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HCImage

ORCA®-Flash4.0 V2 Camera Guide

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GETTING STARTED

This guide explains how to install, setup and run the ORCA®-Flash4.0 V2 (C11440-22CU) in HCImage and HCImage Live. In order for the camera to achieve its maximum performance, the frame grabber/interface card must be properly installed and the current versions of DCAM-API® and HCImage used. The ORCA®-Flash4.0 V2 can be connected using Camera Link or USB 3.0, both interface options are covered below.

Note: The current version of DCAM-API® is available for download at https://dcam-api.com/. For HCImage, registered users can download the latest version from https://hcimage.com/download/login/ (login required). For access to HCImage downloads, complete the software registration form (https://hcimage.com/register/), including a valid dongle number and email address, and an email will be sent with the HCImage download details. For HCImage Live, please contact hcsupport@hamamatsu.com and request a download link.

Interface Options

Camera Link Active Silicon FireBird (AS-FBD-1XCLD-2PE8)

- Recommend that the frame grabber be installed in PCIe x8 Gen2 or better.
- HC Demo Computer Dell Precision T5810, it is recommend that the frame grabber be installed in SLOT1_PCIe3x8 and the Raid Controller be installed in SLOT4_PCIe3x16.
- Highly recommended to adjust the following BIOS settings:
  - Disable (uncheck) SpeedStep and C-State under the performance section.
  - Enable (check) Turbo Boost and Hyper-Threading under the Performance section.

Note: For more information about the PC configuration, please see the PC Recommendations for ORCA®-Flash4.0 V2.

USB 3.0 IOI Technology (U3-PCIE-1XG205-10)

- The USB 3.0 card must be installed on a PCIe x1 Gen 2 (5GT/s) slot or better.
- HC Demo Computer Dell Precision T5810, it is recommend that the frame grabber be installed in SLOT3_PCIe2x1 and the Raid Controller be installed in SLOT4_PCIe3x16.
- Highly recommended to adjust the following BIOS settings:
  - Disable (uncheck) SpeedStep and C-State under the performance section.
  - Enable (check) Turbo Boost and Hyper-Threading under the Performance section.
- Camera must be connected to a USB 3.0 compliant bus, the drivers for the USB 3.0 chipset controller must be operational in the Device Manager.
- For Windows 8 and 8.1, USB xHCI compliant drivers are included in the OS. For Windows 7 get drivers from manufacturers website - Renesas USB3.0 Driver (ftp://60.248.38.84/cat_106/30230_dr.zip)

Accessory

Trigger Cable for the ORCA®-Flash4.0 V2, ORCA®-Flash4.0 LT and ImagEM® X2 (CAMRA-4303-000)

- Single cable with one SMA to one BNC connection. Typically only one cable is included with a kit.

HCImage Document Types and File Formats

To understand the workings of the application it is important to be familiar with the HCImage document types:

Image Documents (.tif and .dcimg) refer to a single or sequence of uncompressed images saved as TIFF files (.tif), MPTIFF files (.tif) or DCAM Image files (.dcimg).

- **DCAM Image File (.dcimg)** is the Hamamatsu image file format created when using high speed streaming to disk.
- **Tagged Image File Format (.tif)** is an uncompressed, tag-based, single image file format.
- **Multi-page TIFF or MPTIFF (.tif)**, contain multiple TIFF images. MPTIFF files have a 65 000 image limit or a 4 GB size limit.

Data Documents (.cxd) are HCImage's proprietary file format that utilize a hierarchical file structure, allowing images and measured data to be easily displayed in a variety of graphical formats.

Additional Notes

Note: The ORCA®-Flash4.0 V2 and the ORCA®-Flash4.0 LT are not supported under Windows XP.

Note: USB 3.0 camera operation is not plug-n-play enabled, connect the camera to the PC before turning the camera power ON. Also, avoid connecting any other devices to the cameras USB 3.0 card.

INSTALLATION

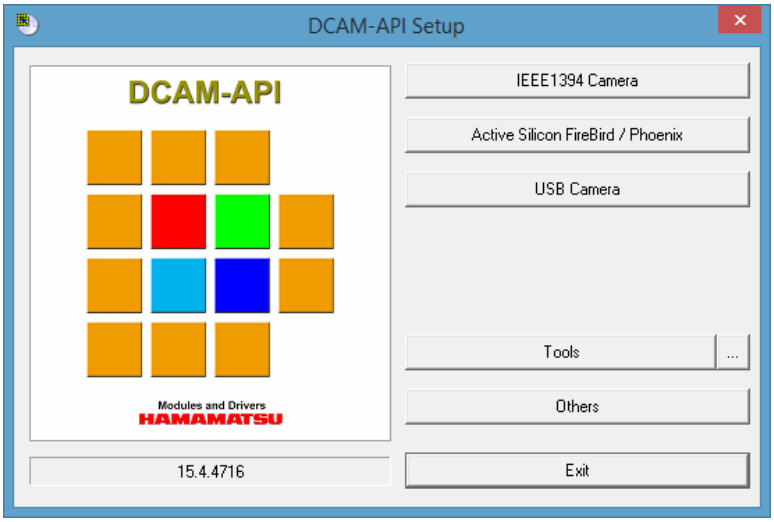
HCImage Live

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.

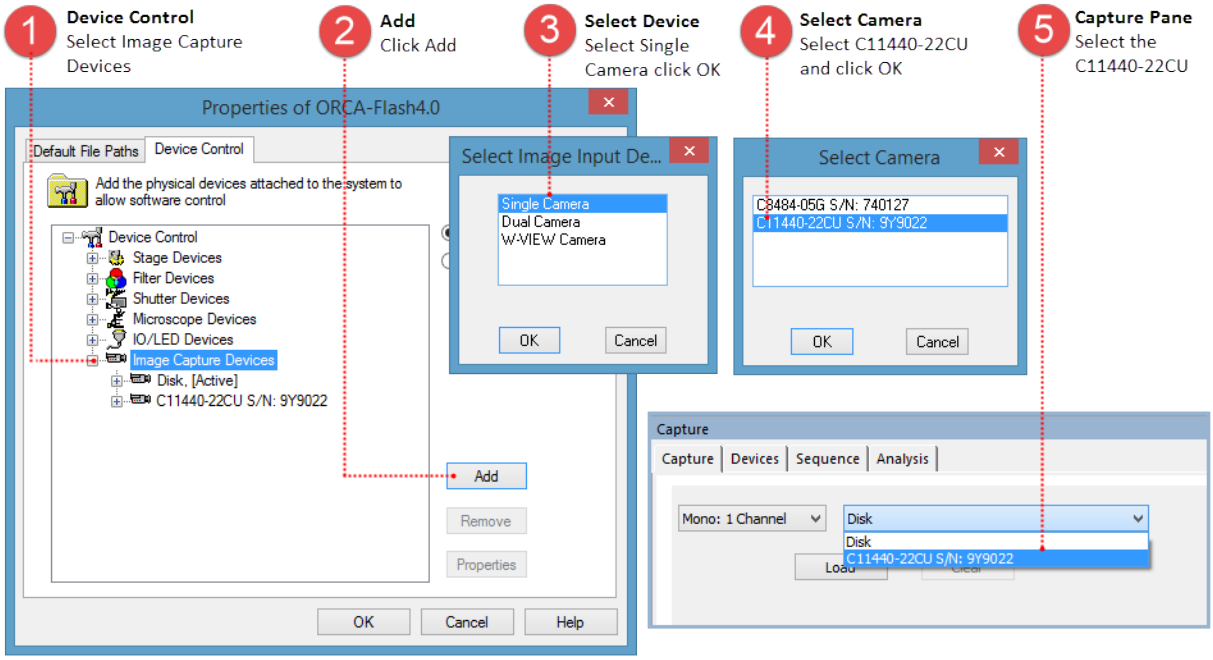


HCImage

1. Insert the HCImage installation DVD into the DVD-ROM drive. If autoplay is enabled, the HCImage setup will run automatically. If autoplay fails to start, locate your DVD-ROM drive and double-click on **setup.exe**.
2. Click **Yes**, if prompted by the User Account Controls.
3. To begin the installation wizard, click **Next**.
4. Follow the instructions on each installation page.
5. Securely connect the dongle () to a USB port after the software installation has finished.
6. Install the appropriate DCAM-API drivers, please see "**DCAM-API Drivers**" on the previous page.
7. Turn the camera power on prior to launching HCImage.
8. Click the **HCImage** icon on your Desktop to launch HCImage.
9. Register the software to receive technical support, please go to https://www.hcimage.com/ and click **Register**.

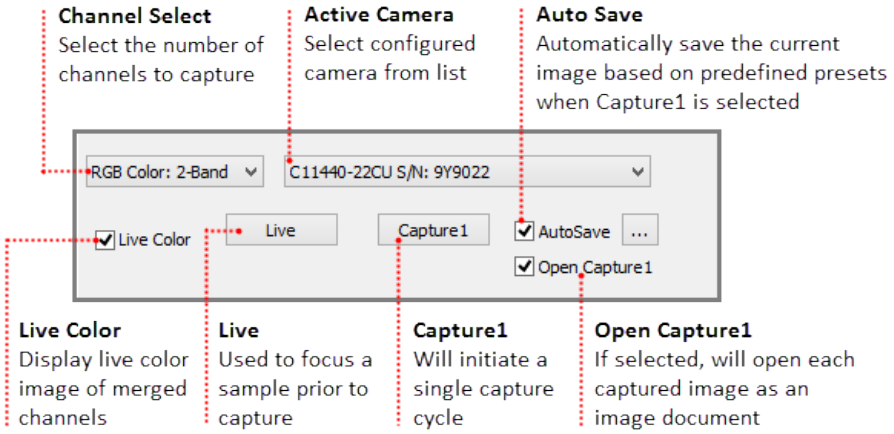
Add the camera

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



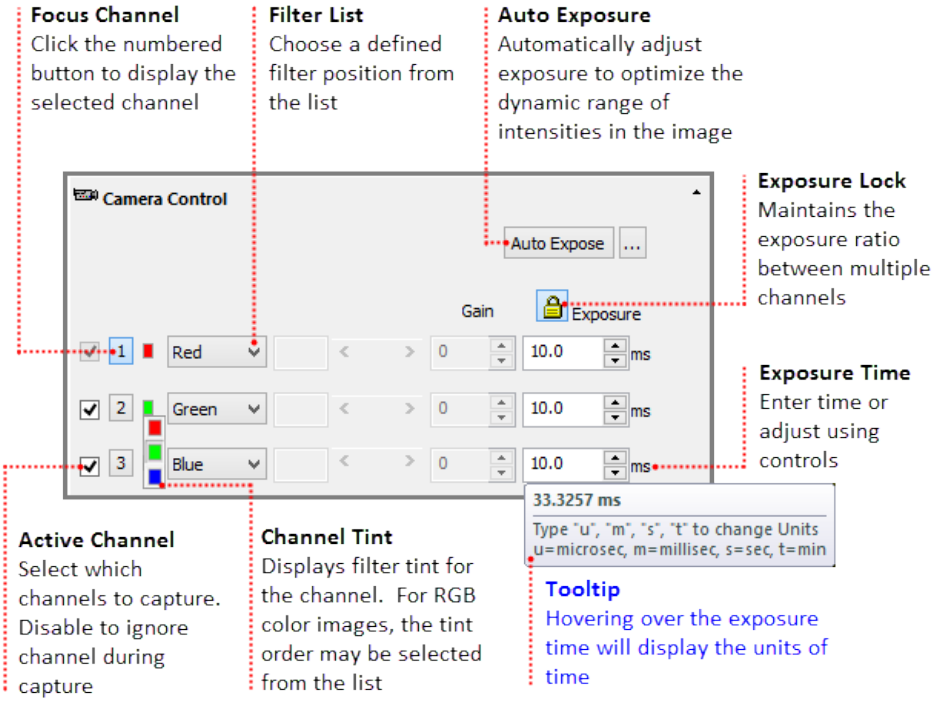
THE CAPTURE PANE

The Capture Pane provides a flexible and comprehensive method to access the ORCA®-Flash4.0 V2 features and functionality. The Capture Pane is organized by functionality into five 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.



Camera Control

Manage capture settings using the individual channel and exposure controls.

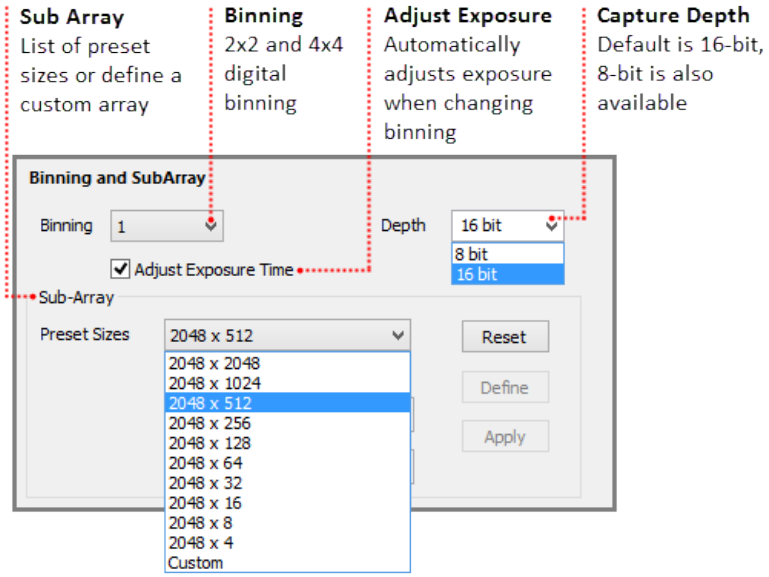


Hint: In order to achieve the best possible acquisition speed when acquiring color images, set the same exposure for each channel. Once the exposures have been entered, click the Exposure Lock icon () to lock the exposure settings. Now any exposure adjustments will be made to all of the channels.

Binning and SubArray

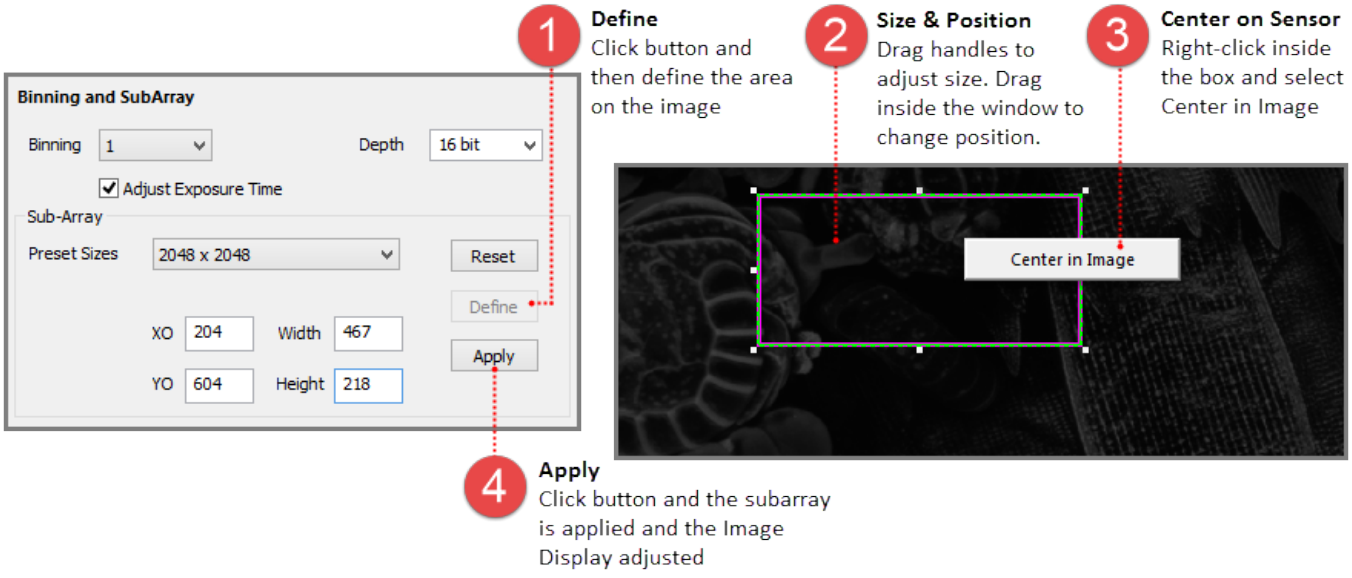
Digital binning can be used to increase the signal to noise ratio but does not increase the speed performance. Adjust the spatial resolution using a subarray preset for increased speed and less data throughput. A subarray must be centered on the camera sensor in order to achieve maximum speed. The subarray preset sizes in the list are automatically centered but custom arrays are not. To center a custom array, see the example below.

Note: On a CCD, 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. On a sCMOS 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.



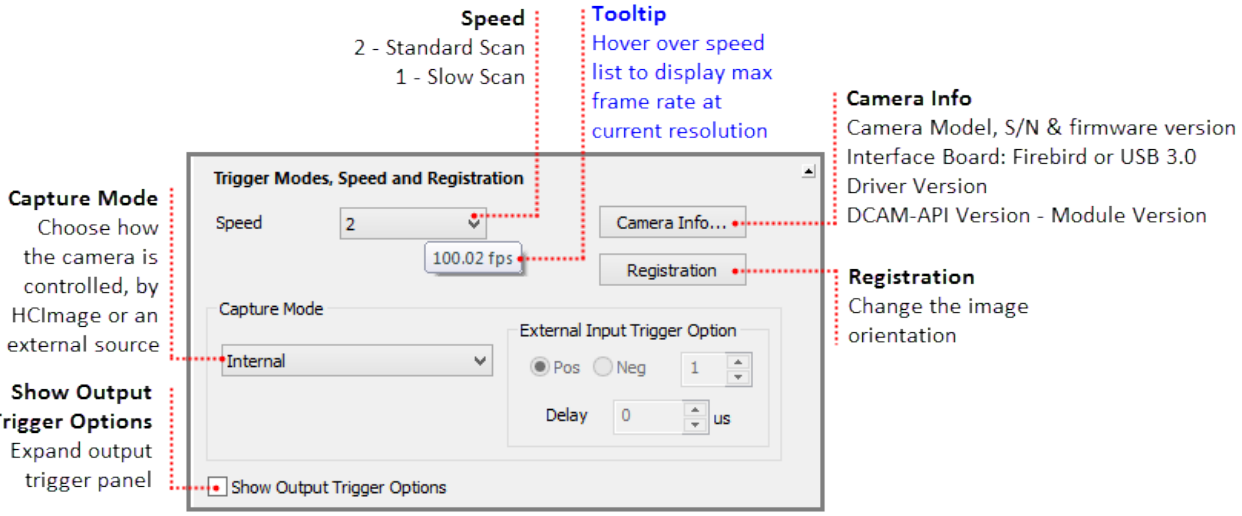
Define a Custom SubArray for Maximum Speed

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.



