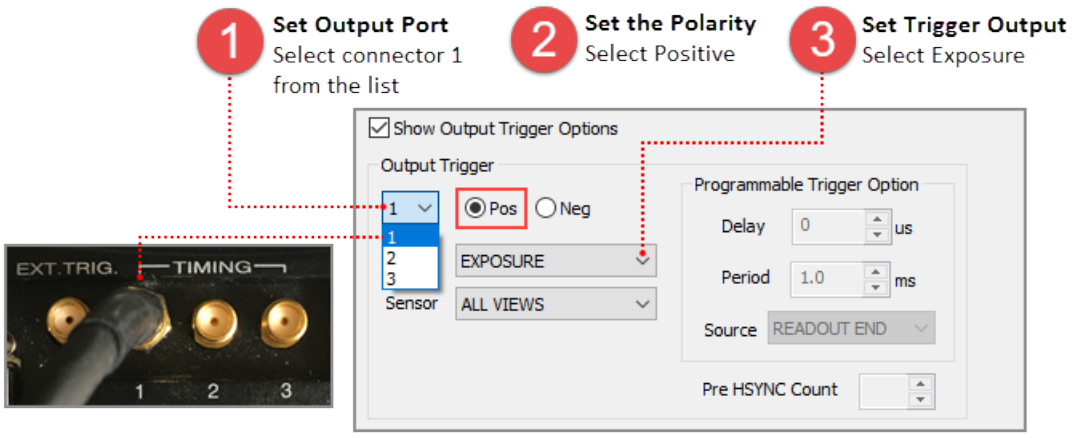


Note: There is no output signal when the exposure time is less than the frame rate.

Electrical shutter mode - Camera Link



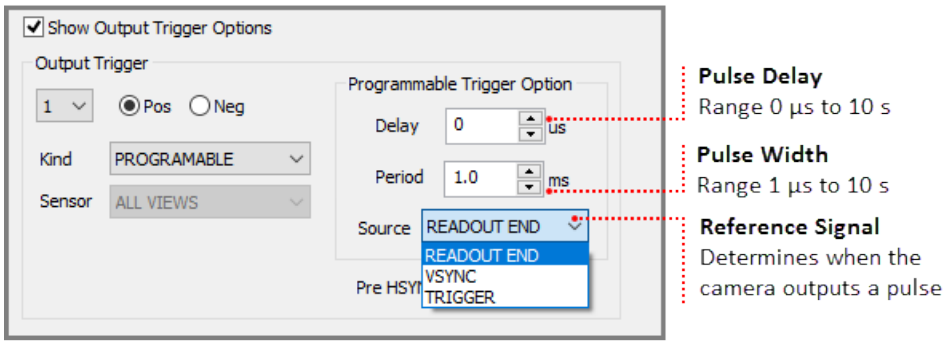
Setup Global Exposure Output Trigger



Programmable Timing Output

By using the programmable timing output, synchronizing external devices is simple. A system that needs simple timing signal does not require a delay unit or pulse generator. It is possible to program and output a pulse that has an optional pulse width and an optional delay time to the end of readout timing or Vsync. The range for delay time is 0 µs to 10 s, and the range for pulse width is 1 µs to 10 s (1 µs steps).

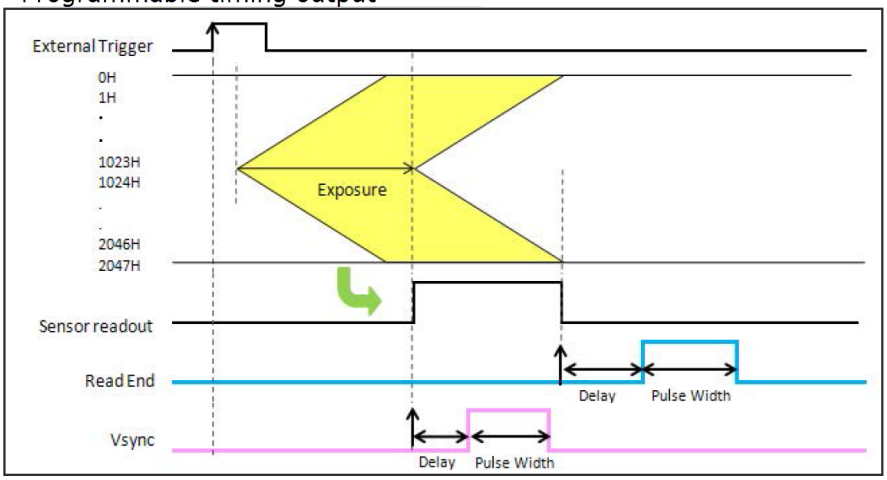
Note: Programmable triggers occur after the frame that triggers it.



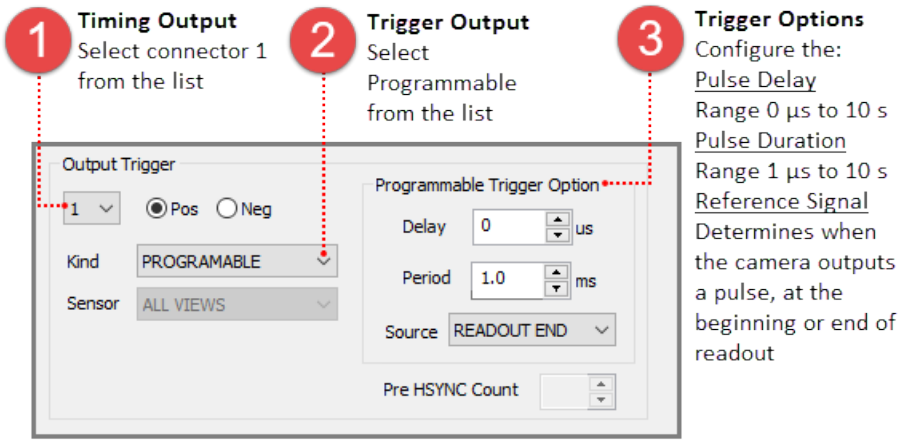
The relation between the parameter which can be set with each reference signal, and an output signal becomes below.

Reference Signal	Output Signal
Readout End	Camera outputs a pulse after certain delay from the end of sensor readout.
Vsync	Camera outputs a pulse after certain delay from the beginning of readout.
Trigger	Camera outputs a pulse after a certain delay, from the master pulse.

Programmable timing output



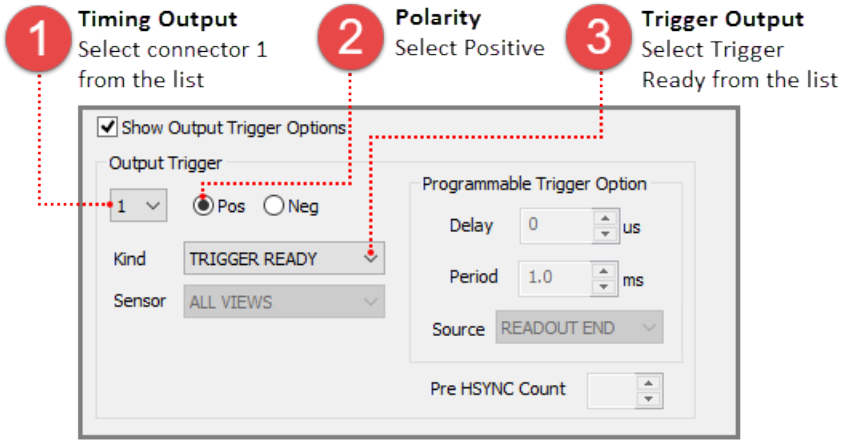
Setup Programmable Output Trigger



Trigger Ready Output

The trigger ready output is useful to make the frame intervals as short as possible in external trigger mode. For example, when the camera is working in the edge trigger mode, the next frame can start after the previous frame exposure is done. Thus, the camera can not accept a trigger for the next frame during the exposure period. The trigger ready output shows the trigger ready period when the camera can accept an external trigger in the external trigger mode.

