

HCImage Live

ORCA[®]-Fusion Guide



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INSTALLATION

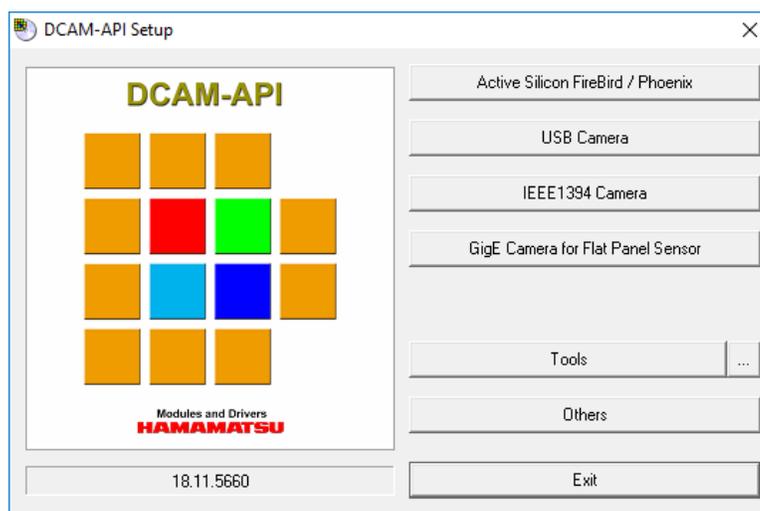
HCIImage Live

1. Insert the HCIImage Live installation DVD into the DVD-ROM drive. If autoplay is enabled, the HCIImage 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 HCIImage Live. If the drivers have not been installed, or the camera is not turned on before launching HCIImage Live, the camera will not be available in the software.
6. Click the **HCIImage Live** icon on the Desktop to launch HCIImage Live.

DCAM-API[®] Drivers

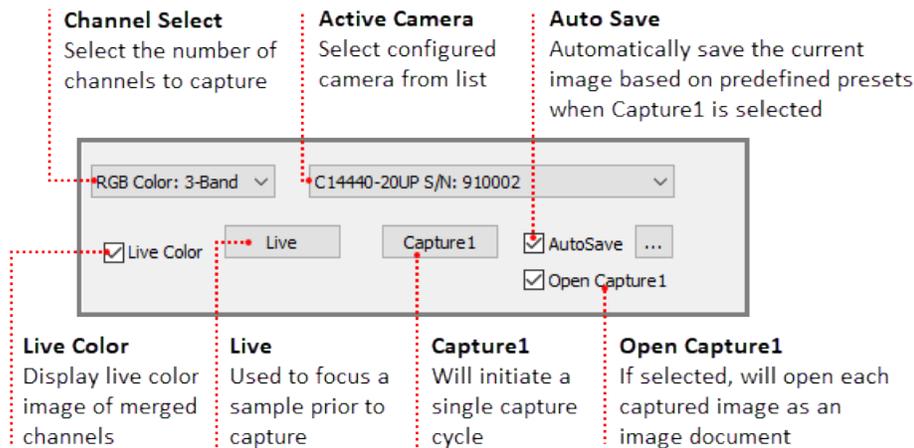
Before installing the camera driver, make sure that the camera is turned off.

1. After installing HCIImage Live from the DVD, you will be prompted to install DCAM-API[®], click **Yes**. If you downloaded HCIImage 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. [CoaXPress] Select the **Active Silicon FireBird / Phoenix** 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.



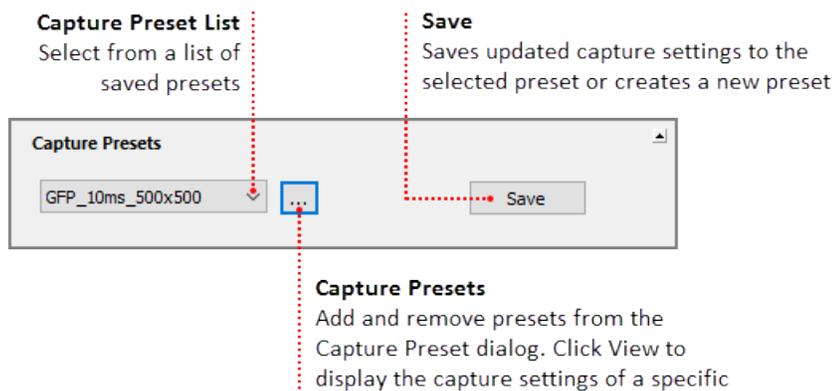
THE CAPTURE PANE

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 easily 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. HCIImage will load the capture settings from the previous session when launched.



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

Camera Control

Manage capture settings using the individual channel and exposure controls.