Trigger Modes, Speed and Registration

By default the camera is controlled through software but advanced triggering features allow the camera to control external devices or be controlled by them. The speed, capture mode and output trigger settings can be adjusted based on the needs of the application.



Speed

The camera has two readout speeds, Speed 2 is the default Standard Scan and Speed 1 is the Slow Scan with lower read noise. The tables below provide a summary of the camera speed specifications, for a more detailed specification, please see "**Speed and Resolution**" on page 43.

Camera Speed

Camera Speed Internal Mode	Speed 2 (Standard Scan)		Speed 1 (Slow Scan)	
	Camera Link	USB 3.0	Camera Link	USB 3.0
Full Resolution	100	30	30	30
Subarray (8 lines at center)	25 655	7894	7696	7696

Readout Time

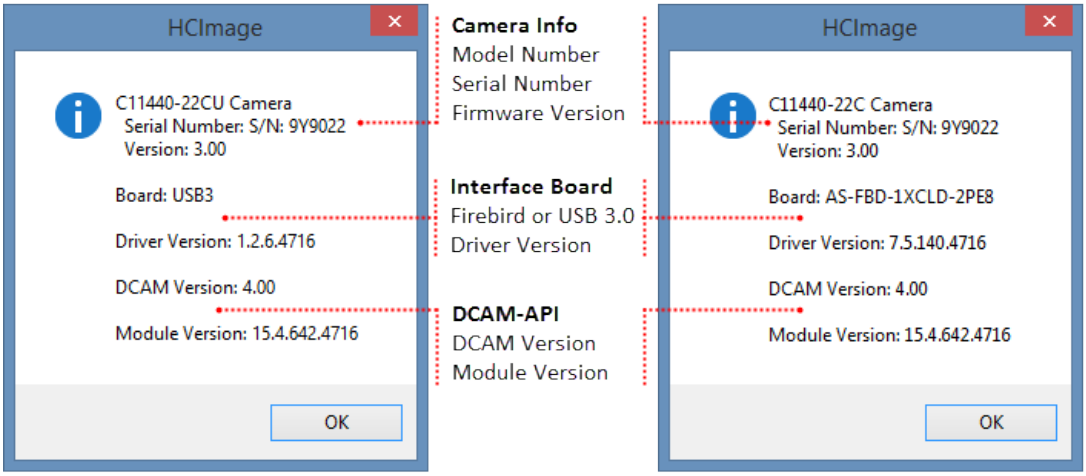
Readout Speed	Readout Time	Read Noise (rms)
Speed 2 (Standard Scan) at 100 fps Camera Link and 30 fps USB 3.0	10 ms	1.6 electrons (1.0 electrons median)
Speed 1 (Slow Scan) at 30 fps for both Camera Link and USB 3.0	33 ms	1.4 electrons (0.8 electrons median)

Exposure Time

Exposure Time	Speed 2 (Standard Scan)	Speed 1 (Slow Scan)
Internal	1 ms to 10 s	3 ms to 10 s
Internal with Subarray	38.96 µs to 10 s	129.99 µs to 10 s
External Trigger	1 ms to 10 s	3 ms to 10 s

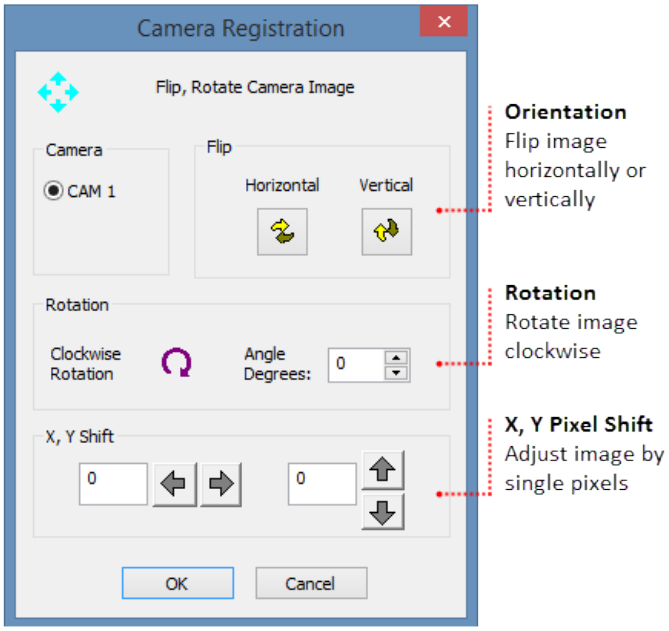
Camera Info

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



Registration

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

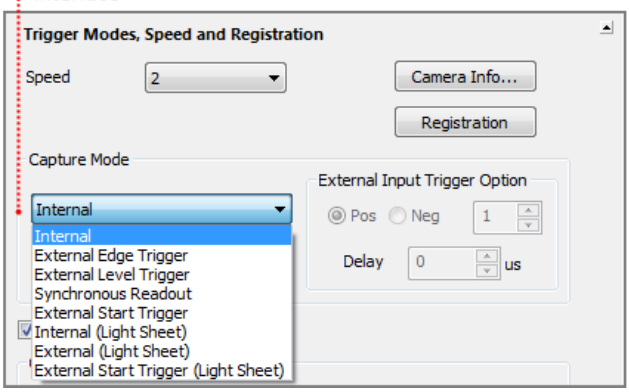


Note: High Speed Streaming does not support multiple channel acquisition, camera registration features (e.g., flip, rotation and pixel shift) or software processing operations (e.g., shade correction and rolling average).

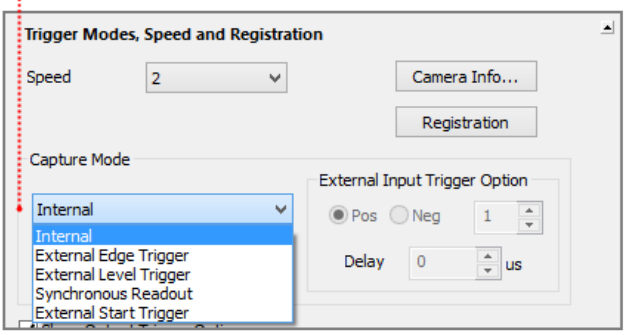
Capture Mode

By default, the camera is in internal "free running" mode, where the software controls exposure and readout timing. The ORCA®-Flash4.0 V2 provides a range of external input trigger modes to synchronize with an external instrument where the external instrument becomes the master and the camera becomes the slave. For a detailed description of each of the input trigger options, please see "**External Input Trigger Modes**" on page 23.

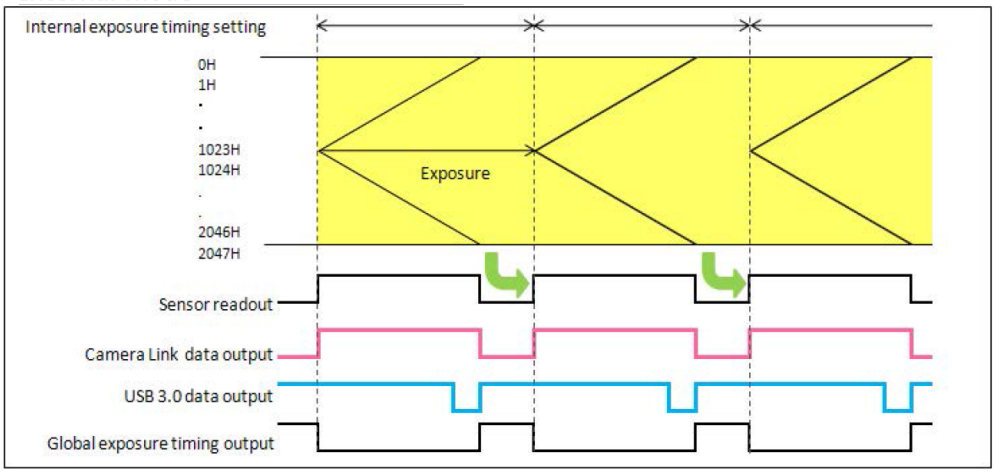
Camera Link
Interface



USB 3.0
Interface

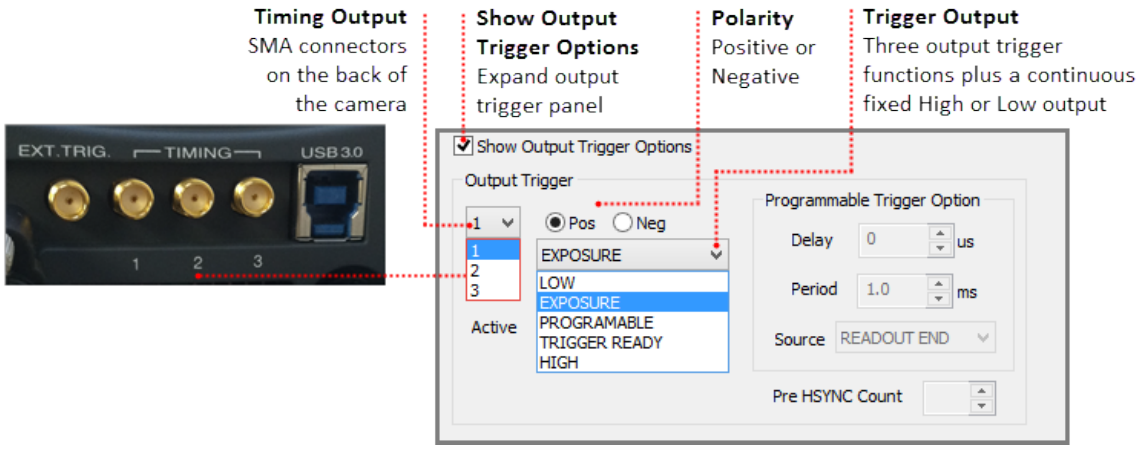


Internal mode



Output Trigger Options

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). For a detailed description of each of the output trigger options, please see "**Camera Trigger Output**" on page 29.



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 in some cases provide access to additional functionality based on the capture mode. These properties are referenced in text and screenshots as needed for setting specific camera modes. Most of the camera properties in the list display values that cannot be changed and appear grayed out.

Processing

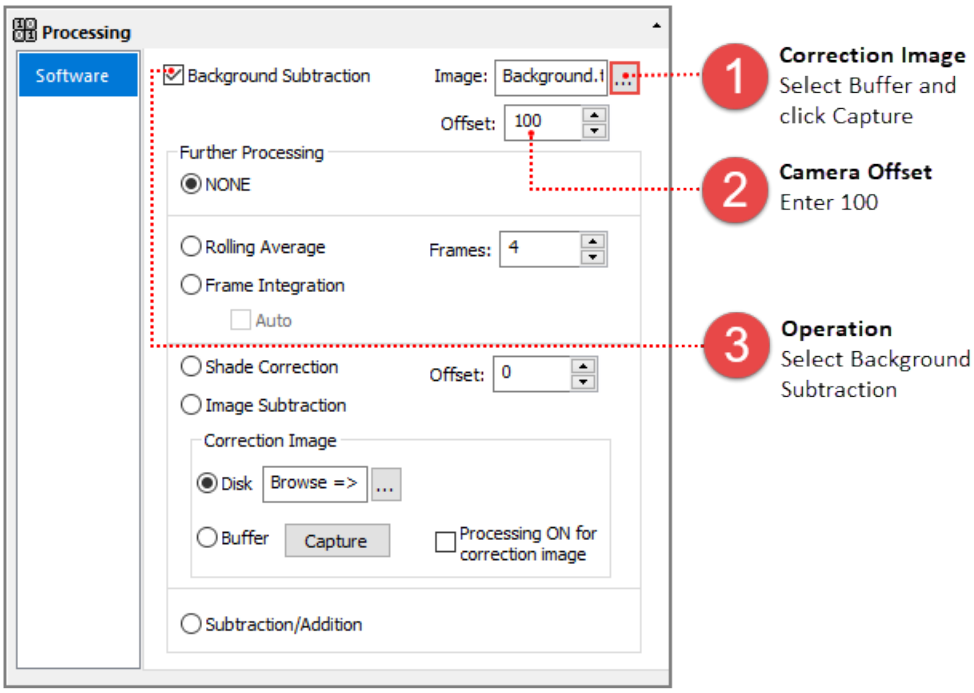
The Processing Panel provides the opportunity to enhance images during focus and acquisition by incorporating image-processing operations during or immediately after image Capture. To select an Image Processing operation, first expand the Process Pane and then select the Operation Type. Rolling Average 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.

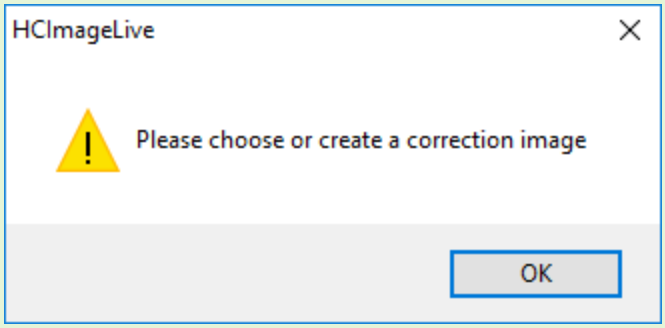
Hint: Enable Processing ON for correction image when you would like to capture a correction image using Rolling Average or Frame Integration. When you are ready to capture the correction image, select Rolling Average and enter the number of frames, enable Processing ON for correction image and then click 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 perfrom 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.

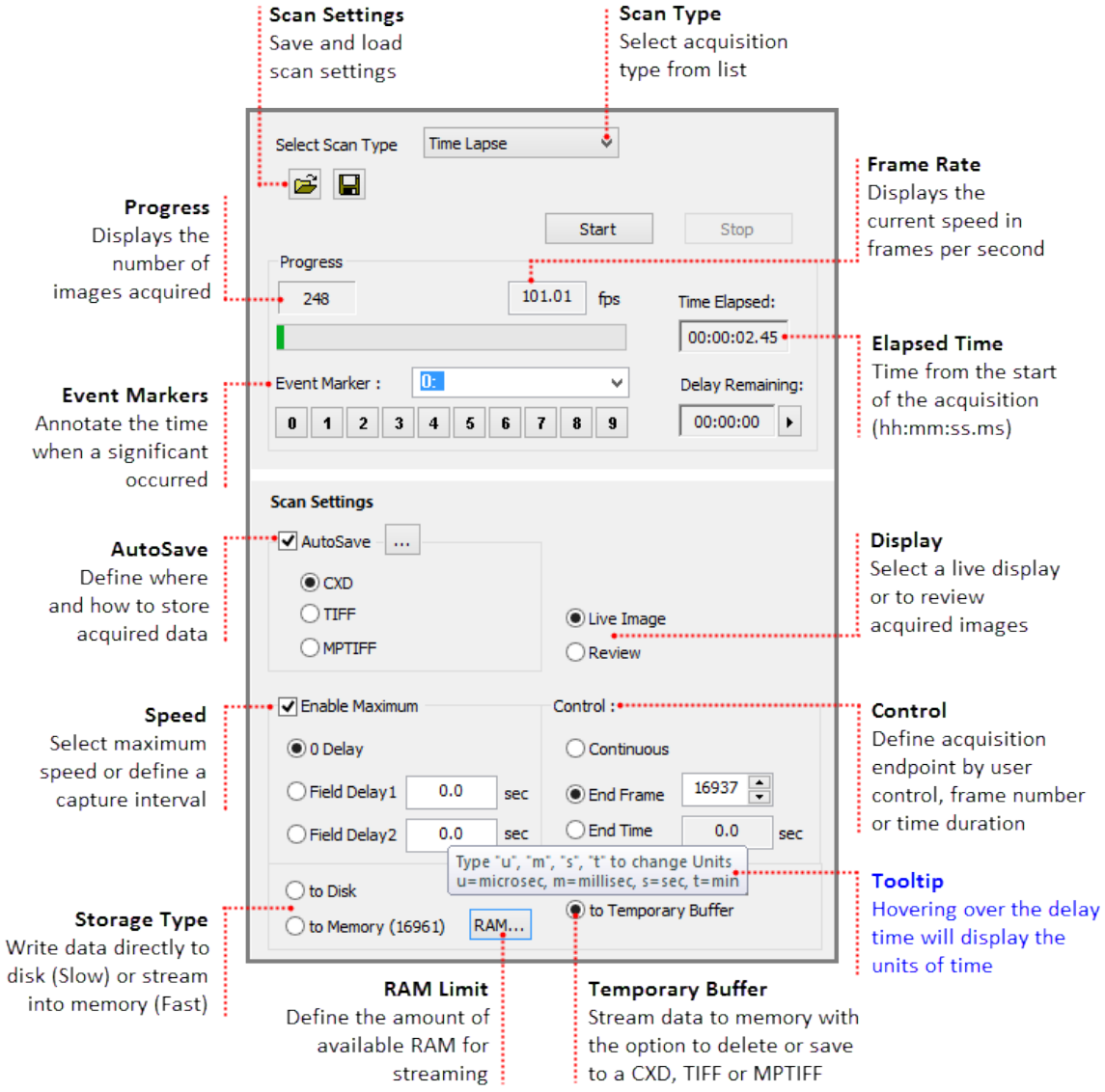


Hint: HCImage remembers the capture settings from the previous session, if background subtraction was left enabled, the following message will appear the next time HCImage is launched.