Setup Trigger Ready Output Trigger



GLOBAL RESET

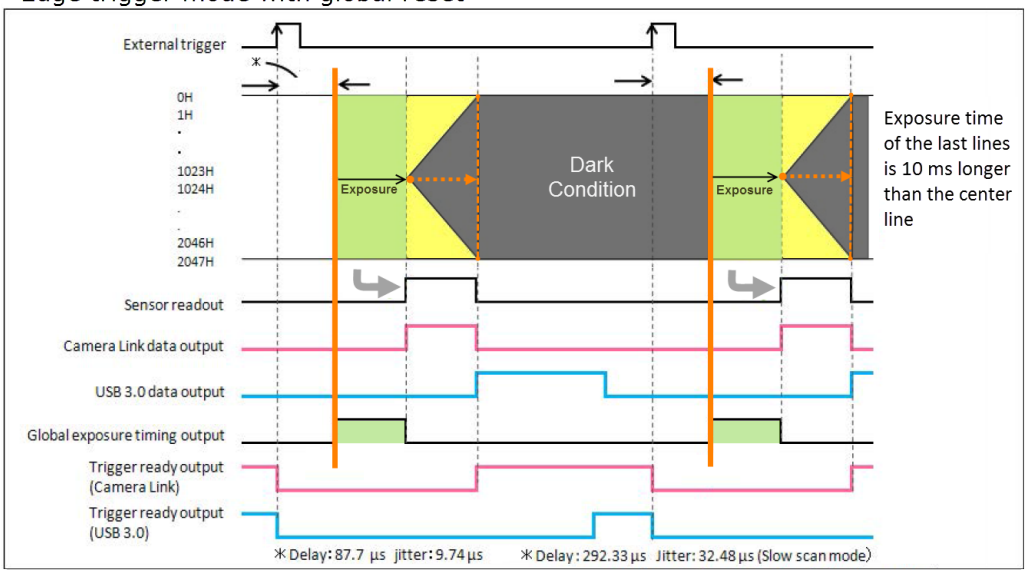
Global reset function is used to reset the electric charge of all of the pixels at the same time, just before acquiring an image. This allows all of the pixels to start exposing at the same time. The first line of the sensor exposes for the exposure time. The subsequent lines expose for the exposure time plus the readout time. This means that the last lines of the sensor will expose for 10 ms longer than the middle line. Global reset will work with External Edge and Level trigger modes.

Note: For optimal results, a dark condition is required during the readout of all of the lines.

Edge Trigger with Global Reset

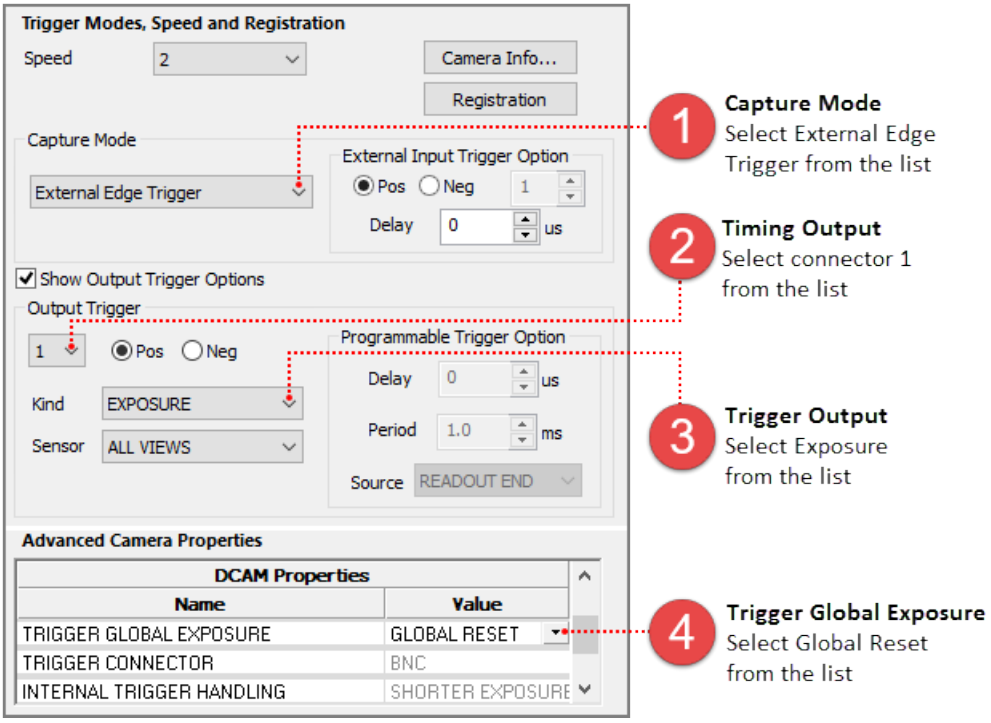
This mode is used with Global Exposure Synchronization. On the edge of the voltage change of the external trigger input, all of the lines start exposing. The first line of the sensor exposes for the exposure time set in HCImage. The subsequent lines expose for the exposure time plus readout time. This mode allows for external equipment to be the master and to precisely control the start of the global exposure timing.

Edge trigger mode with global reset



Setup Capture Mode for External Edge Trigger

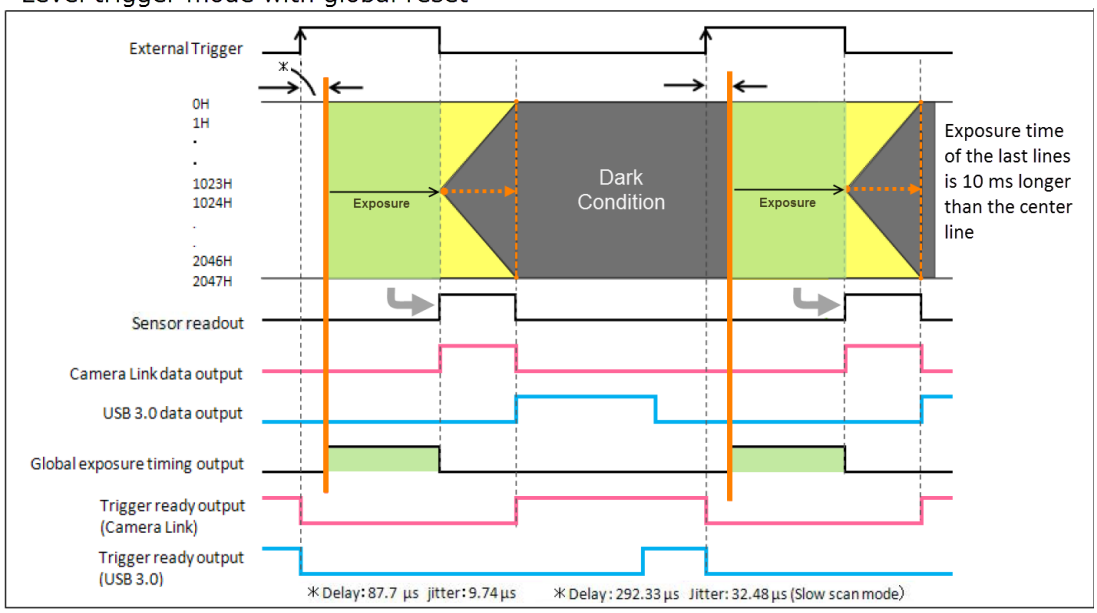
Follow the steps below to enable external edge trigger mode in HCImage. Enter the exposure time in the Camera Control panel. Click Live and the software will wait until the camera receives the external signal before displaying an image.