Temperature
Reports the current temperature of the sensor

Auto Exposure
Automatically adjust exposure to optimize the dynamic range of intensities in the image

Exposure Lock
Maintains the exposure ratio between multiple channels

Exposure Time
Enter time or adjust using controls

Filter List
Choose a defined filter position from the list

Focus Channel
Click the numbered button to display the selected channel

Active Channel
Select which channels to capture. Disable to ignore channel during capture

Channel Tint
Displays filter tint for the channel. For RGB color images, the tint order may be selected from the list

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

The screenshot shows the 'Camera Control' window with a 'Cooling' section displaying 'Temp: -5.00 °C'. Below this are three channels: '1 Red', '2 Green', and '3 Blue', each with a checked checkbox and a 'Focus' button. To the right, there are 'Gain' and 'Exposure' controls, with a lock icon on the exposure field. A tooltip is visible over the exposure time field, showing '33.3257 ms' and instructions to type 'u', 'm', 's', or 't' to change units.

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.

Binning
2x2 and 4x4 digital binning

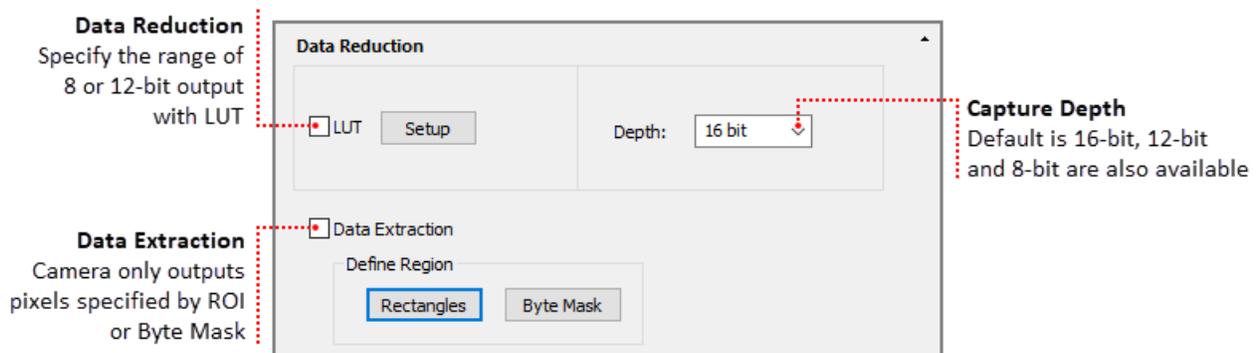
Adjust Exposure
Automatically adjusts exposure when changing binning

Sub Array
List of preset sizes or define a custom array

The screenshot shows the 'Binning and SubArray' window. It features a 'Binning' dropdown menu set to '1' and an 'Adjust Exposure Time' checkbox. Below this is a 'Sub-Array' section with a 'Preset Sizes' dropdown menu set to '1024 x 512'. To the right of the dropdown are 'Reset', 'Define', and 'Apply' buttons. At the bottom, there are input fields for 'X0' (512), 'Width' (1024), 'Y0' (768), and 'Height' (512).

Data Reduction

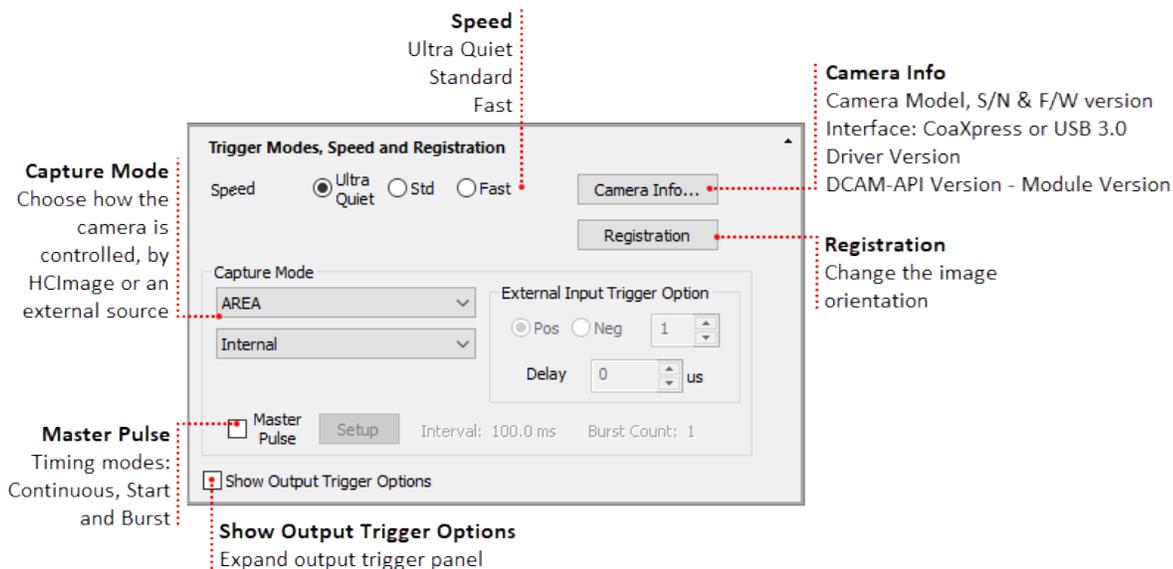
Reduce the amount of image data by using the LUT. LUT is a customizable Look Up Table that allows the user to specify the range for 12 or 8-bit output. Data Reduction not only reduces the amount image data that will be acquired, it allows for higher frame rates through USB 3.0 with 12 or 8-bit output. For more information and examples on using data reduction, see "**Data Reduction**" on page 18.