TIME LAPSE IMAGE SEQUENCE

The Time Lapse scan provides flexibility and a variety of options for defining a time lapse to fit the needs of your application.



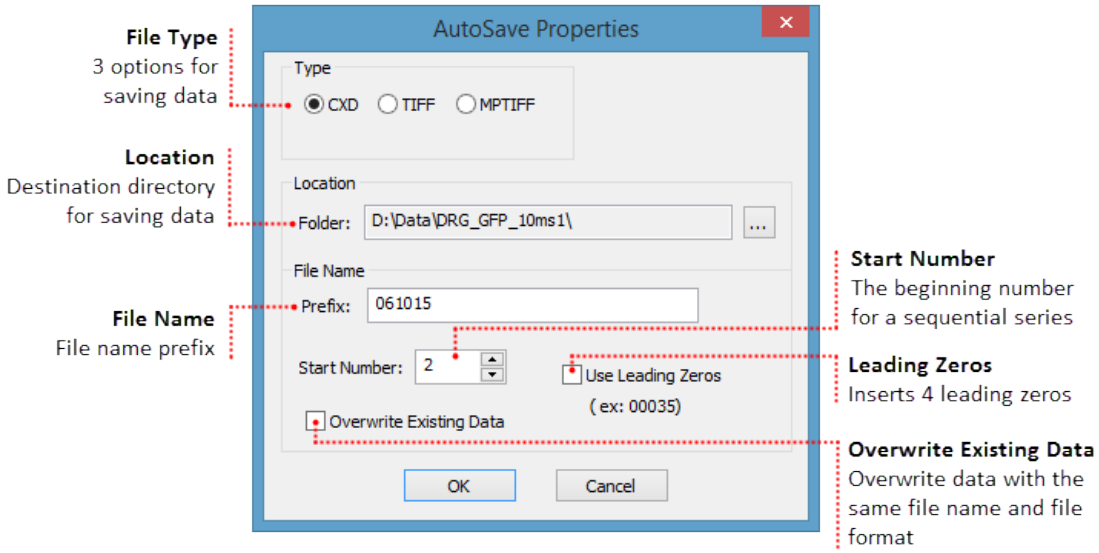
Scan Settings

The Scan Settings panel provides multiple options for defining speed, storage, duration and output settings. Scan settings can be saved for future use.

Note: Select Enable Maximum to acquire at maximum speed. During maximum speed, items which slow down acquisition will be ignored.

Auto Save

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

Storage Options

The three options for storing acquired data during a time lapse include saving to Disk, Memory or Temporary Buffer.

Save to Disk

Acquired data is written directly to the hard drive. Frame rates vary based on the PC configuration, including the type and speed of the hard drive(s) being used.

Save to Memory

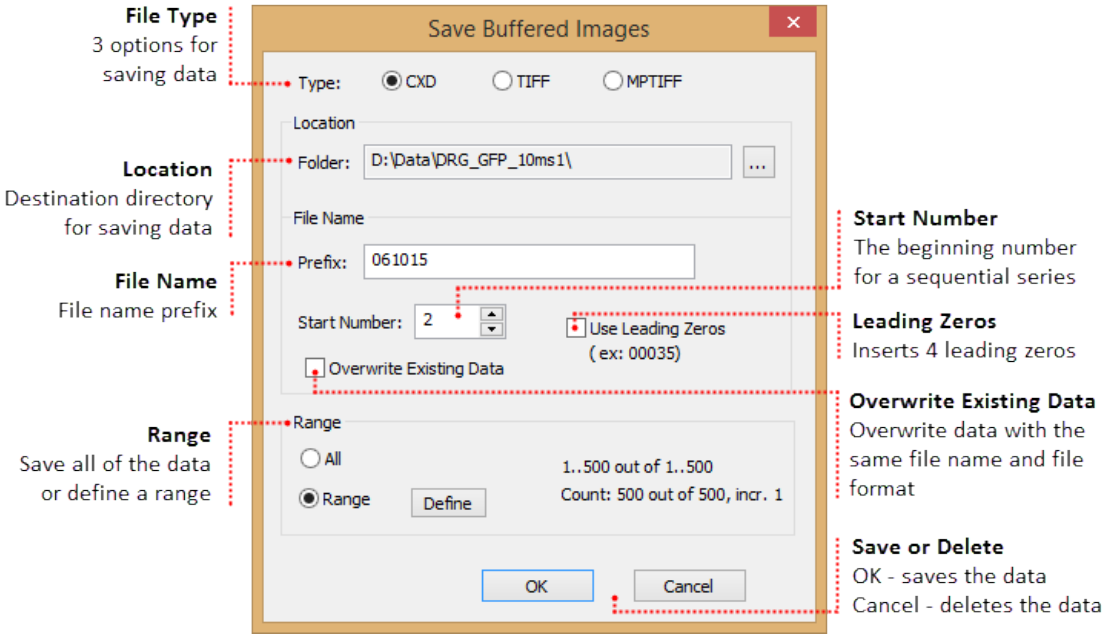
Acquired data is stored in memory and then written to disk when the time lapse is complete or stopped. When the system runs out of memory during a time lapse, acquired data is written to disk for the remainder of the sequence. Saving to memory typically provides a higher frame rate with less timing variation then saving to disk. The maximum number of images that can be acquired depends upon the amount the RAM in the system and the RAM limit set in HCImage. This number is displayed to the right of the memory storage option. When Memory is selected, End Frame automatically displays the maximum number of frames that can be streamed to memory, although any number less than the max can be entered. The Status Bar, located in the bottom left corner of the application window, displays the maximum number of frames that can be streamed to memory.

Save to Temporary Buffer

Acquired data is stored in memory with the option to review the image sequence before saving or deleting it. Storage is limited to the amount of system memory without the option to write to disk when the memory is full. The maximum number of images that can be acquired depends upon the

amount the RAM in the system and the RAM limit set in HCImage. When Temporary Buffer is selected, End Frame is automatically enabled and display the maximum number of frames that can be streamed to memory, although any number less than the max can be entered.

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.

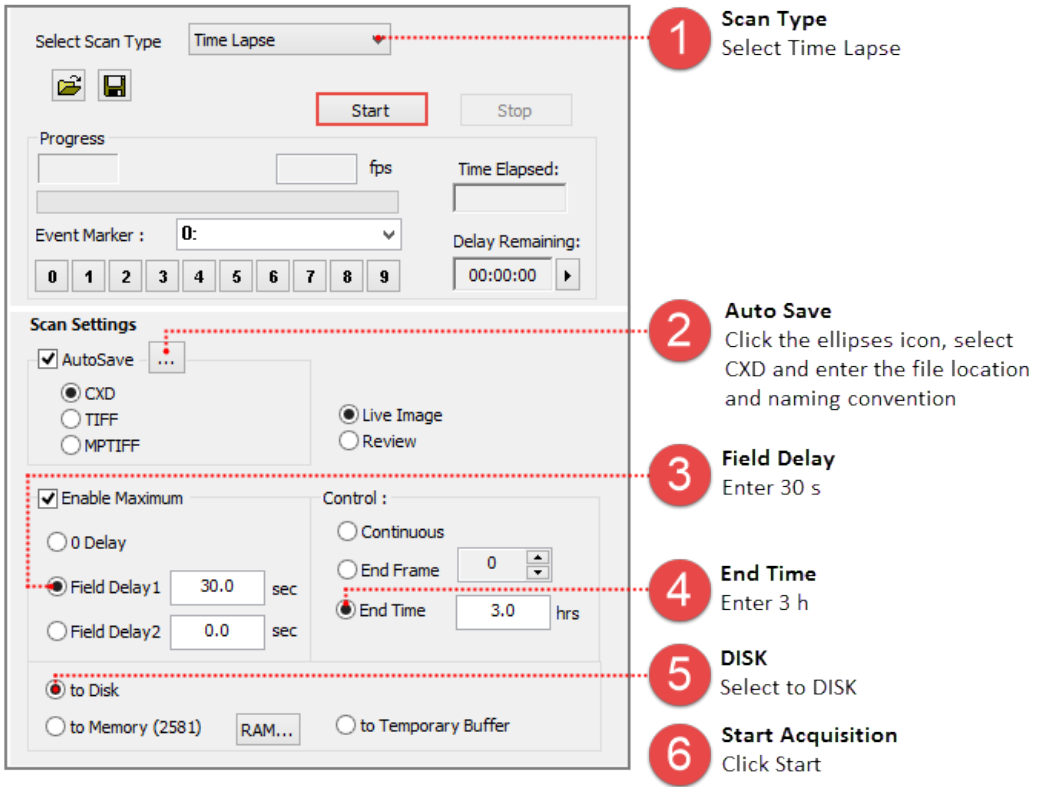


Setting up a Time Lapse

This section provides three examples of typical time lapse settings, using each of the storage options.

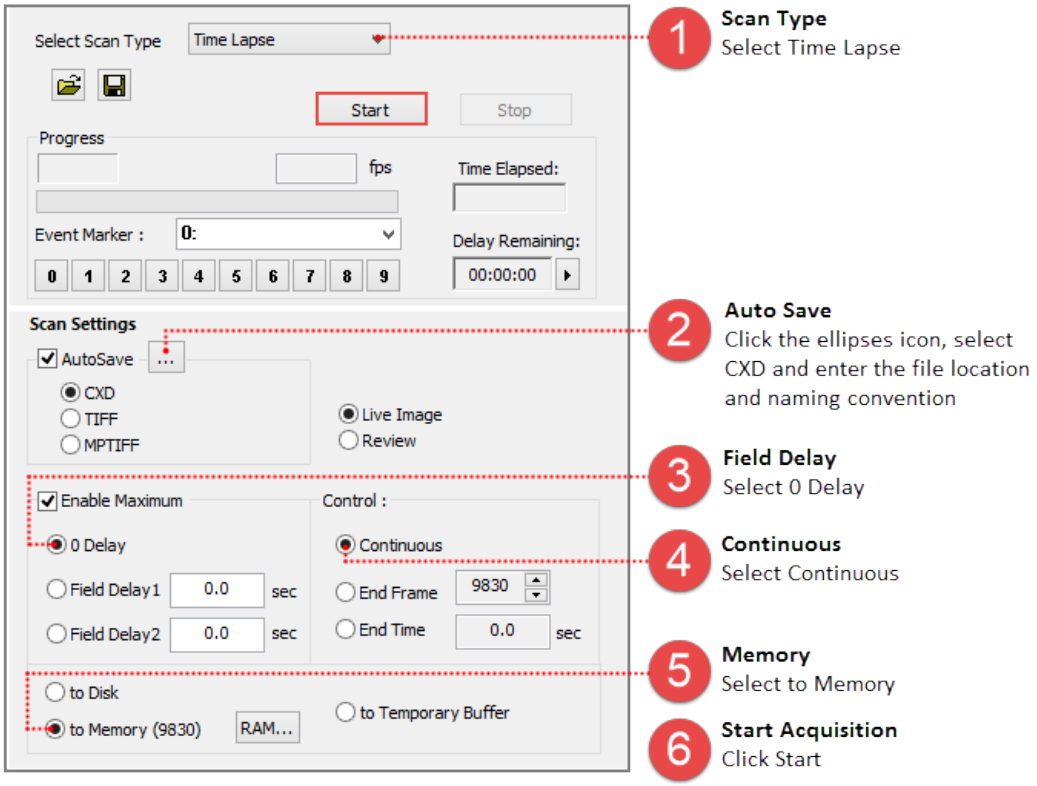
Setup a Time Lapse - Save to Disk

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.



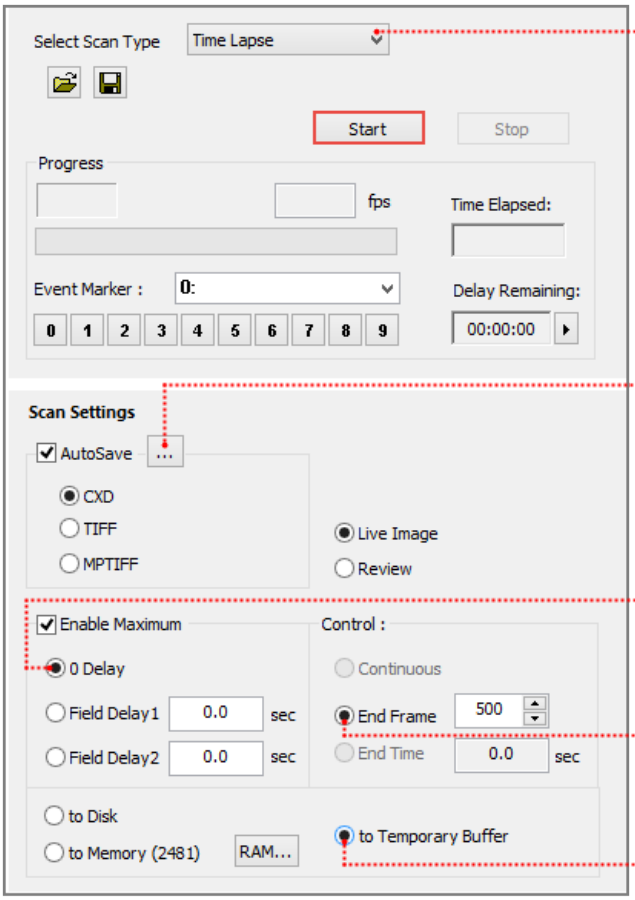
Setup a Time Lapse - Save to Memory

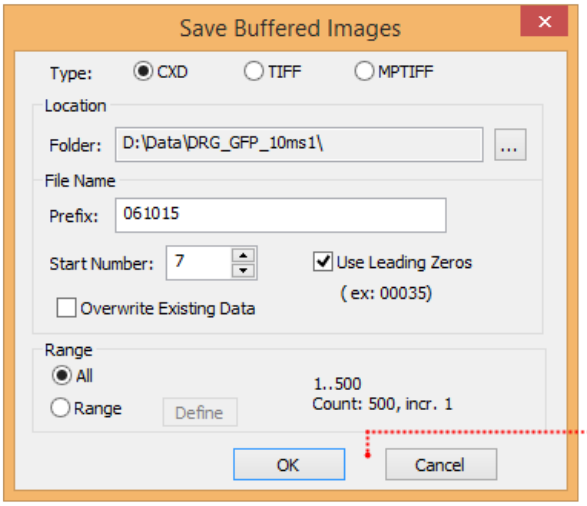
The time lapse in this example will store images in memory until the acquisition is stopped or runs out of memory at which point the acquired images are saved to disk for the remainder of the time lapse. Once your are satisfied with capture settings and the sample is in focus, go to the Sequence pane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



Setup a Time Lapse - Save to the Temporary Buffer

Once your are satisfied with capture setting and the sample is in focus, go to the Sequence pane and follow the steps below.



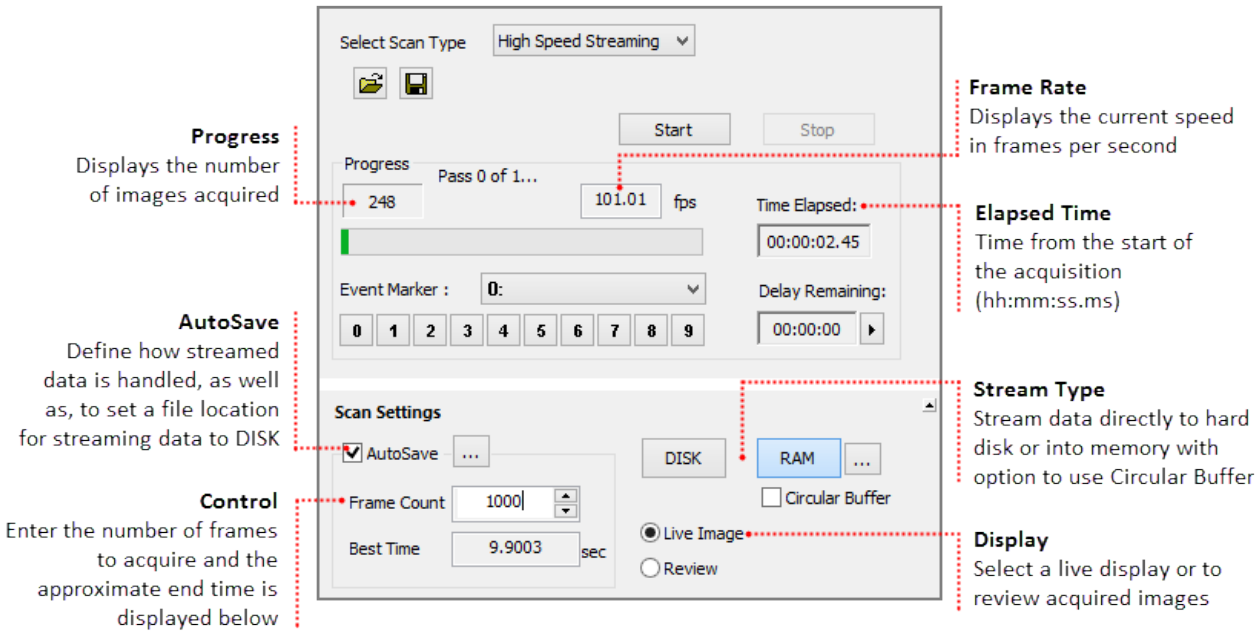


1. **Scan Type**
   Select Time Lapse
2. **Auto Save**
   Click the ellipses icon, select CXD and enter the file location and naming convention
3. **Field Delay**
   Select 0 Delay
4. **End Frame**
   Enter 500
5. **Temporary Buffer**
   Select to Temporary Buffer
6. **Start Acquisition**
   Click Start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

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 V2.



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).