Level Trigger with Global Reset

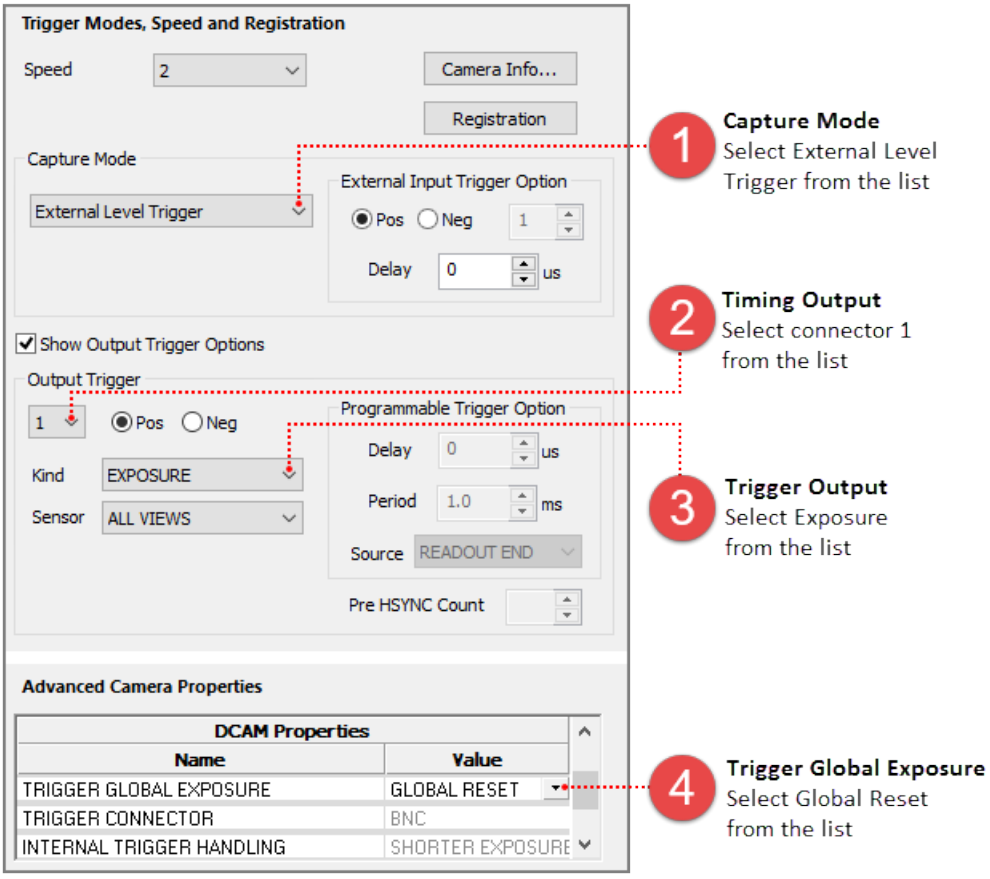
This mode is used with Global Exposure Synchronization. On the edge of the voltage change of the external trigger input, all of the lines start exposing. The first line of the sensor exposes until the edge of the voltage of the external trigger input. The subsequent lines expose for the exposure time plus readout time. This mode allows for external equipment to be the master and to precisely control the start of the global exposure timing.

Level trigger mode with global reset



Setup Capture Mode for External Level Trigger

Follow the steps below to enable external level trigger mode in HCImage. Click Live and the software will wait until the camera receives the external signal before displaying an image.

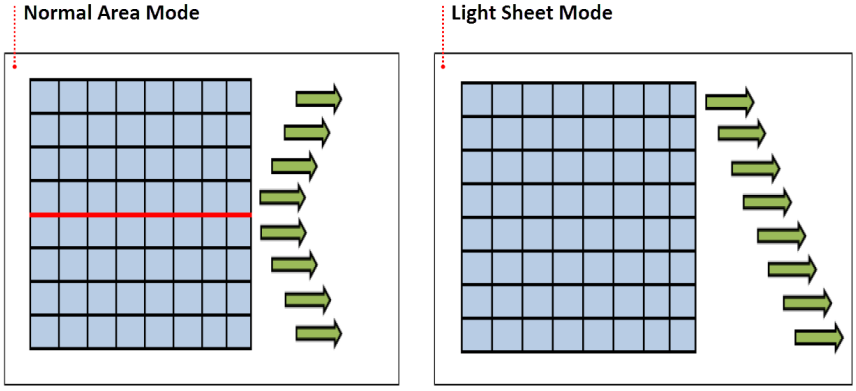


LIGHT SHEET MODE

Light Sheet Microscopy, sometimes referred to as SPIM (Selective Plane Illumination Microscopy) requires synchronizing image acquisition with the movement of light as it sweeps across a sample. The ORCA®-Flash4.0 V3 Light Sheet Mode incorporates specific timing features and a unified readout direction allow for this synchronization to occur.

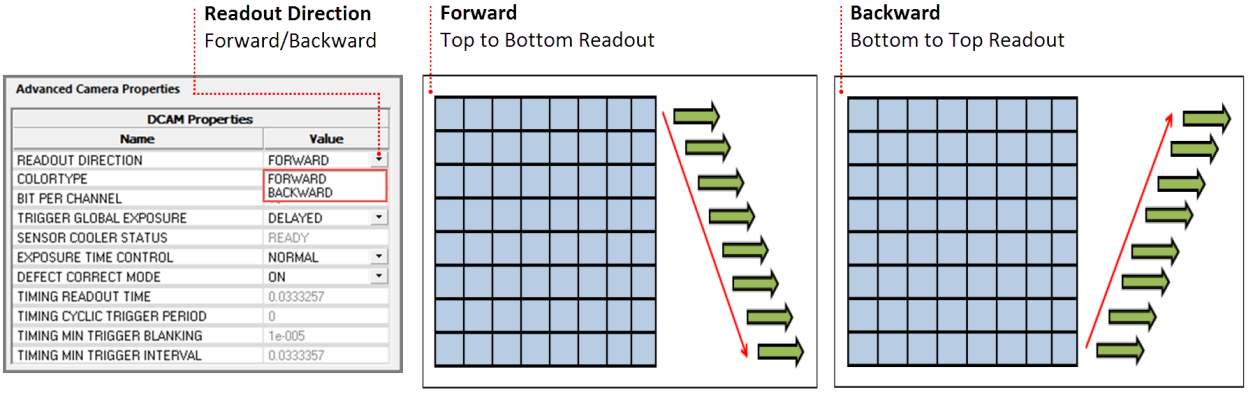
Readout Direction

With normal area mode, the camera readout is from the center line to the top line and to the bottom line simultaneously. With light sheet mode, the camera readout is from the top to the bottom line or from the bottom to the top line.



How to Change Readout Direction

With a light sheet mode enabled, expand the Advanced Camera Properties panel and under DCAM Properties, select Forward or Backward from Readout Direction list.

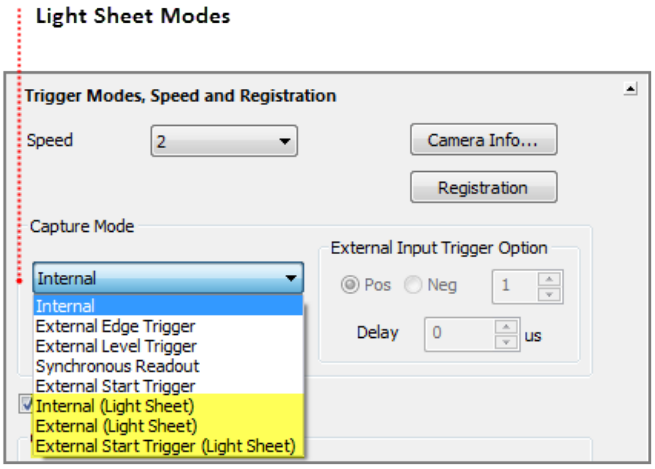


The size and position of the sub-array can be configured according to the table below.