Note: In order to achieve a reduced file size using 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.

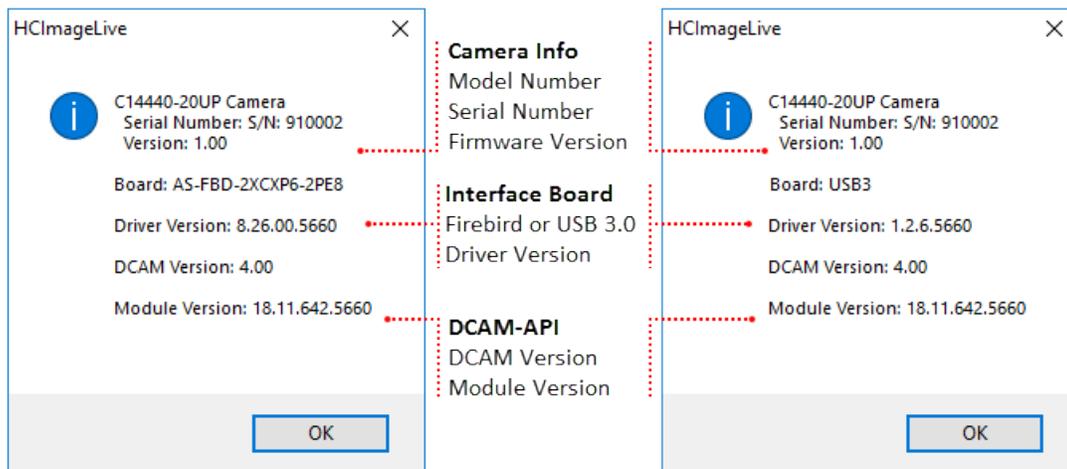
Trigger Modes, Speed and Registration

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[®]-Fusion.