Stream to RAM

When streaming to RAM, the image data is stored in memory and then the user has the option to save as either CXD, TIFF, MPTIFF or to delete the data. Up to 80% of the systems available memory will be used for storing streamed data. The Status Bar, located in the bottom left corner of the application window, displays the maximum number of frames that can be streamed to memory. In the AutoSave Properties dialog, the user can determine how and where to store the acquired data. Once the acquisition is complete, the data stored in memory can automatically be saved as a CXD, TIFF or MPTIFF.

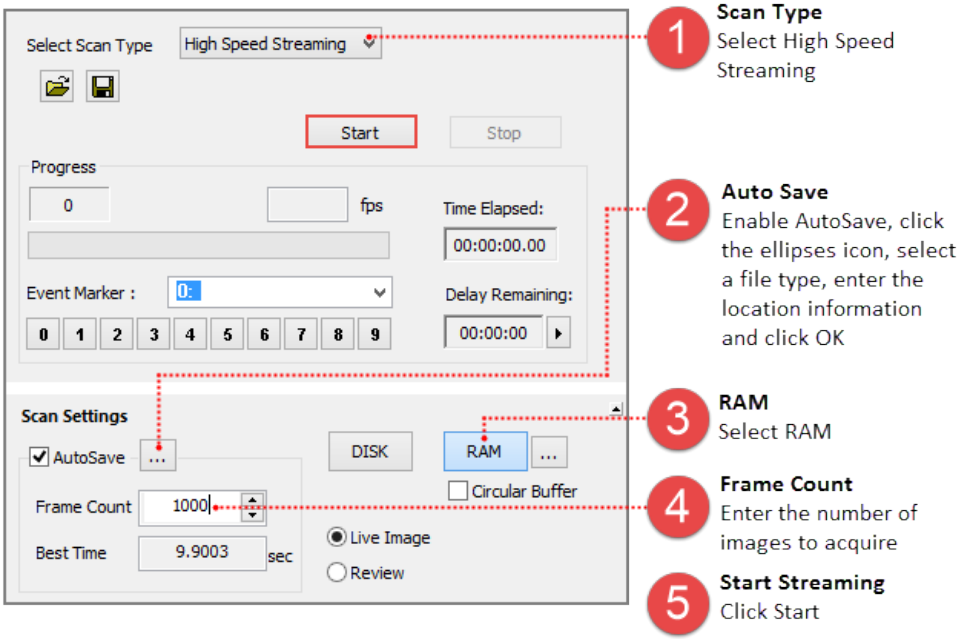
Note: MPTIFF files have a 65 000 image limit or 4 GB size limit. For image sequences having more than 65 000 images or larger than 4 GB, multiple MPTIFF files will be saved and numbered sequentially.

Circular Buffer

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.

Steps for Streaming to RAM

Once your are satisfied with capture settings and the sample is in focus, go to the Sequence pane and follow the steps below.

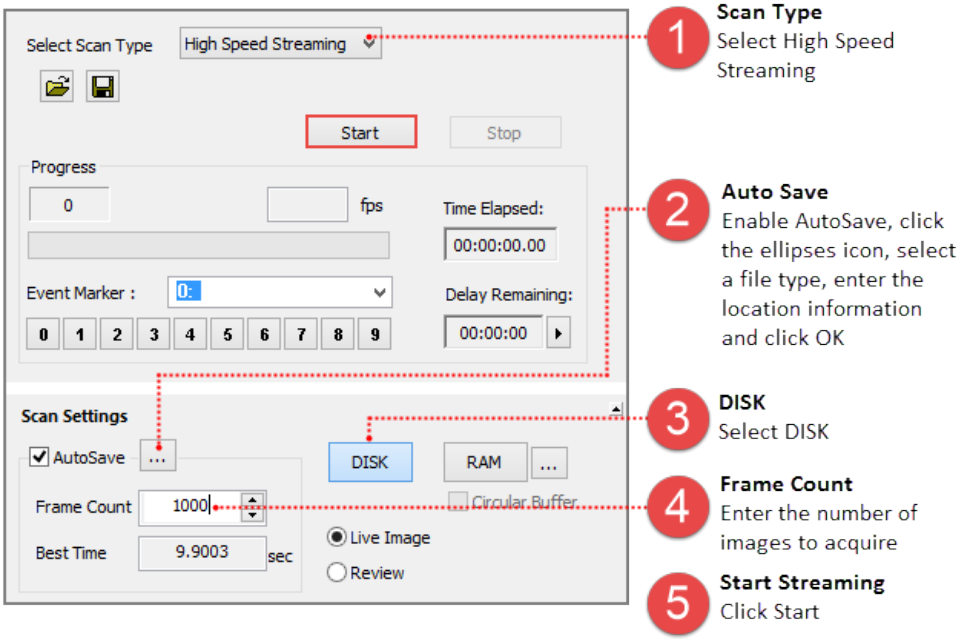


Stream to Disk

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.

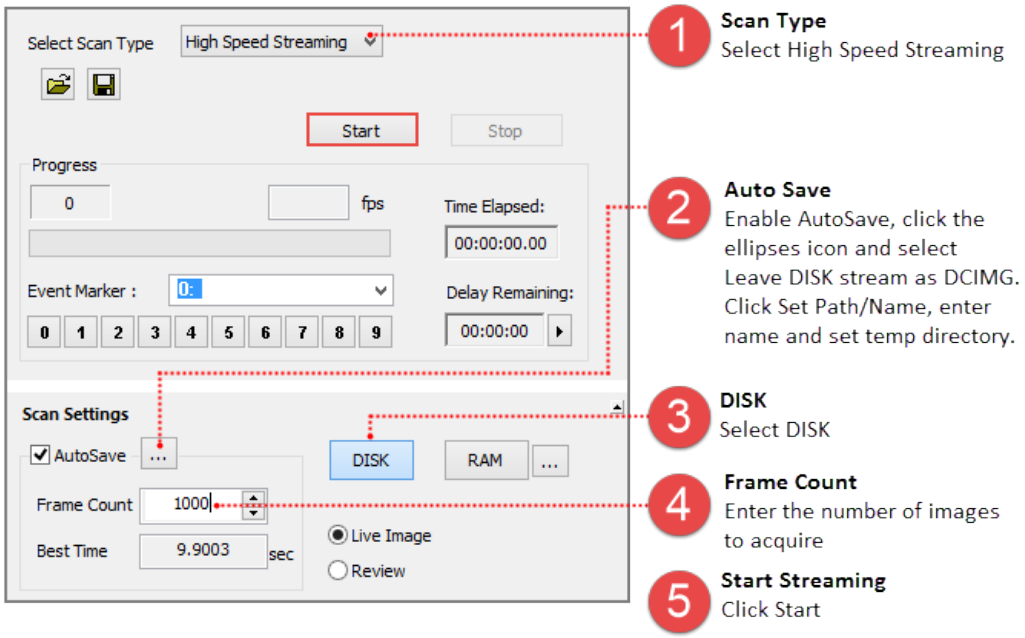
Steps for Streaming to Disk

Configure the capture settings, go to the Sequence pane and follow the steps below.



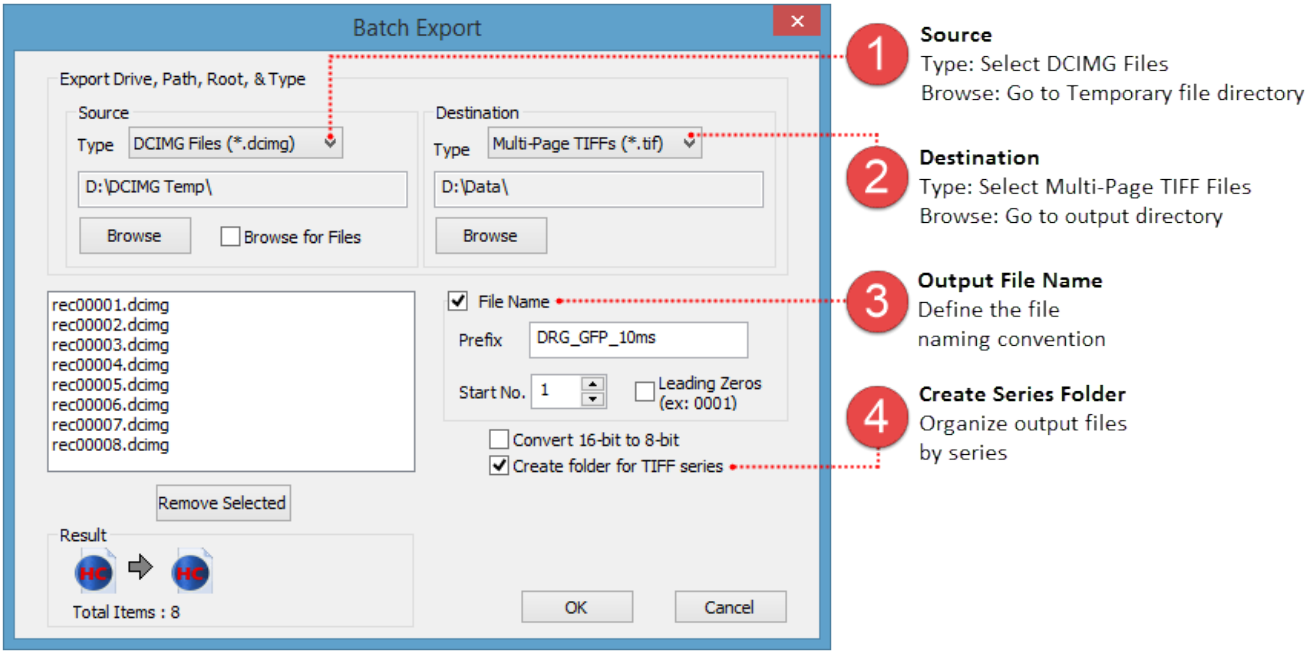
Steps for Streaming to Disk and Leave as DCIMG

Once your are satisfied with capture settings and the sample is in focus, go to the Sequence pane and follow the steps below.



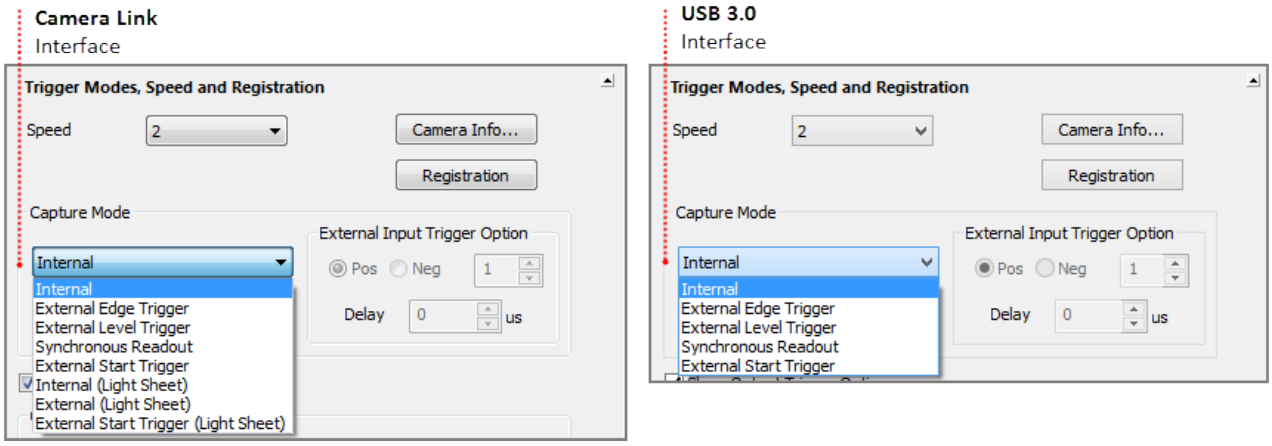
Steps for Batch Export DCIMG to MPTIFF

Go to the File menu, select Batch Export and follow the instructions below.



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 35.



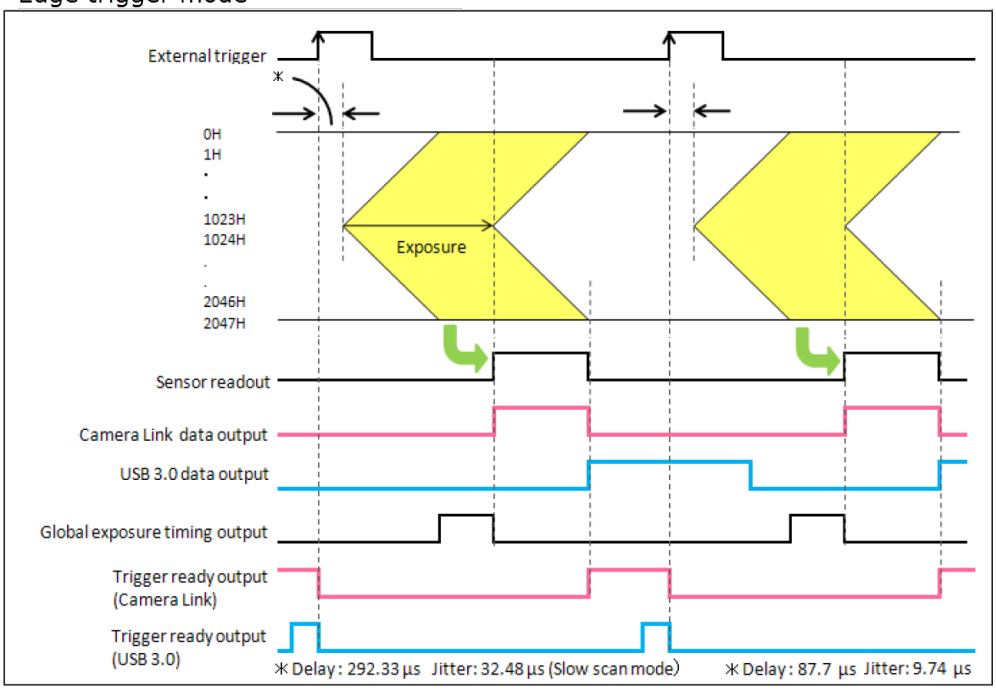
External Trigger Delay Function

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.

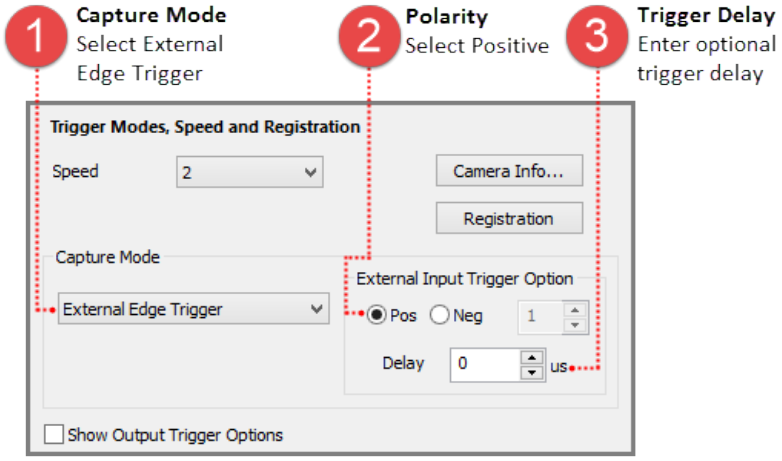
Edge trigger mode



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

Setup Capture Mode for External Edge Trigger

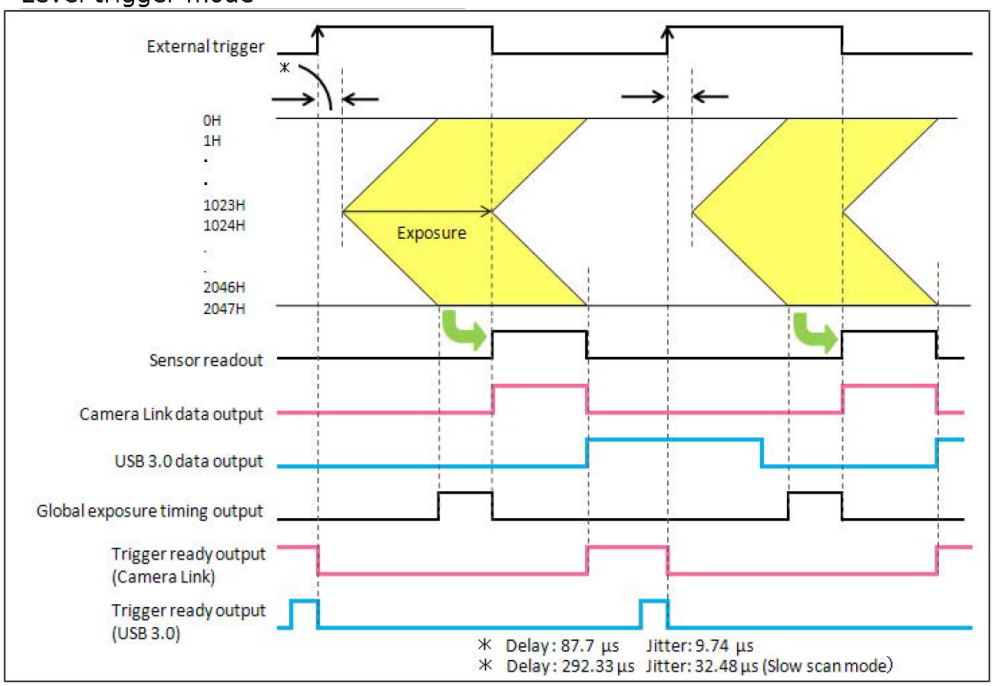
Follow the steps below to enable external edge trigger mode in HCImage. Enter the exposure time in the Camera Control panel. Click Live and the software will wait until the camera receives the external signal before displaying an image. A stream of triggers are required for continuously updated images.



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.