Interface	Horizontal		Vertical	
	Size	Position	Size	Position
Camera Link	128 pixels	4 pixels	4 line steps	
USB 3.0	128 pixels	4 pixels	4 line steps	

Light Sheet Capture Modes

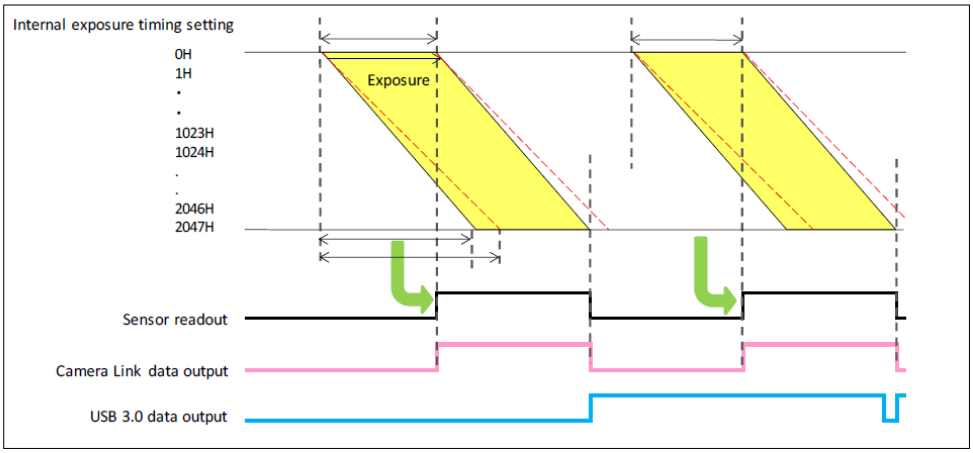
Light Sheet Modes are accessible from the Capture Mode list in the Trigger Modes, Speed and Registration panel shown below. The ORCA®-Flash4.0 V3 supports three modes for light sheet microscopy as described below. Along with each description is a basic set of steps for enabling that particular light sheet mode in HCImage.



Internal "Free Running" Mode

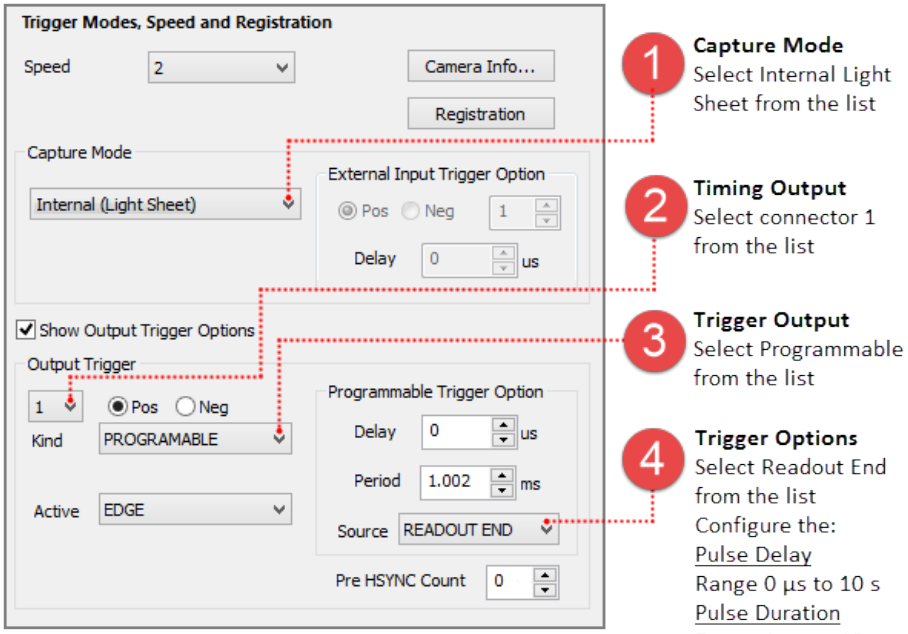
Synchronization is determined by the empirically matching the rate of the sweep of the light sheet and the camera readout to each other. There is no hardware or software triggering involved.

Light sheet internal mode



How to Setup Light Sheet Internal Mode

Follow the steps below to enable Internal Light Sheet Mode and to have the camera output an external trigger for every frame.

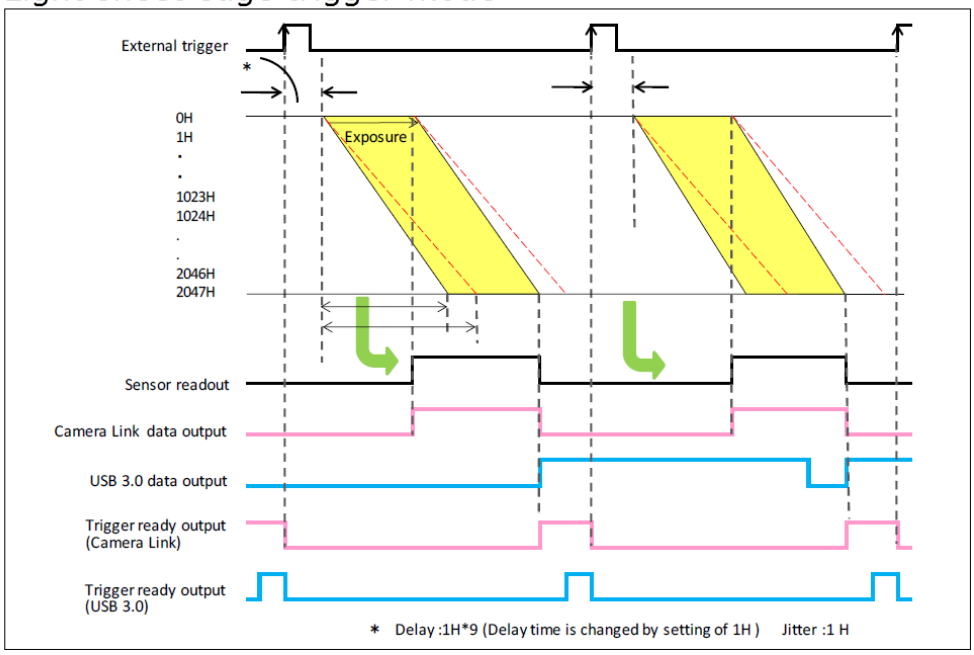


External "Edge" Trigger Mode

An external device triggers the camera at the start of each image frame. The exposure time is set by HCImage. This provides synchronization between the readout of the camera and the subsequent sweep of the light sheet. This method provides the most control over the camera and light sheet synchronization.

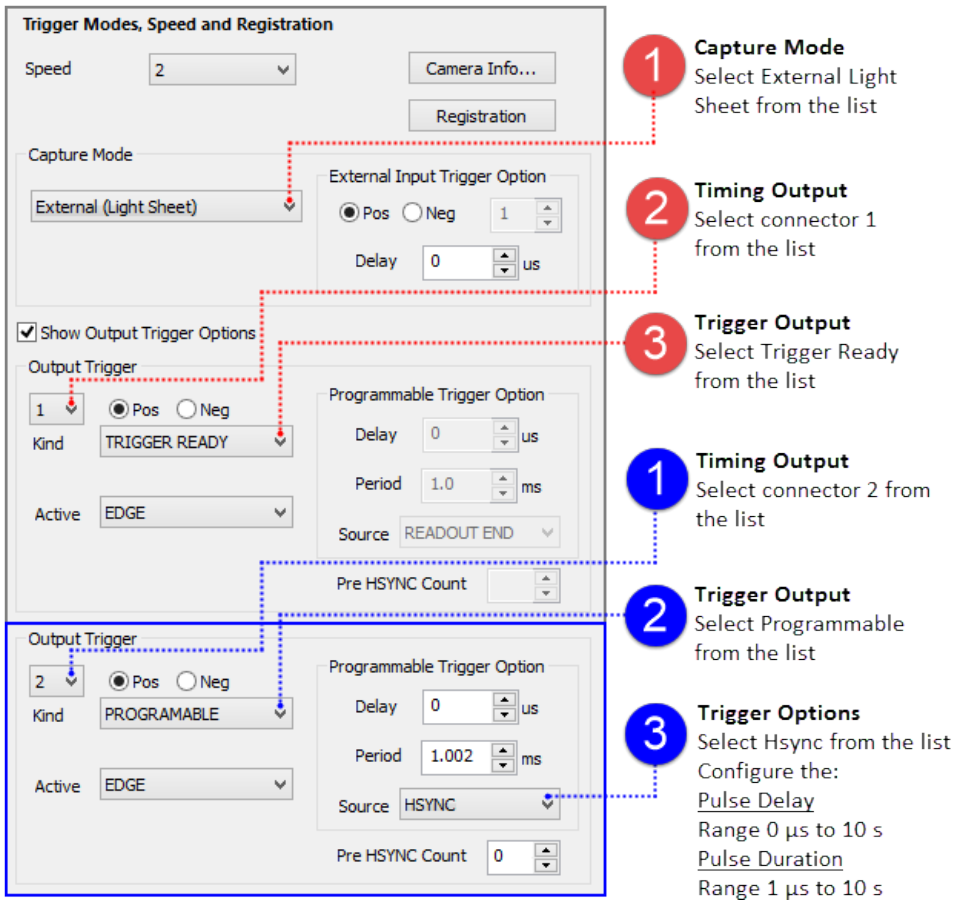
Note: Light Sheet External Trigger Mode is a frame trigger not a line trigger.

Light sheet edge trigger mode



Setup Light Sheet External "Edge" Trigger Mode

Follow the steps in Part 1 below in RED, to enable External Light Sheet Mode and to enable the camera trigger ready output for connector 1. Next, follow the steps in Part 2 (Blue) to have the camera output an external trigger from connector 2 for every line.



1 **Capture Mode**
Select External Light Sheet from the list

2 **Timing Output**
Select connector 1 from the list

3 **Trigger Output**
Select Trigger Ready from the list

1 **Timing Output**
Select connector 2 from the list

2 **Trigger Output**
Select Programmable from the list

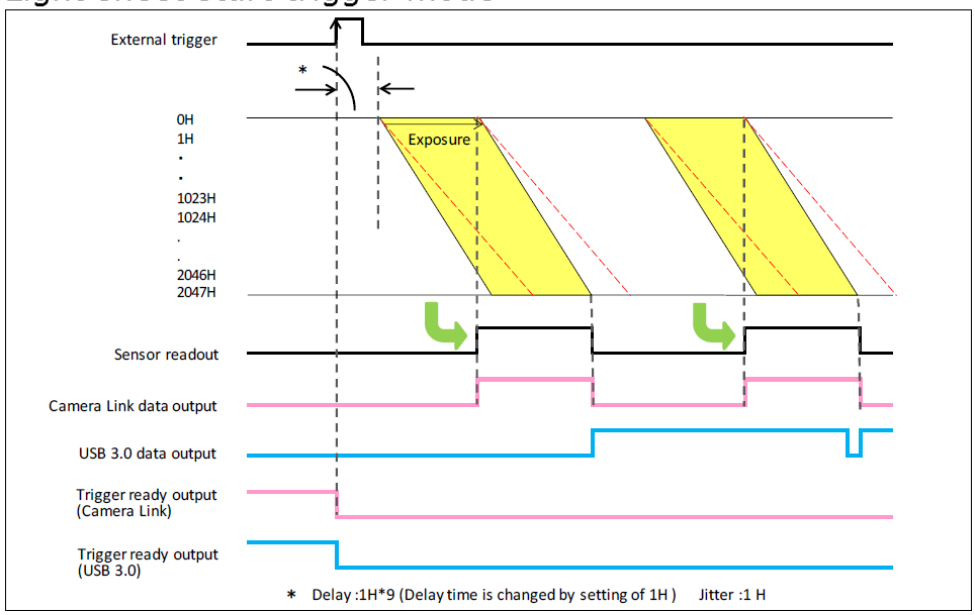
3 **Trigger Options**
Select Hsync from the list
Configure the:
Pulse Delay
Range 0 µs to 10 s
Pulse Duration
Range 1 µs to 10 s

External Start Trigger Mode

An external device triggers the camera to begin acquiring images in internal mode. As in "free running" operation the sweep of the light sheet and the readout of the camera need to have been previously matched empirically.

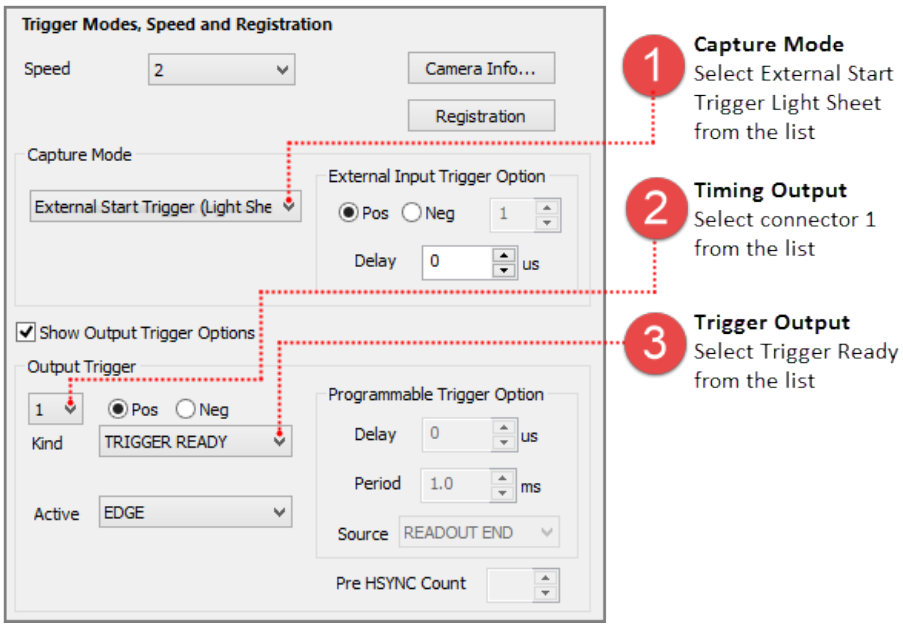
Note: Light Sheet External Start Trigger Mode is only available when acquiring a single channel.