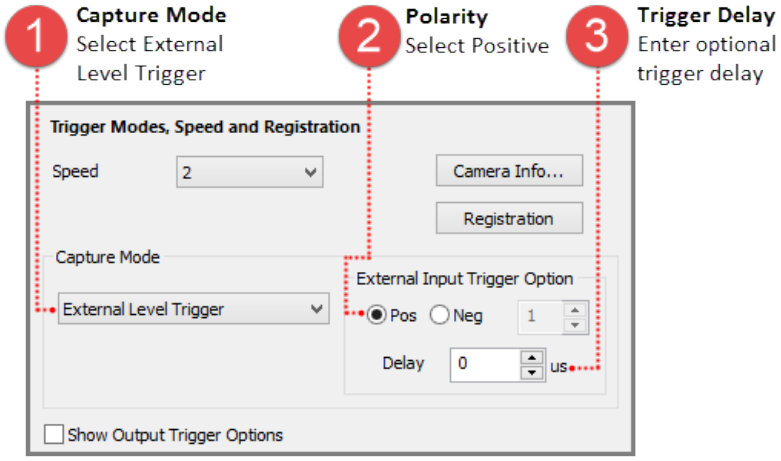
Level trigger mode



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.

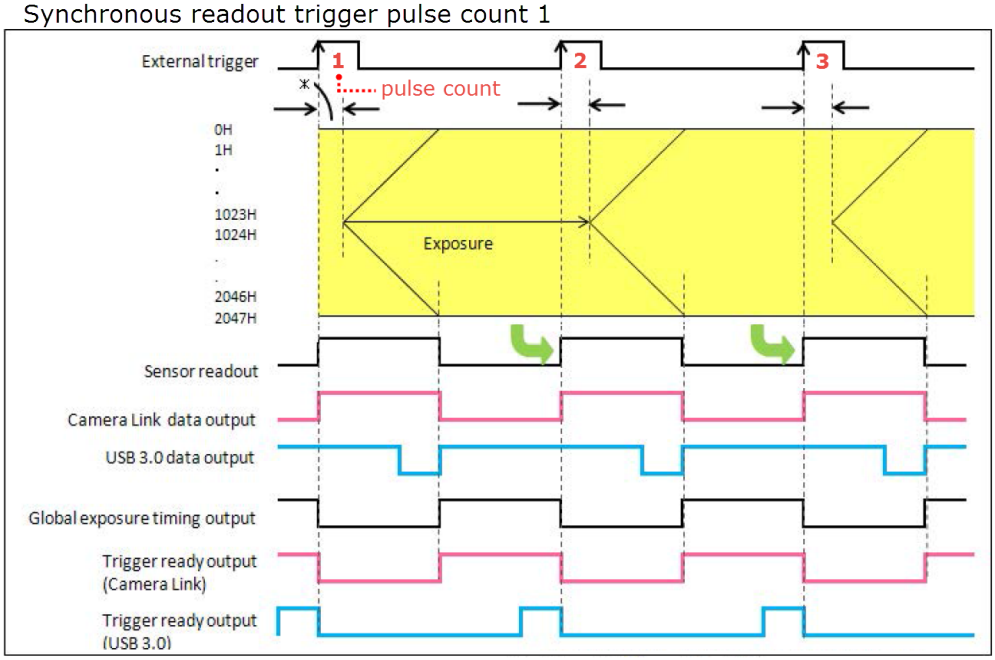


Synchronous Readout Trigger

(1) Normal operation (pulse count 1)

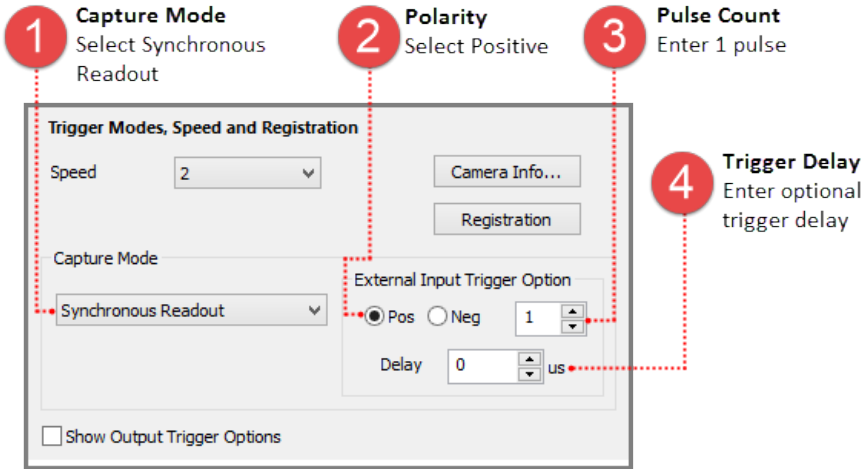
The synchronous readout trigger mode is used for continuous imaging when it is necessary to control the exposure start timing of each frame from an outside source and also when it is necessary to secure as long exposure time as possible. In the synchronous readout trigger mode, the camera ends each exposure, 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)

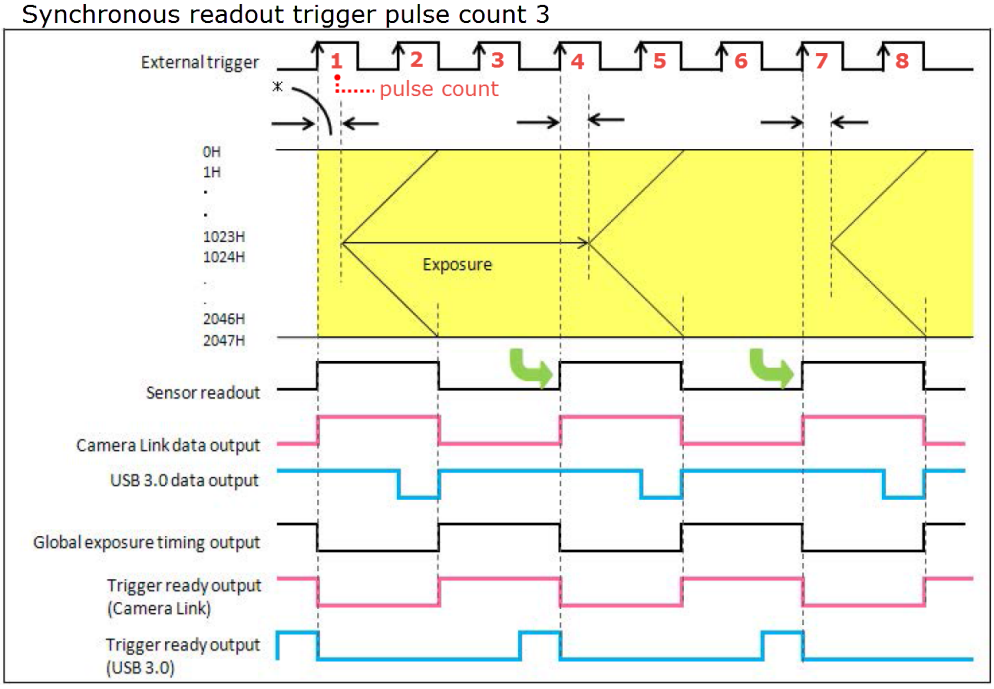
Follow the steps below to enable synchronous readout mode in HCImage. Click Live and the software will wait until the camera receives the external signal before displaying an image. A stream of pulses are required for continuously updated images or at least 2 pulses are required to capture a single image.



(2) Pulse count

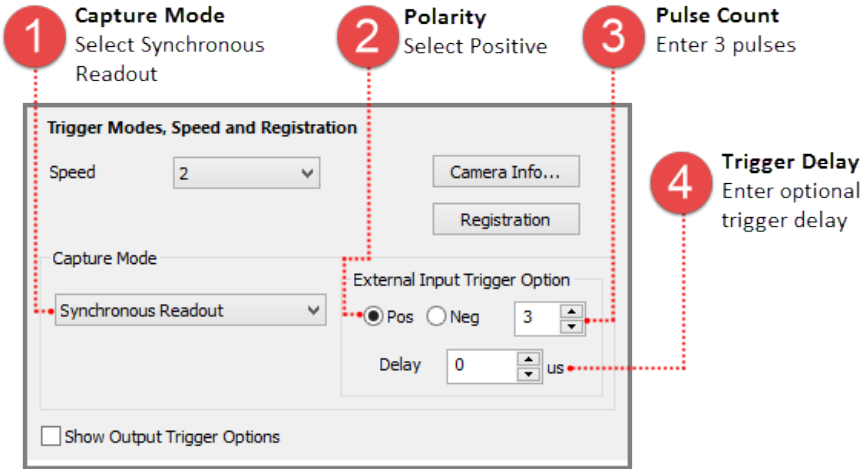
Useful for synchronizing the camera with a spinning disk confocal, the exposure time is determined by a specified number of timing pulses or pulse count. For example, the pulse count is set to 3. The first pulse starts exposing the first frame, the fourth pulse stops the exposure and starts the readout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

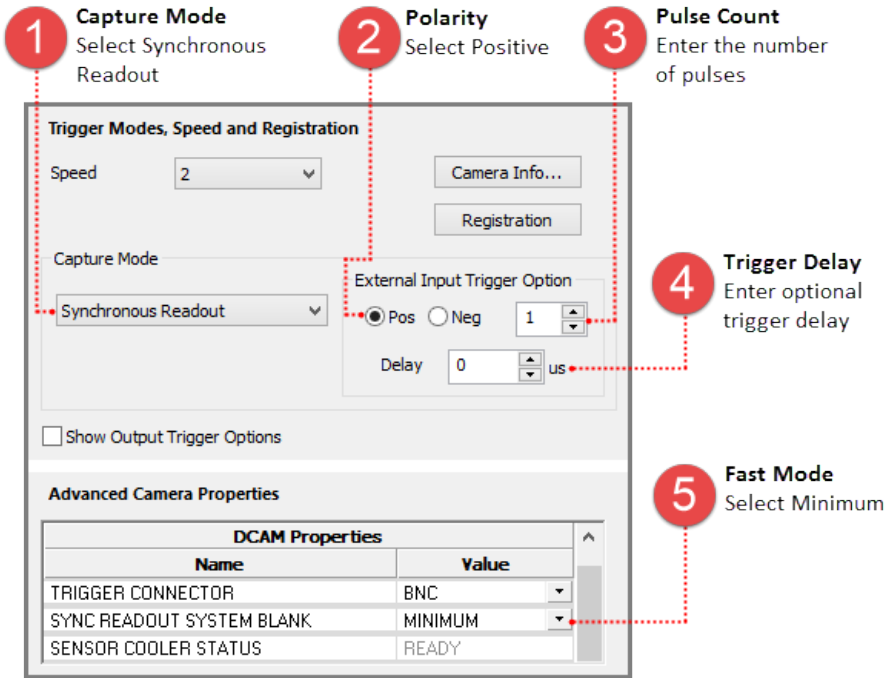


Fast Synchronous Readout Trigger

This mode allows the user to work with a shorter time interval when using Synchronous Readout mode with the Camera Link interface. To enable this mode, set the Capture Mode to Synchronous Readout, expand the Advanced Camera Properties panel and under DCAM Properties set Sync Readout System Blank to Minimum.

Setup Capture Mode for Fast Synchronous Readout Trigger

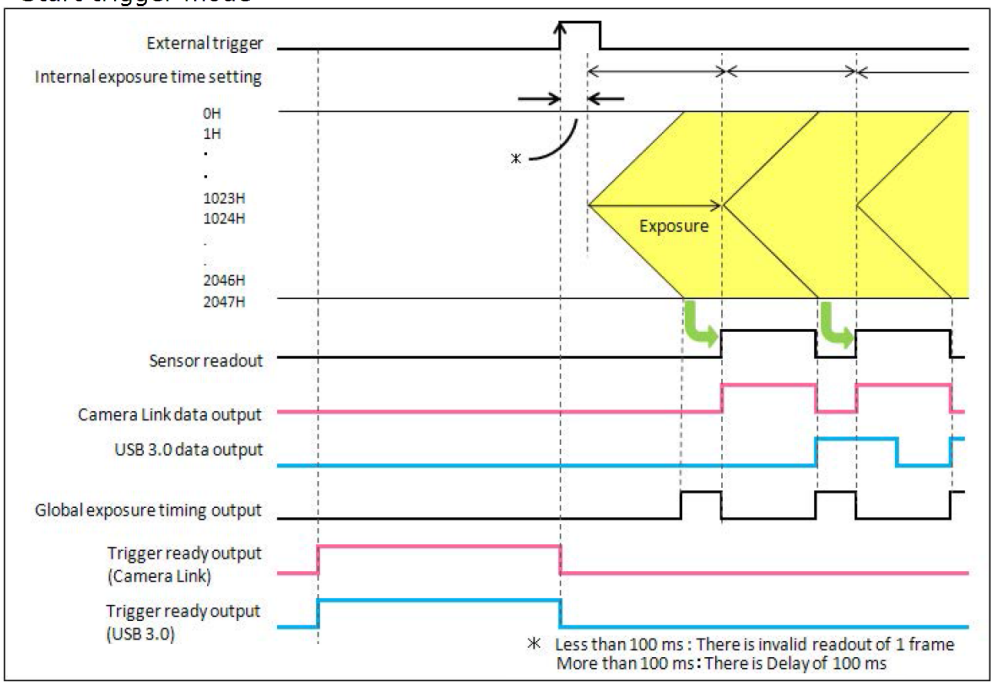
Follow the steps below to enable fast synchronous readout mode in HCImage. Click Live and the software will wait until the camera receives the external signal before displaying an image.



Start Trigger

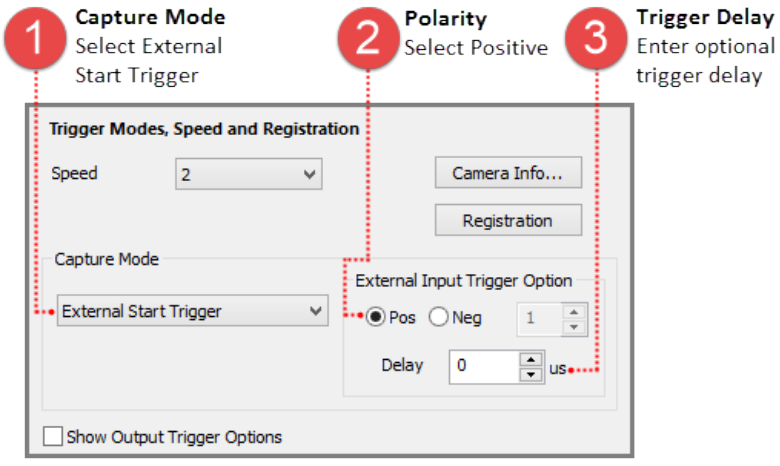
External start trigger mode utilizes a single trigger pulse to start acquiring images. An external signal triggers the start of exposure and then the camera acquires images at max frame rate in internal "free running" mode. This mode is only available when acquiring a single channel.

Start trigger mode



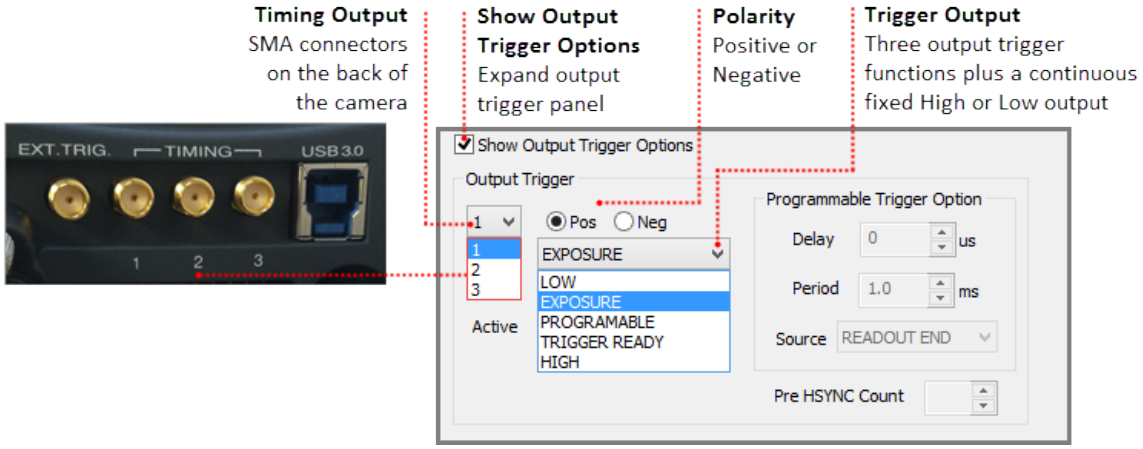
Setup Capture Mode for External Start Trigger

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.



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.

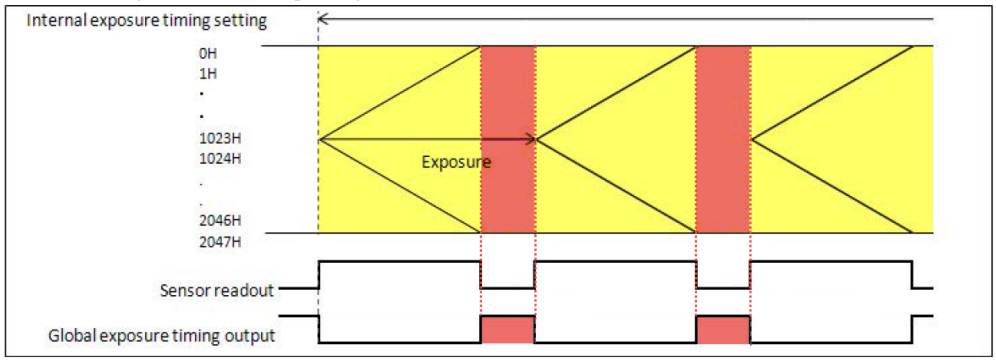


Global Exposure Output

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.