Light sheet start trigger mode



Setup Light Sheet External Start Trigger Mode

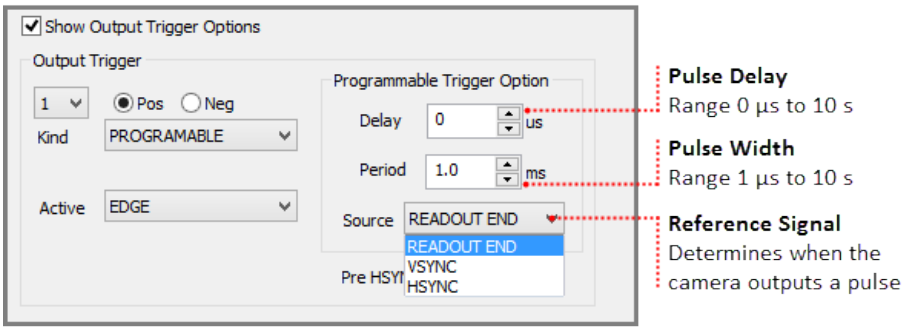
Follow the steps below to enable External Start Trigger Light Sheet Mode and to enable the camera trigger ready output for connector 1.



Programmable Timing Output

By using the programmable timing output, synchronizing with external devices is simple. A system which needs simple timing signal does not require a delay unit or pulse generator. It is possible to program and output a pulse that has an optional pulse width and an optional delay time to the end of readout timing, Vsync or Hsync. The range of delay is 0 µs to 10 s, and the range of pulse width is 1 µs to 10 s (1 µs steps).

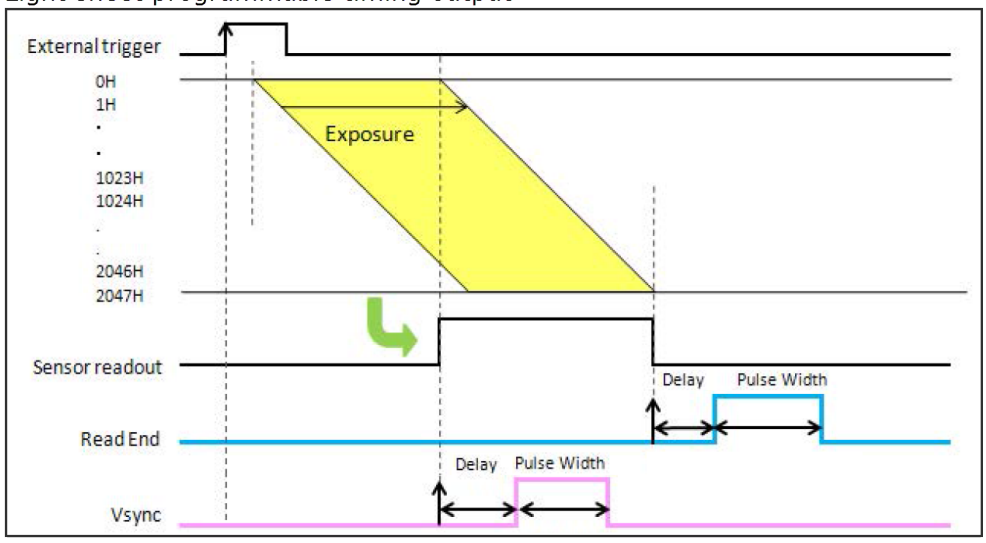
Note: In Light Sheet mode, programmable timing out can output an external trigger every line (Hsync) and frame (Readout End, Vsync). The camera can be a master to control a stage and light source.



The relation between the parameter which can be set with each reference signal, and an output signal becomes as shown below.

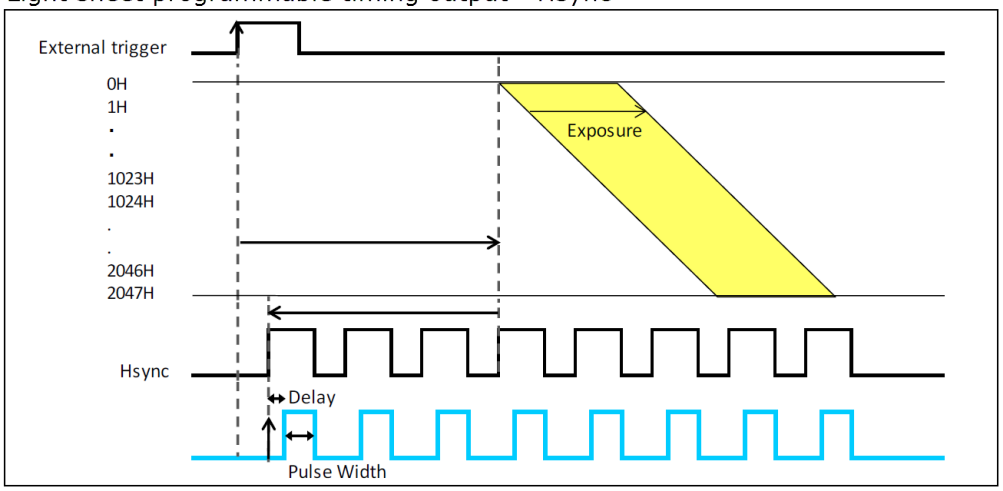
Reference Signal	Output Signal
Readout End	Camera outputs a pulse after certain delay from the end of sensor readout for each frame.
Vsync	Camera outputs a pulse after certain delay from the beginning of readout for each frame.
Hsync	Camera outputs a pulse after certain delay from the end of readout for each line.

Light sheet programmable timing output



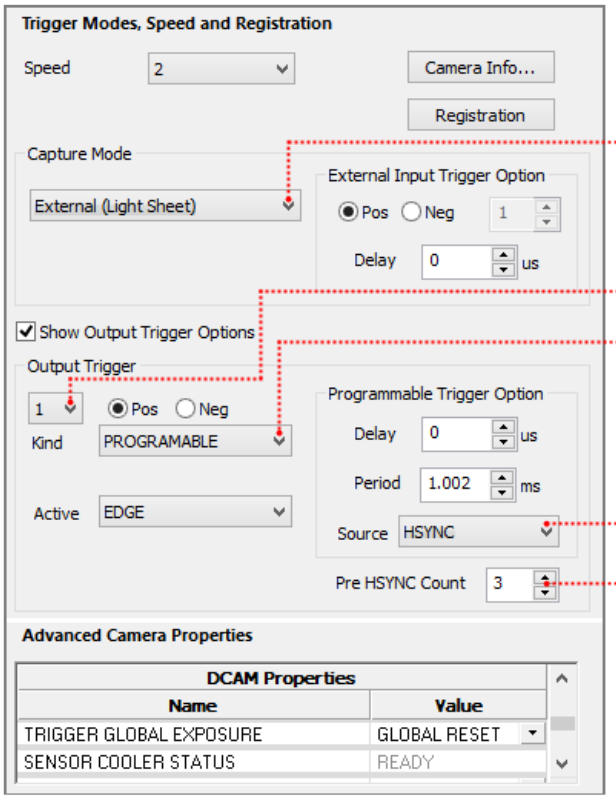
When you choose Hsync for the reference of programmable timing output, the camera can output a number of pulses before the start of exposure. This is called as Pre-Hsync. The Pre-Hsync range is 0 to 2047.

Light sheet programmable timing output - Hsync



How to Setup Pre-Hsync Pulses

Follow the steps below to enable External Light Sheet Mode and then to have the camera output a number of Pre-Hsync pulses prior to the start of exposure, as well as, send an external trigger for every line.



1 **Capture Mode**
Select External Light Sheet from the list

2 **Timing Output**
Select connector 1 from the list

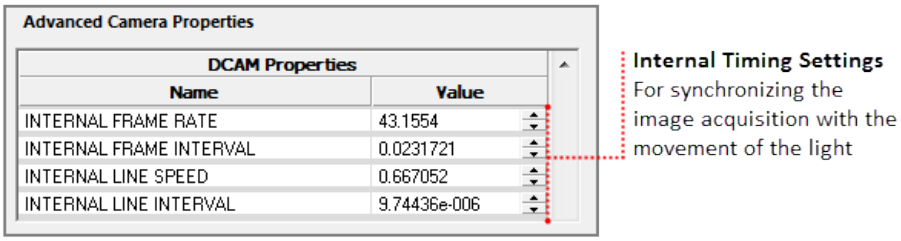
3 **Trigger Output**
Select Programmable from the list

4 **Trigger Options**
Select Hsync from the list
Configure the:
Pulse Delay
Range 0 µs to 10 s
Pulse Duration
Range 1 µs to 10 s

5 **Pre-Hsync Count**
Enter the number of pulses

Advanced Camera Properties

DCAM Properties provide a list of camera parameters reported by DCAM. The camera properties and reported values are specific to the connected camera and provide access to additional functionality based on the capture mode. Most of the camera properties in the list display values that cannot be changed and appear grayed out. In light sheet mode, readout direction and internal timing controls are available for synchronizing with external equipment. The camera readout direction can be set to forward or backward as was described at the beginning of this section, see "**Readout Direction**" on page 37.

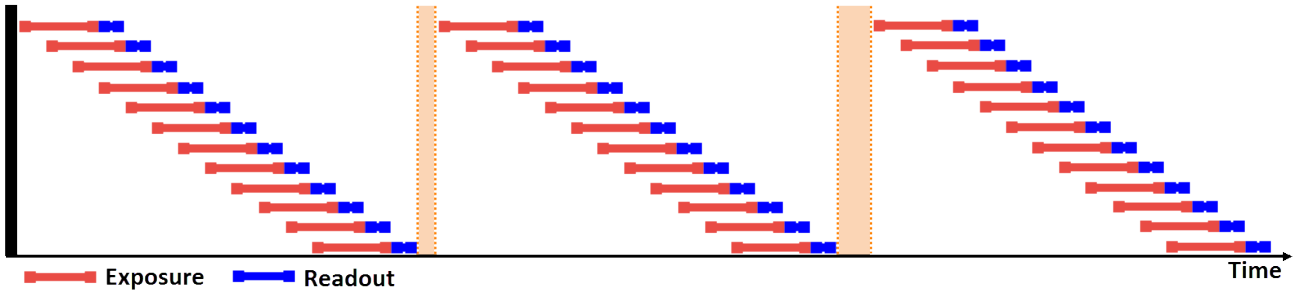


Internal Timing Settings

The internal timing settings are needed for synchronizing the image acquisition with the sweeping movement of the light.

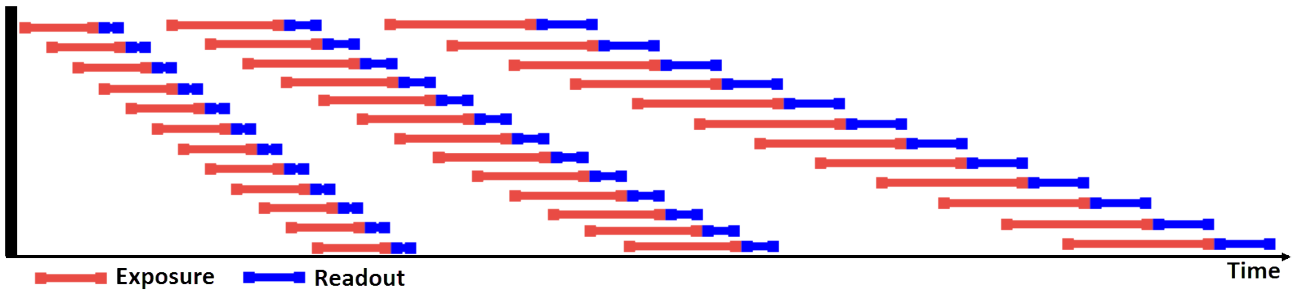
Internal Frame Rate - The number of frames per second that the camera acquires.

Internal Frame Interval - Is the period of time between the start of two frames. The internal frame interval is reported in seconds and can range from (0.998 ms) 1 ms to 10 s. This period can be adjusted to allow for the light sheet to return to the starting position.



Internal Line Interval - Is the readout slope, i.e., the period of time between the readout of two lines. The internal line interval is reported in seconds and can range from 9.7 µs to 100 ms.

Note: The time to readout a single line 1H = 9.7 µs in a standard scan. The exposure time should be set to a minimum of the number of sensor lines that the light beam covers, times the internal line interval.



Internal Line Speed - Is the vertical speed from top to bottom or bottom to top of the sensor readout. The internal line speed is reported in meters per second and can range from 0.0065 to 0.667 meters per second.

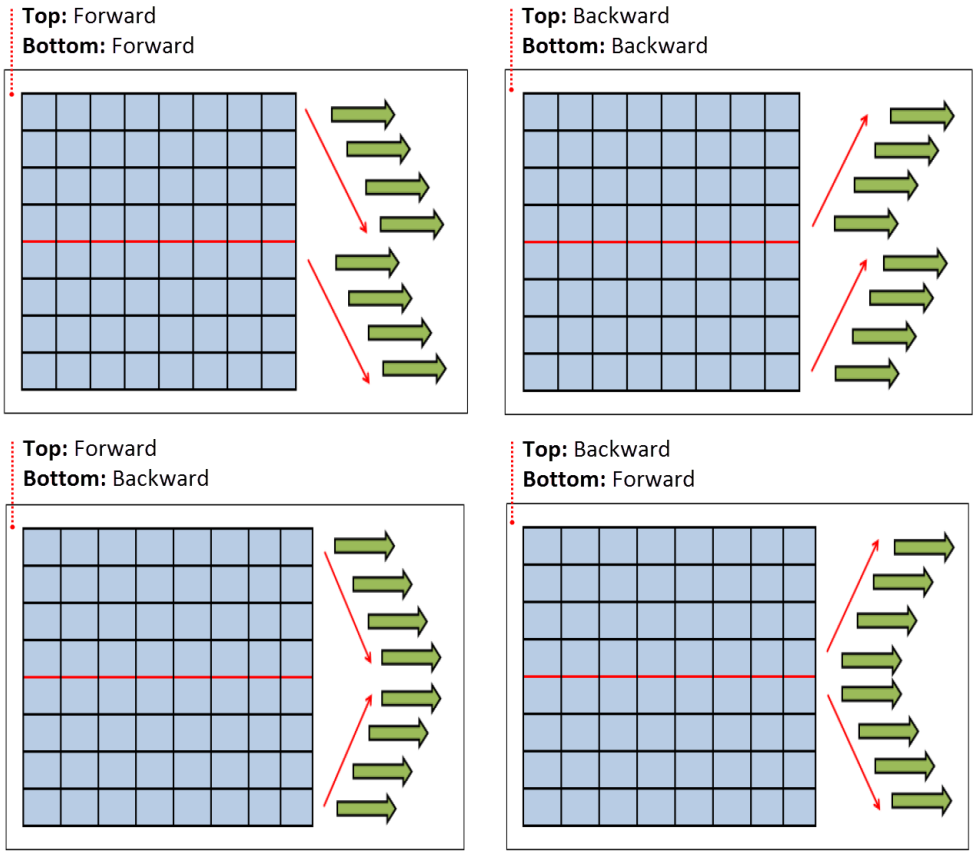
Note: Internal Line Speed = Pixel Size ÷ Internal Line Interval. So for the ORCA®-Flash4.0 V3, this would be 6.5 µm ÷ 9.7 µs = 6.5 x 10^{-6} ÷ 9.7 x 10^{-6} = 0.67 meters per second.

W-VIEW

For use with the W-VIEW Gemini image splitting optics for simultaneous dual wavelength image acquisition. The W-VIEW mode allows for independent exposure time settings, independent readout directions and separate position offset for subarray. W-VIEW capture modes include: Mono 1 Channel, RGB Color 2-Band and Mono 2 Channel.