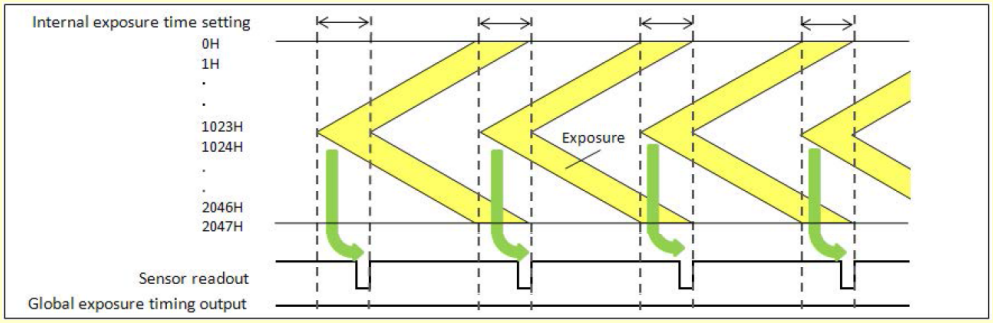
Global Exposure Time = Exposure Time - Readout Time

Global exposure timing output

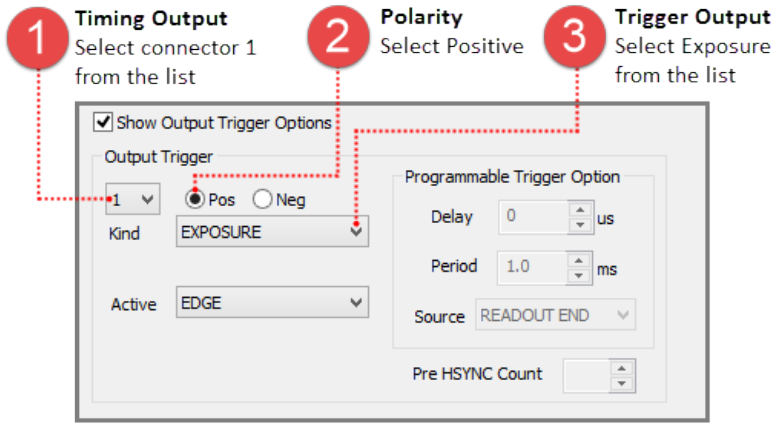


Note: There is no output signal when the exposure time is less than the frame rate.

Electrical shutter mode - Camera Link



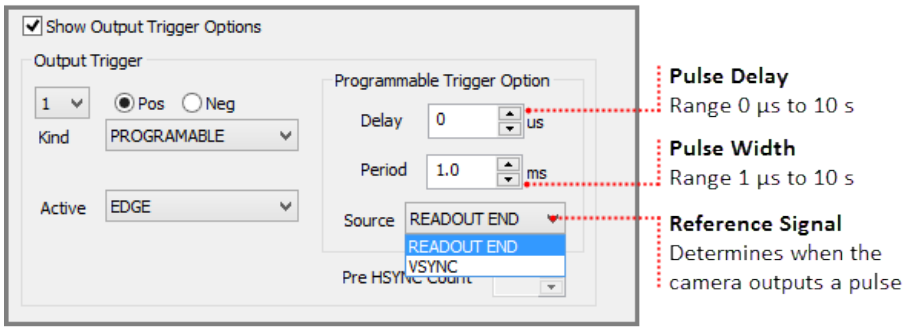
Setup Global Exposure Output Trigger



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).

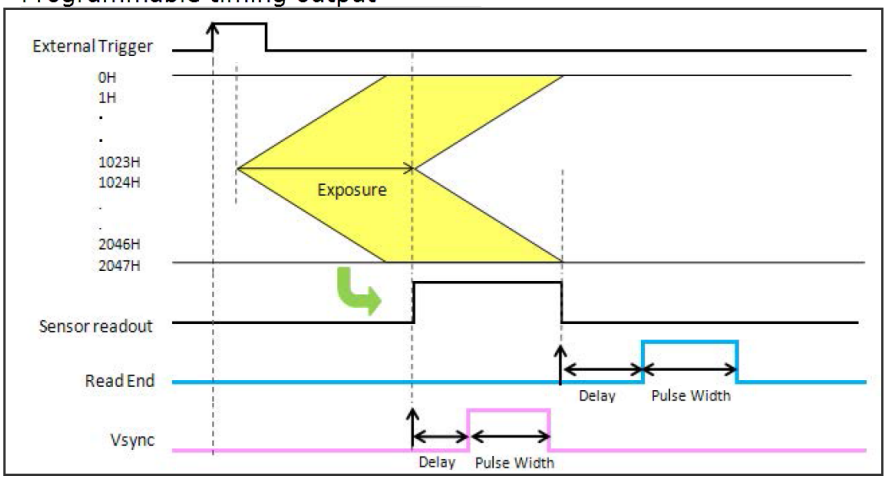
Note: Programmable triggers occur after the frame that triggers it.



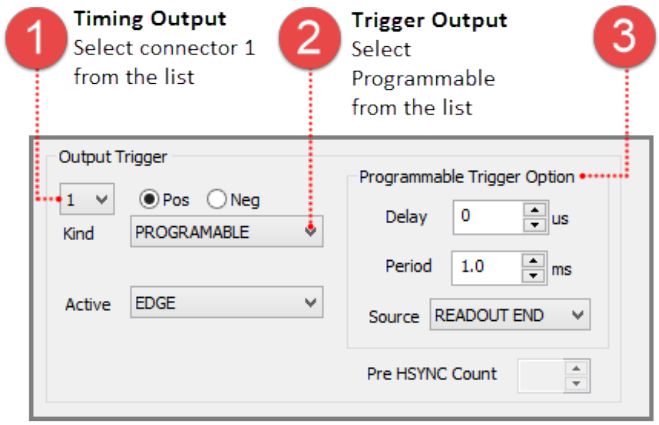
The relation between the parameter which can be set with each reference signal, and an output signal becomes below.

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.

Programmable timing output



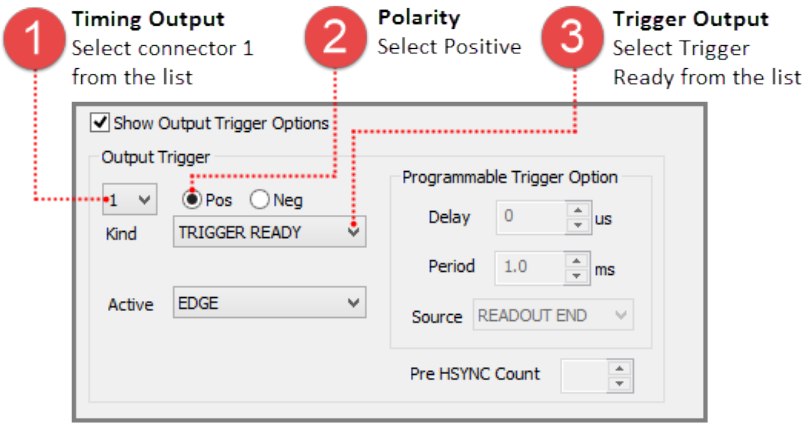
Setup Programmable Output Trigger



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

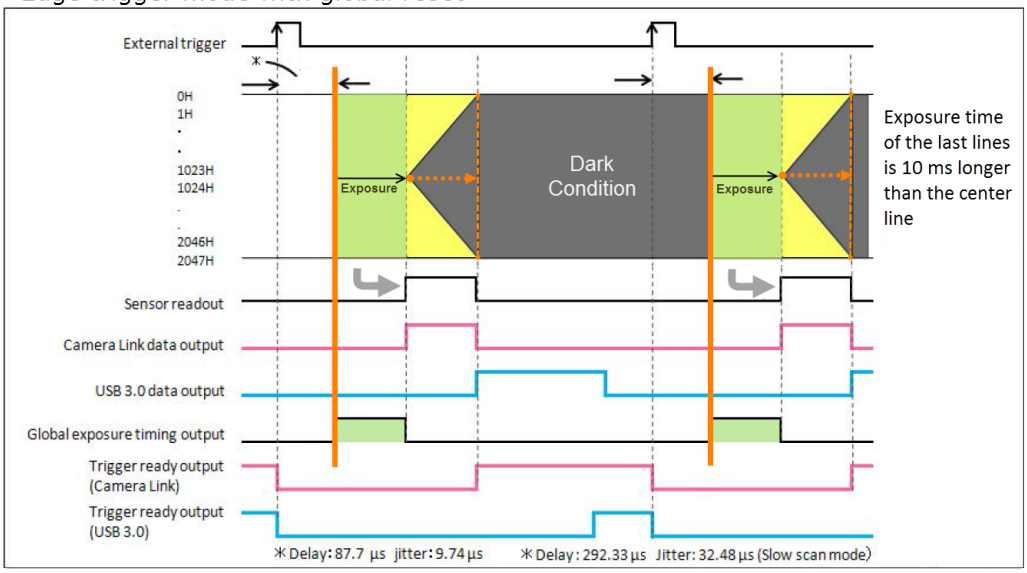
Global reset function is used to reset the electric charge of all of the pixels at the same time, just before acquiring an image. This allows all of the pixels to start exposing at the same time. The first line of the sensor exposes for the exposure time. The subsequent lines expose for the exposure 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

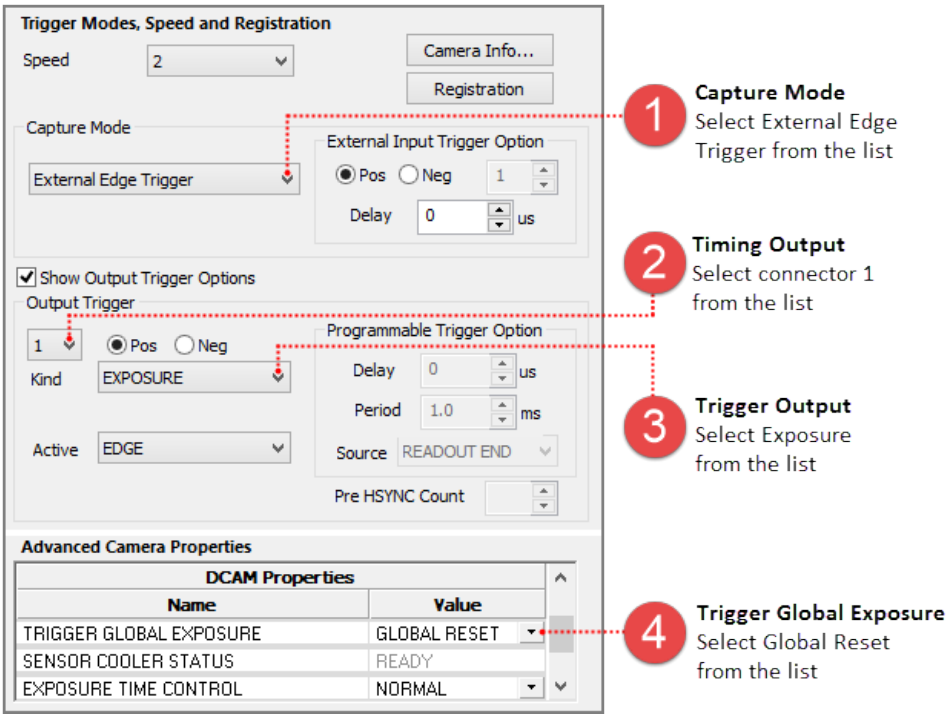
This mode is used with Global Exposure Synchronization. On the edge of the voltage change of the external trigger input, all of the lines start exposing. The first line of the sensor exposes for the exposure time set in HCImage. The subsequent lines expose for the exposure time plus readout time. This 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

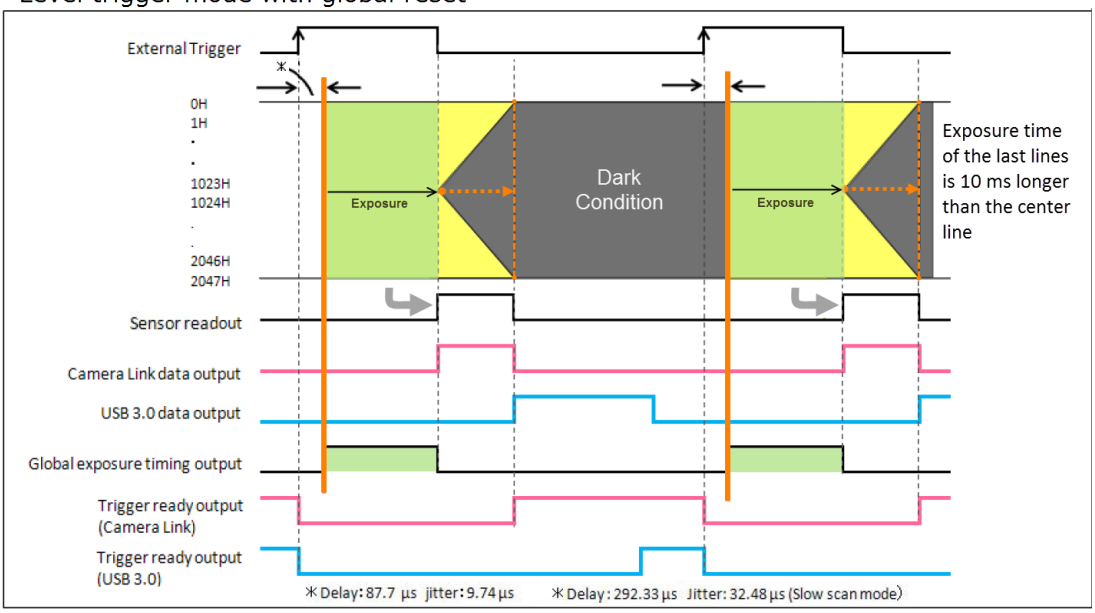
Follow the steps below to enable external edge trigger mode in HCImage. Enter the exposure time in the Camera Control panel. Click Live and the software will wait until the camera receives the external signal before displaying an image.



Level Trigger with Global Reset

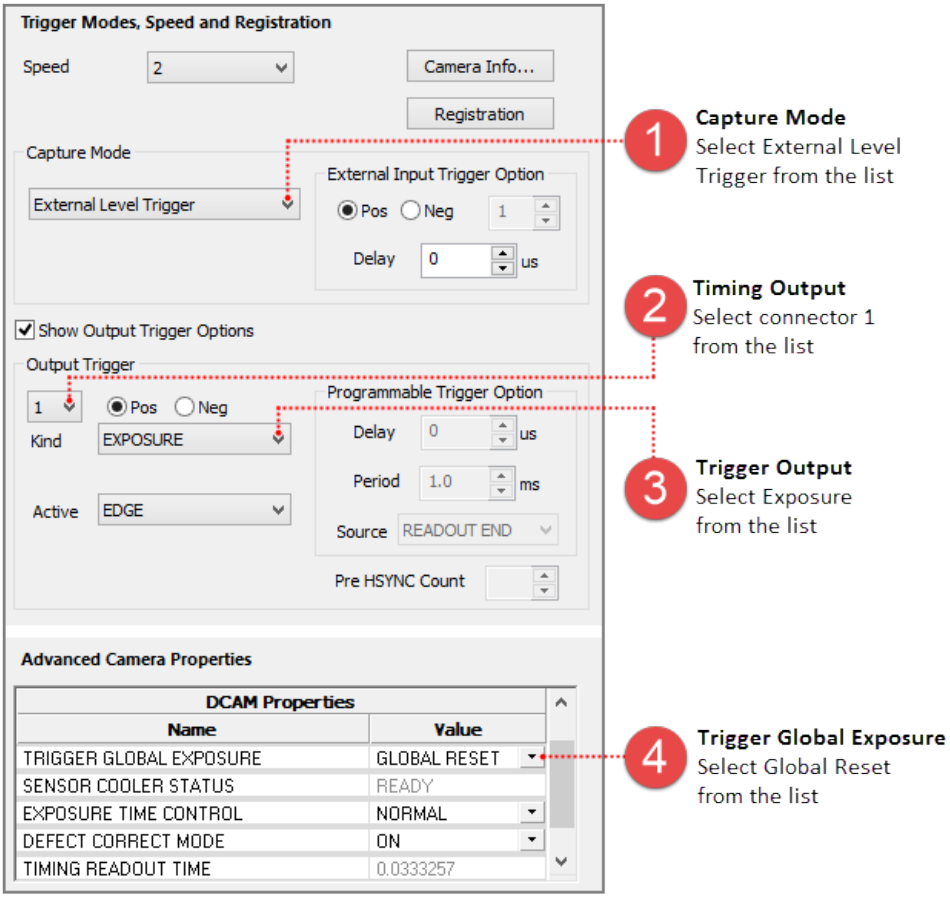
This mode is used with Global Exposure Synchronization. On the edge of the voltage change of the external trigger input, all of the lines start exposing. The first line of the sensor exposes until the edge of the voltage of the external trigger input. The subsequent lines expose for the exposure time 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.



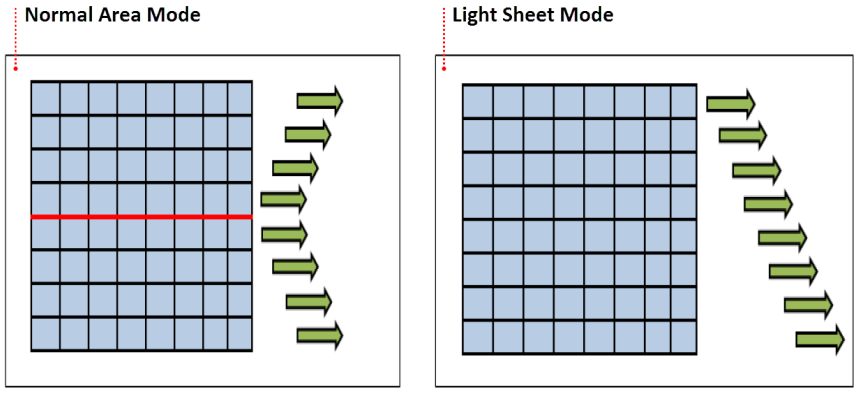
LIGHT SHEET MODE

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 V2 Light Sheet Mode incorporates specific timing features and a unified readout direction allow for this synchronization to occur.

Note: Light sheet mode is only available with the Camera Link interface operation. The fastest frame rate is 49 frames per second.

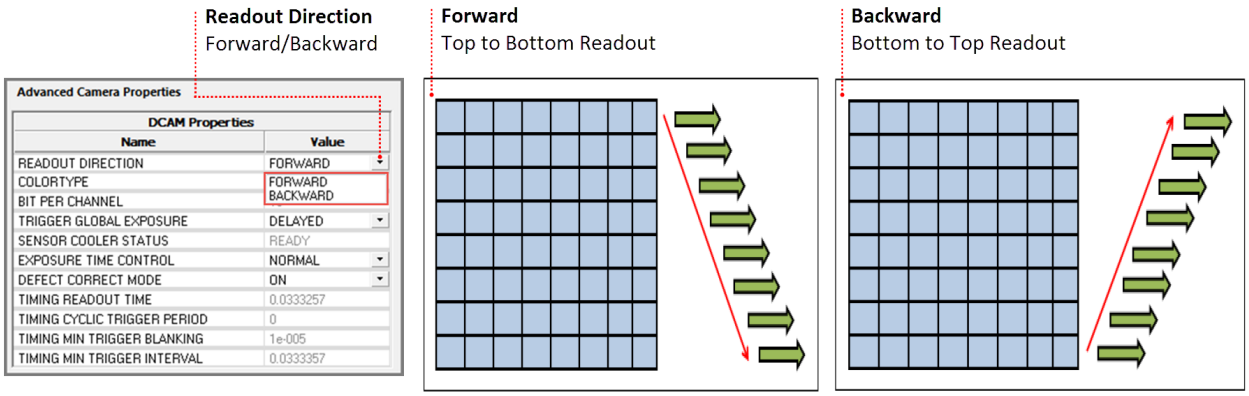
Readout Direction

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.



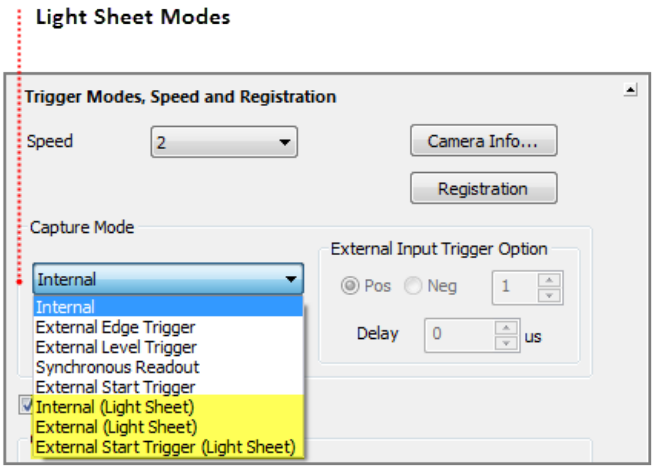
How to Change Readout Direction

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



Light Sheet Capture Modes

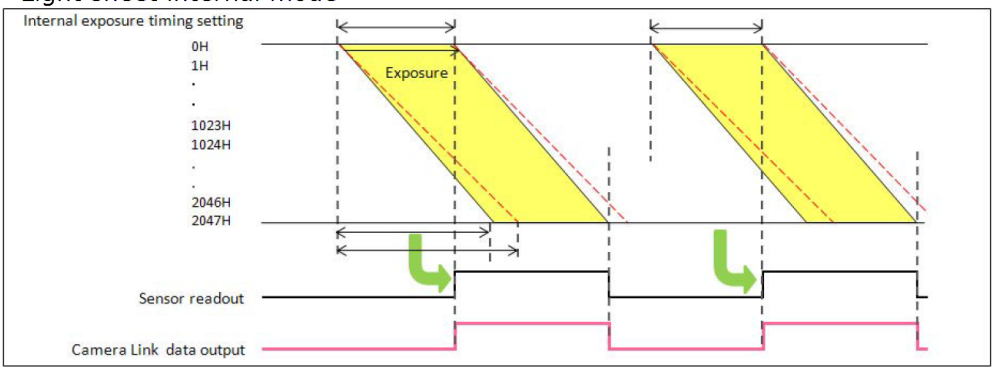
Light Sheet Modes are accessible from the Capture Mode list in the Trigger Modes, Speed and Registration panel shown below. The ORCA®-Flash4.0 V2 supports three modes for light sheet microscopy as described below. Along with each description is a timing diagram taken from the camera manual followed by a basic set of steps for enabling that particular light sheet mode in HCImage.



Internal "Free Running" Mode

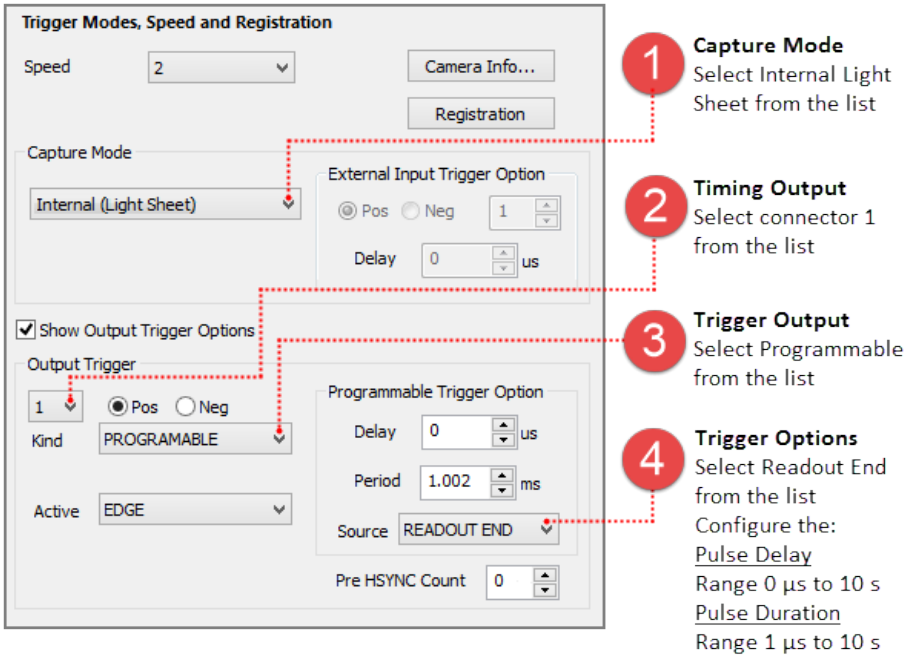
Synchronization is determined by the empirically matching the rate of the sweep of the light sheet and the camera readout to each other. There is no hardware or software triggering involved.

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.

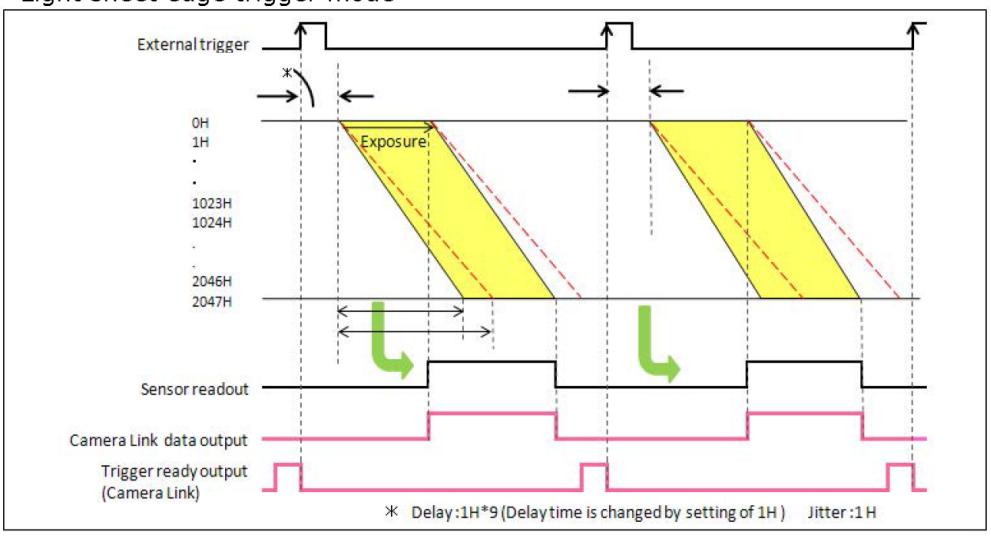


External "Edge" Trigger Mode

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.

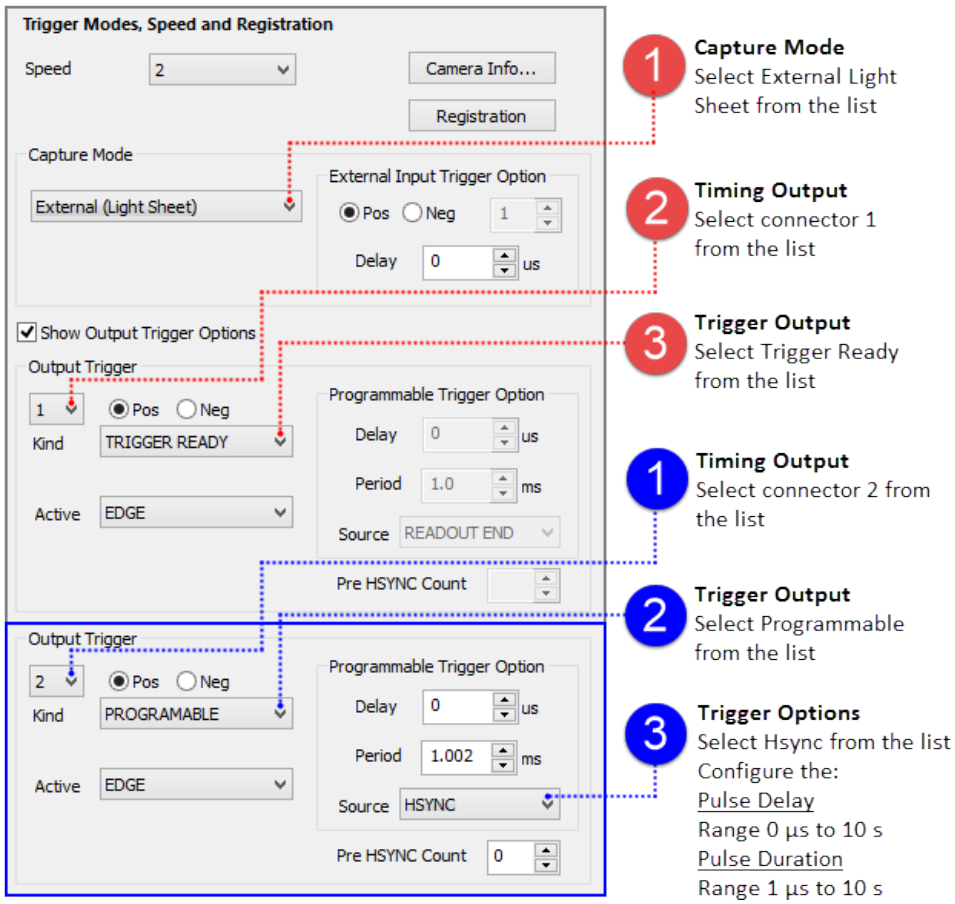
Note: Light Sheet External Trigger Mode is a frame trigger not a line trigger.

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

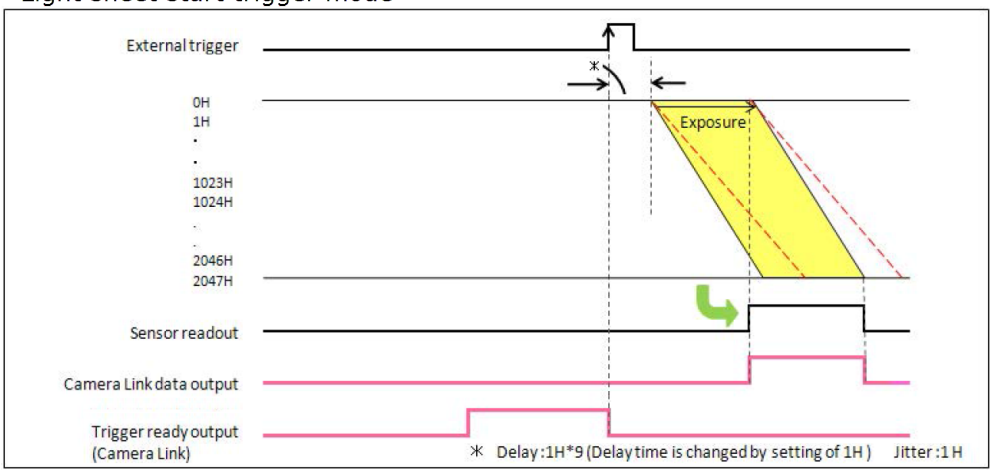
3 Trigger Options
Select Hsync from the list
Configure the:
Pulse Delay
Range 0 µs to 10 s
Pulse Duration
Range 1 µs to 10 s

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.

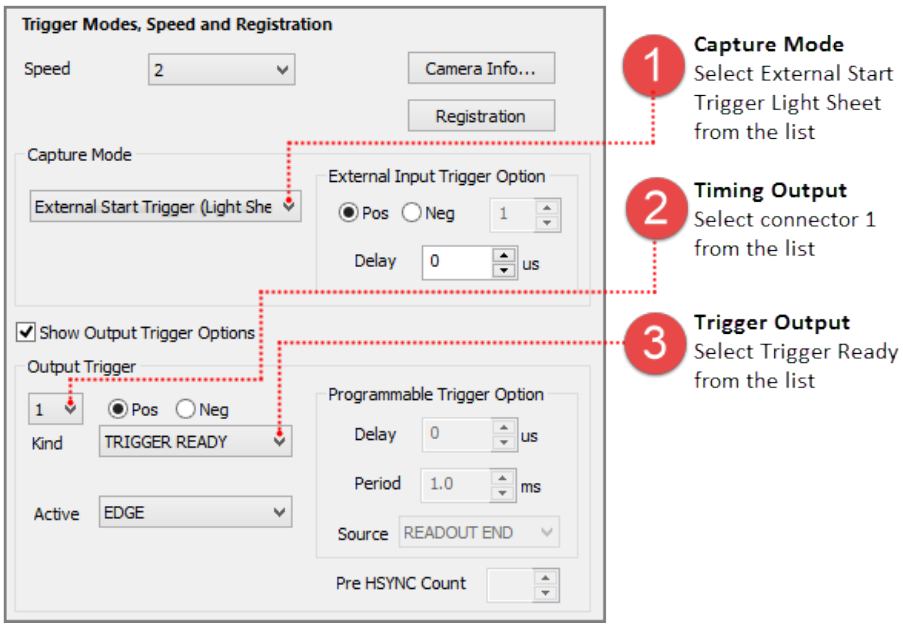
Note: Light Sheet External Start Trigger Mode is only available when acquiring a single channel.

Light sheet start trigger mode



Setup Light Sheet External Start Trigger Mode

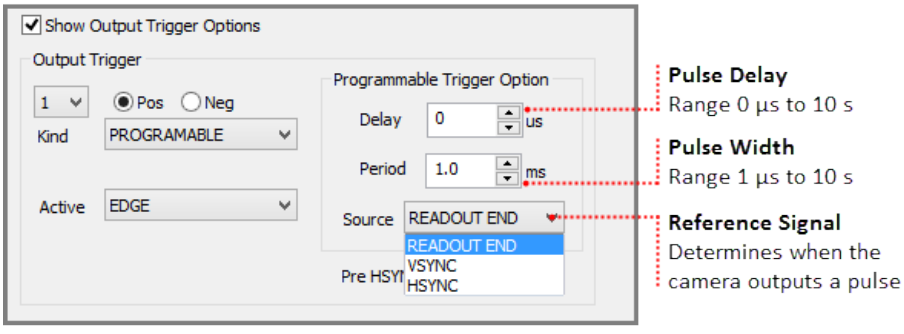
Follow the steps below to enable External Start Trigger Light Sheet Mode and to enable the camera trigger ready output for connector 1.



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).

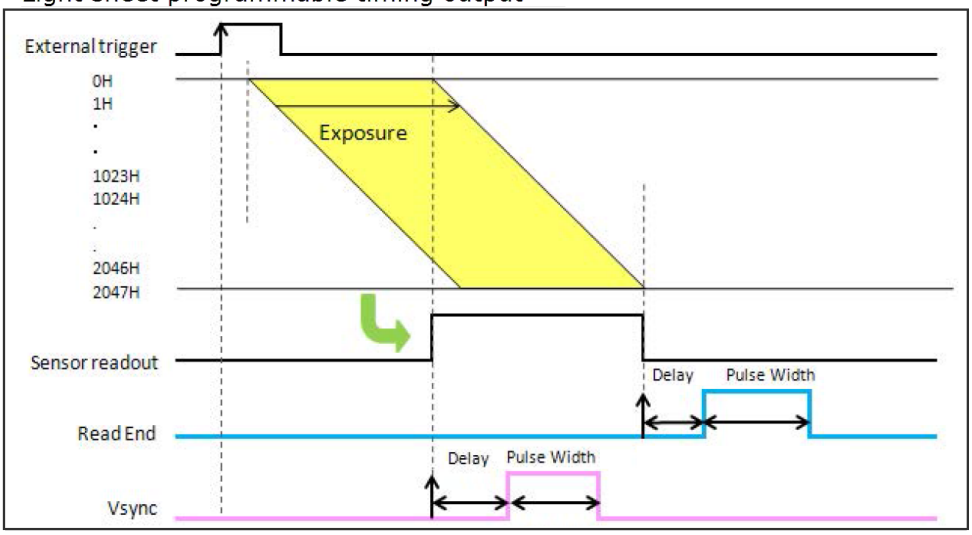
Note: In Light Sheet mode, programmable timing out can output an external trigger every line (Hsync) and frame (Readout End, Vsync). The camera can be a master to control a stage and light source.