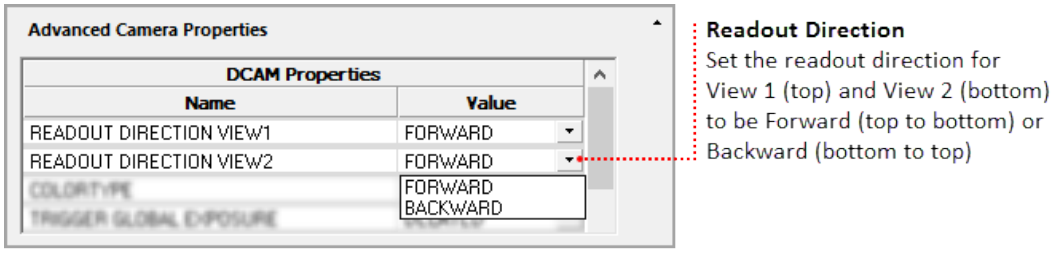
W-VIEW Readout Direction

With W-VIEW mode, the readout direction in the upper and lower half of the sensor can be setup separately.



Set Readout Direction

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.

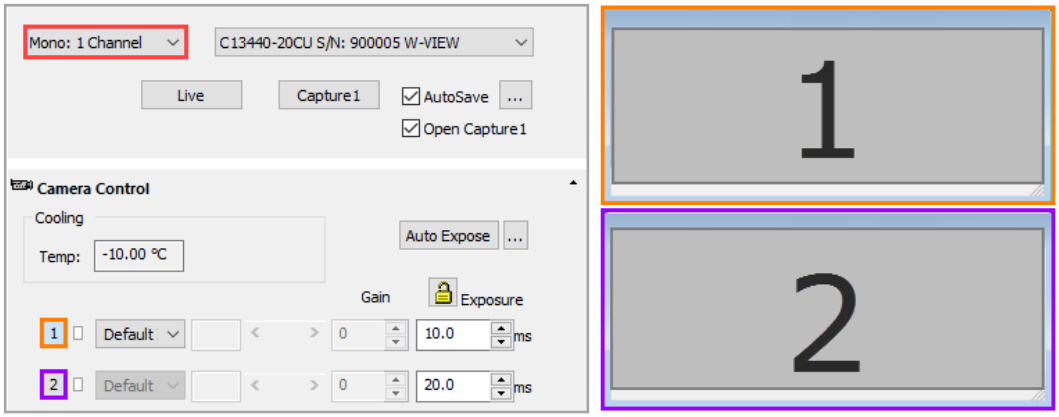


W-VIEW Capture Modes

HCImage Live will automatically detect the ORCA®-Flash4.0 V3 as two cameras, a normal camera and as a camera in W-VIEW mode. Select C13440-20CU S/N: ## for normal mode or C13440-20CU S/N:## W-VIEW for W-VIEW mode from the Capture Device list. W-VIEW capture modes include: Mono 1 Channel, RGB Color 2-Band and Mono 2 Channel. The capture modes are explained below.

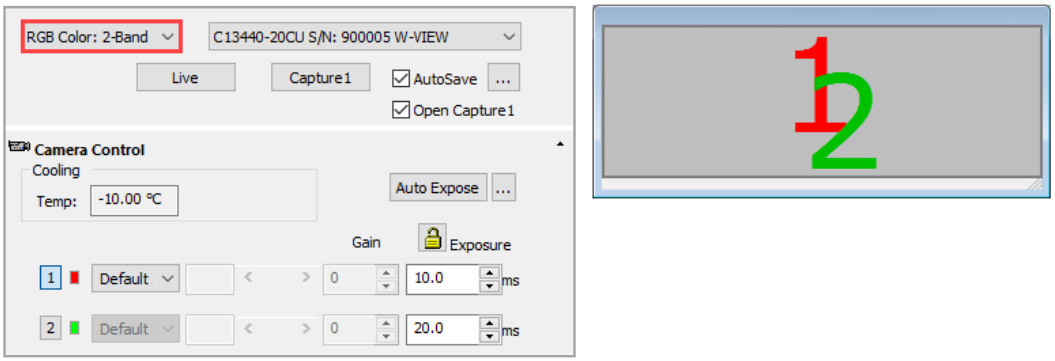
Mono 1 Channel

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).

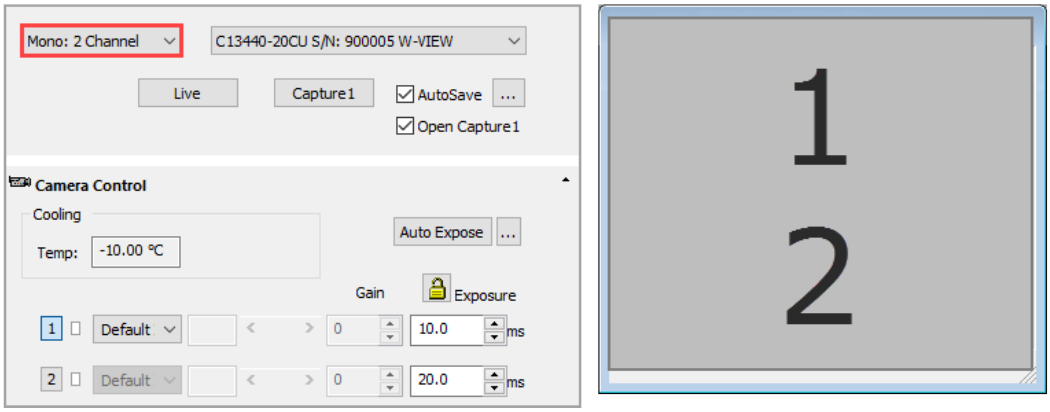
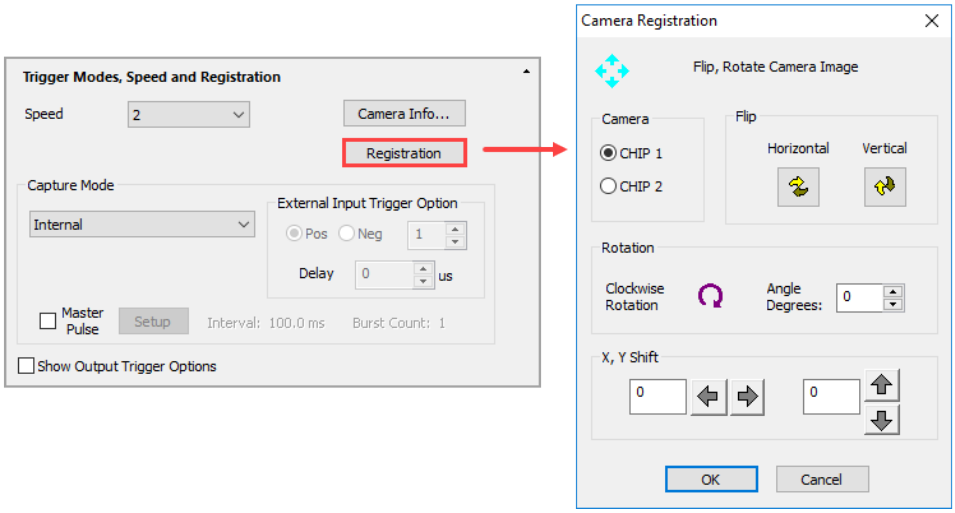


Image Alignment

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.



Dual Light Sheet Readout Mode

In Dual Light Sheet Readout Mode, the exposure and readout for both halves (top/bottom) is done simultaneously.

Basic Settings for Internal Light Sheet Mode