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

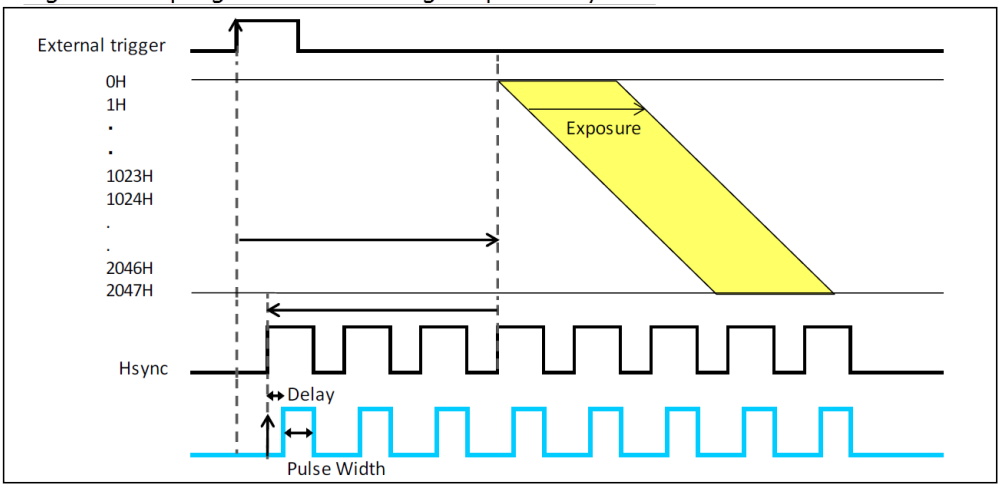
Reference Signal	Output Signal
Readout End	Camera outputs a pulse after certain delay from the end of sensor readout for each frame.
Vsync	Camera outputs a pulse after certain delay from the beginning of readout for each frame.
Hsync	Camera outputs a pulse after certain delay from the end of readout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

Light sheet programmable timing output



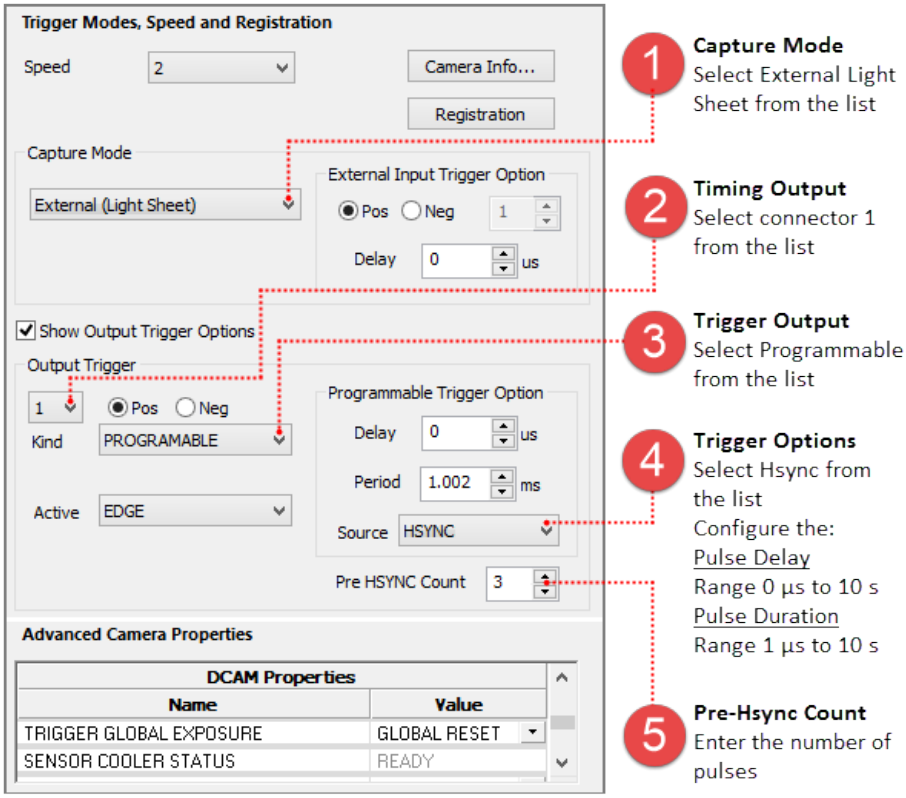
When you choose Hsync for the reference of programmable timing output, the camera can output a number of pulses before the start of exposure. This is called as Pre-Hsync. The Pre-Hsync range is 0 to 2047.

Light sheet programmable timing output - Hsync



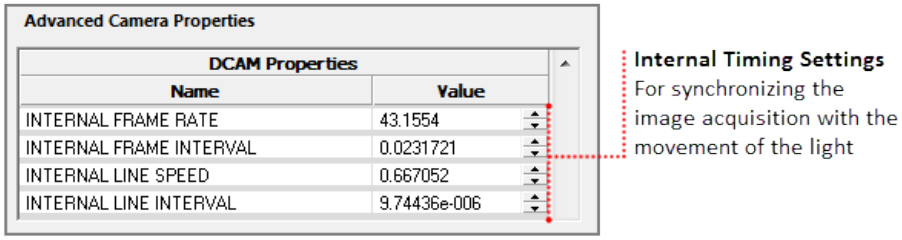
How to Setup Pre-Hsync Pulses

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.



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 35.

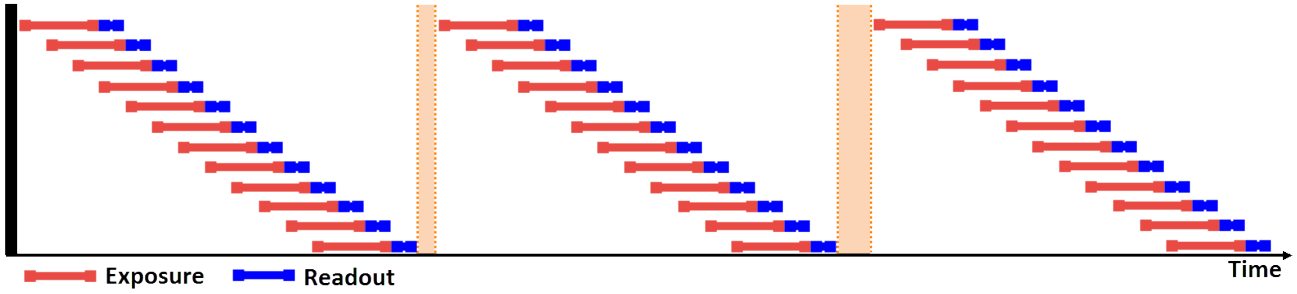


Internal Timing Settings

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

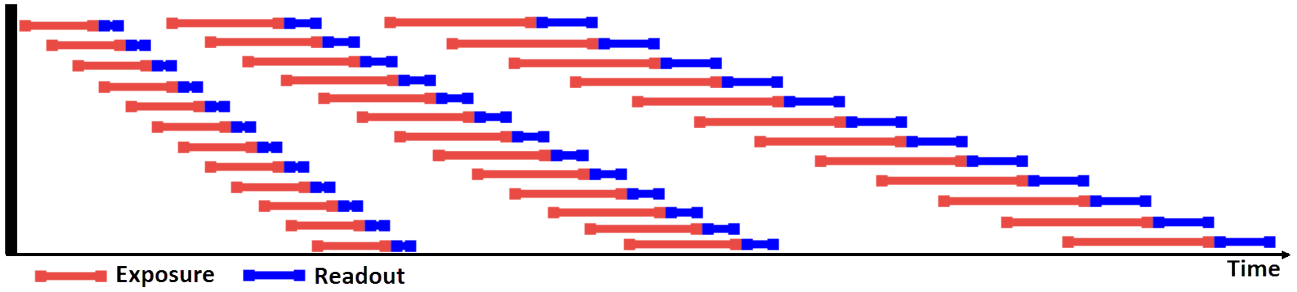
Internal Frame Rate - The number of frames per second that the camera acquires.

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 V2, this would be 6.5 µm ÷ 9.7 µs = $6.5 \times 10^{-6} \div 9.7 \times 10^{-6}$ = 0.67 meters per second.

SPEED AND RESOLUTION

The camera specifications reported are based on firmware version 3.20.A and 4.00.A.

Normal Mode

Readout Time

Readout Speed	Readout Time	Read Noise (rms)
Speed 2 (Standard Scan) at 100 fps Camera Link and 30 fps USB 3.0	10 ms	1.6 electrons (1.0 electrons median)
Speed 1 (Slow Scan) at 30 fps for both Camera Link and USB 3.0	33 ms	1.4 electrons (0.8 electrons median)

Exposure Time

Exposure Time	Speed 2 (Standard Scan)	Speed 1 (Slow Scan)
Internal	1 ms to 10 s	3 ms to 10 s
Internal with Subarray	38.96 µs to 10 s	129.99 µs to 10 s
External Trigger	1 ms to 10 s	3 ms to 10 s

Speed 2 (Standard Scan) - Camera Link and USB 3.0

Capture Mode		Camera Link	USB 3.0		
*Readout speed at center position (fps)	Horizontal Vertical	2048/1536/1024/512	2048/1536/1024	512	Binning 2x2, 4x4
Internal "Free Running" Mode	2048	100	30	100	100
	1024	200	60	200	200
	512	400	120	400	400
	256	801	240	801	801
	128	1603	481	1603	1603
	64	3206	968	3206	3206
	8	25 655	7894	25 655	25 655
External Trigger Mode: Edge	2048	90	30	90	90
	1024	164	60	164	164
	512	278	120	278	278
	256	427	240	427	427
	128	582	481	583	583
	64	712	712	712	712
	8	884	884	884	884
External Trigger Mode: Level	2048	90	30	90	90
	1024	164	60	164	164
	512	278	120	278	278
	256	427	240	427	427
	128	582	481	583	583
	64	712	712	712	712
	8	884	884	884	884

Capture Mode		Camera Link	USB 3.0		
External Trigger Mode: Synchronous Readout	2048	98	30	98	98
	1024	193	60	193	193
	512	374	120	374	374
	256	702	240	702	702
	128	1251	481	1251	1251
	64	2052	968	2052	2052
	8	4664	4664	4664	4664
External Trigger Mode: Fast Synchronous Readout (Camera Link)	2048	99	n/a	n/a	n/a
	1024	198	n/a	n/a	n/a
	512	393	n/a	n/a	n/a
	256	771	n/a	n/a	n/a
	128	1487	n/a	n/a	n/a
	64	2773	n/a	n/a	n/a
	8	11 402	n/a	n/a	n/a

Speed 1 (Slow Scan) Camera Link and USB 3.0

Capture Mode	Horizontal	Vertical	Frame Rate
Internal "Free Running" Mode	2048	2048	30
		1024	60
		512	120
		256	240
		128	481
		64	962
		8	7696
External Trigger Mode: Edge & Level	2048	2048	27
		1024	50
		512	85
		256	133
		128	185
		64	229
		8	290
External Trigger Mode: Synchronous Readout	2048	2048	29
		1024	58
		512	112
		256	210
		128	375
		64	615
		8	1399
External Trigger Mode: Fast Synchronous Readout (Camera Link)	2048	2048	29
		1024	59
		512	117
		256	231
		128	446
		64	832
		8	3420

Light Sheet Mode

In Light Sheet mode the readout time at full resolution can range from 20 ms to 204.8 s. The exposure time range is from 9.7 µs to 10 s.

Frame Rate

Horizontal width x Vertical width		Frame Rate
2048	2048	49
	1024	99
	512	196
	256	385
	128	743
	64	1386
	8	5701
	4	7330

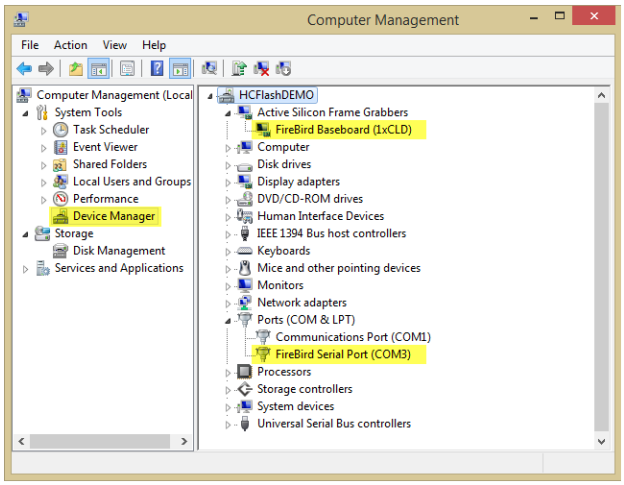
TROUBLESHOOTING

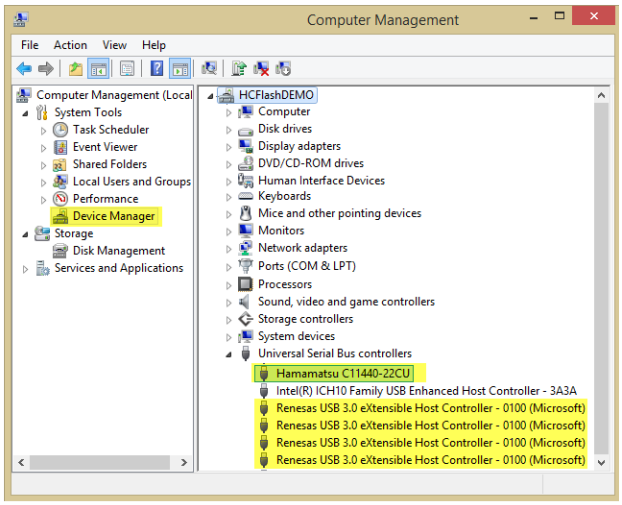
ORCA®-Flash4.0 V2 and card in the Device Manager

What am I looking for in the Device Manager?

(Camera Link) The board is listed under Active Silicon Frame Grabbers as the FireBird Baseboard and under Ports (COM & LPT) as FireBird Serial Port (COM#). The camera make an model numbers are not displayed.

(USB 3.0) The card and camera will show up under Universal Serial Bus Controllers. The USB 3.0 card is identified as Renesas USB 3.0 and the camera as a Hamamatsu C11440-22CU.





Camera Link card is not recognized in the Device Manager

DCAM-API Active Silicon FireBird module installed?

The current version of DCAM-API is available for download at https://dcam-api.com/.

USB 3.0 card is not recognized in the Device Manager

Renesas USB 3.0 driver installed?

The current version of the Renesas USB 3.0 Driver is available for download at ((ftp://60.248.38.84/cat_106/30230_dr.zip)).

Camera is not recognized in the Device Manager

DCAM-API installed?

The current version of DCAM-API is available for download at https://dcam-api.com/. Before installing the camera driver, make sure that the camera is turned off.

Installed the latest updates for Windows?

Check for Windows Updates, press the Windows logo key ⊞+Pause to display the System Properties dialog and select Windows Update located in the lower left corner of under See Also.

USB 3.0 cable connected after turning the camera on?

The camera is not plug-n-play enabled, connect the camera to the PC before turning the camera power ON.

Are there other devices connected to the USB card?

Avoid connecting any devices other than the ORCA®-Flash4.0 V2 to the USB card.

Camera is recognized but not responding

Is the camera connected properly and are the cables secure?

Verify that all of the cables are securely connected and in the proper location. The camera can be connected to computer using either the Camera Link cables or the USB 3.0 cable but not both at the same time. Exit the software and power off the camera before moving, removing or re-seating the cables.

Is the camera in an external input trigger capture mode?

When set in an External Trigger mode and Live, Capture1 or Start is selected, the software will wait until the camera receives the external signal before acquiring an image. Depending on which external mode is selected the exposure time may be grayed out as well. This may give the appearance that the camera is not responding when in fact it is functioning correctly. To switch to internal mode, expand the Trigger Modes, Speed and Registration panel and select Internal from the Capture Mode list.

Camera performance issues, unable to achieve 100 fps (30 fps for USB)

Is the card in the proper PCIe slot?

(Camera Link) Recommended that the board is installed in a PCIe x8 Gen2 or better slot.
(USB 3.0) Recommended that the card is installed in a PCIe x1 Gen 2 (5GT/s) slot or better.

Update the BIOS settings?

Recommended BIOS settings:

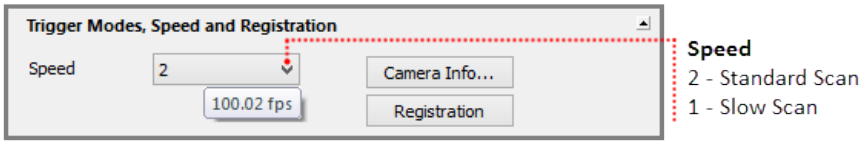
- Disable (uncheck) SpeedStep and C-State under the performance section
- Enable (check) Turbo Boost and Hyper-Threading under the Performance section

Are there other devices connected to the USB card?

Avoid connecting any devices other than the ORCA®-Flash4.0 V2 to the USB card.

What is camera speed setting in HCImage?

In the Capture pane, expand the Trigger Modes, Speed and Registration panel and make sure Speed 2 is selected. Also, the exposure time should be less than 10 ms.



What are the settings for the time lapse?

In the Scan Settings panel, make sure that Enable Maximum and 0 Delay are selected and that storage is set to Memory or to Temporary Buffer.

ORCA®-Flash4.0 V2 Cooling

Turned the camera power on but the fan is not running?

The cooling fan will start running approximately 10 minutes after the camera is turned on to prevent condensation from forming.

What is the cooling temperature?

The temperature for the ORCA®-Flash4.0 V2 is not reported in HCImage. The camera sensor is cooled to around –10°C using a forced air cooling method with the ambient temperature around +20°C.

Is water cooling an option?

The ORCA®-Flash4.0 V2 is equipped for water cooling, please consult the ORCA®-Flash4.0 V2 camera manual for more information.

Why is the camera making a loud noise?

The buzzing alarm indicates that the camera is overheated, exit the software immediately and power off the camera. Remove any material that may be blocking the camera's ventilation. Ensure that there is at least 2 cm of clearance for the intake and exhaust vents.