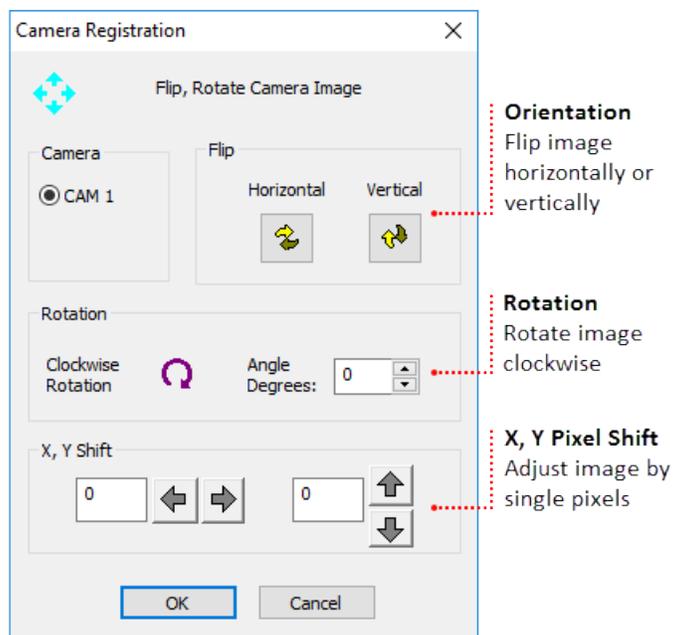
Camera Info

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



Registration

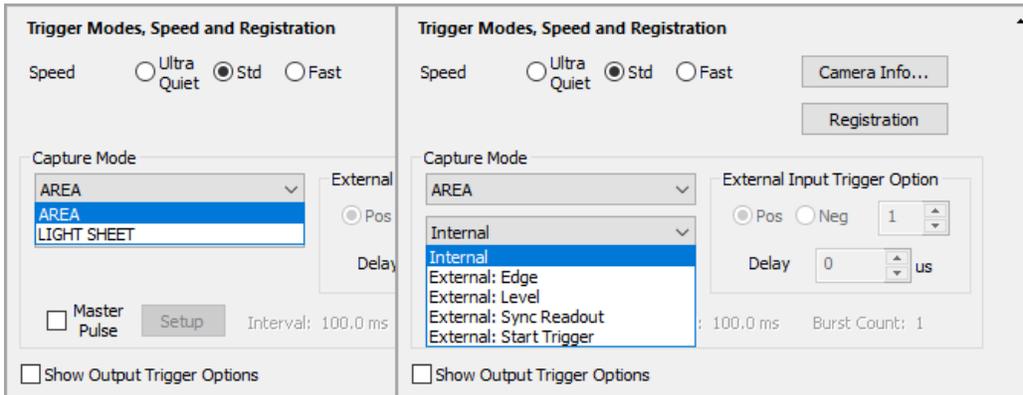
Adjust the orientation, rotation or pixel shift of the camera image.



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).

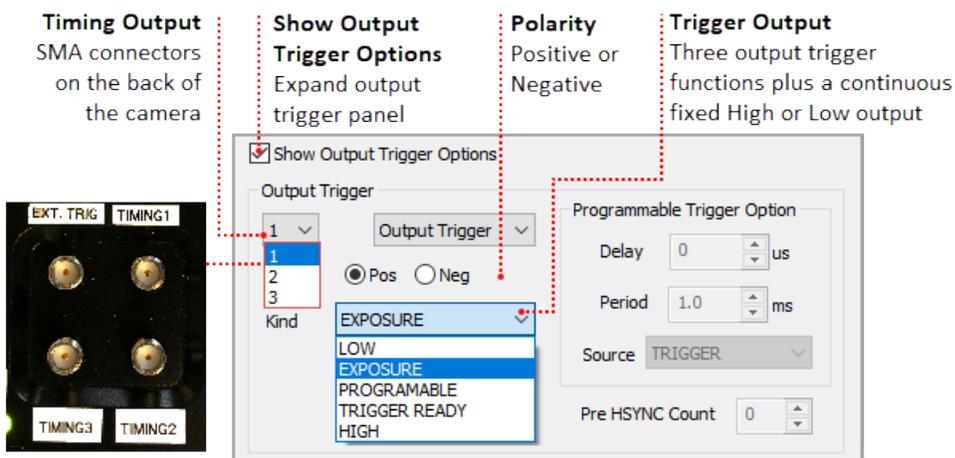
Capture Mode

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[®]-Fusion with the CoaXPRESS and USB 3.0 interfaces. For more information about Capture Modes, please see "**External Input Trigger Modes**" on page 21.



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 29.



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[®]-Fusion supports multiple levels of pixel correction for quantitative and qualitative control of images.

Correction Level for White Spots	Ratio of the number of pixels to be corrected to the total number of pixels
Aggressive	Approximately 0.5 % (~30 000 pixels)
Standard (default)	Approximately 0.05 % (~3000 pixels)
Minimum	Less than 0.0001 %
Off	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.

1 Enable Correction Mode
Select ON from the list

2 Set Correction Level
Select the desired level of hot pixel correction

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.

1 Correction Image
Select Buffer and click Capture

2 Camera Offset
Enter 100

3 Operation
Select Background Subtraction

Hint: HImage 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.

Camera Control

Cooling
Temp: -5.00 °C

Auto Expose ...

Process ON

Gain Exposure

1 Default < > 0 25.0 ms

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.

Scan Settings
Save and load scan settings

Scan Type
Select acquisition type from list

Progress
Displays the number of images

Event Markers
Annotate the time when a significant occurred

Frame Rate
Displays the current speed in frames per second

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

Time Elapsed: 00:00:05.35

Delay Remaining: 00:00:00

43.01 fps

Progress: 231

Select Scan Type: Time Lapse, Time Lapse, High Speed Streaming

Start Stop

Event Marker: 0

0 1 2 3 4 5 6 7 8 9

Time Lapse

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

AutoSave
Define where and how to store acquired data

Speed
Select maximum speed or define a capture interval

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

RAM Limit
Define the amount of available RAM for streaming

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

Display
Select a live display or to review acquired images

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

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

Scan Settings

AutoSave ...

Enable Maximum

Control :

0 Delay

Field Delay 1 0.0 sec

Field Delay 2 0.0 sec

to Disk

to Memory (2555) RAM...

to Temporary Buffer

Live Image

Review

Continuous

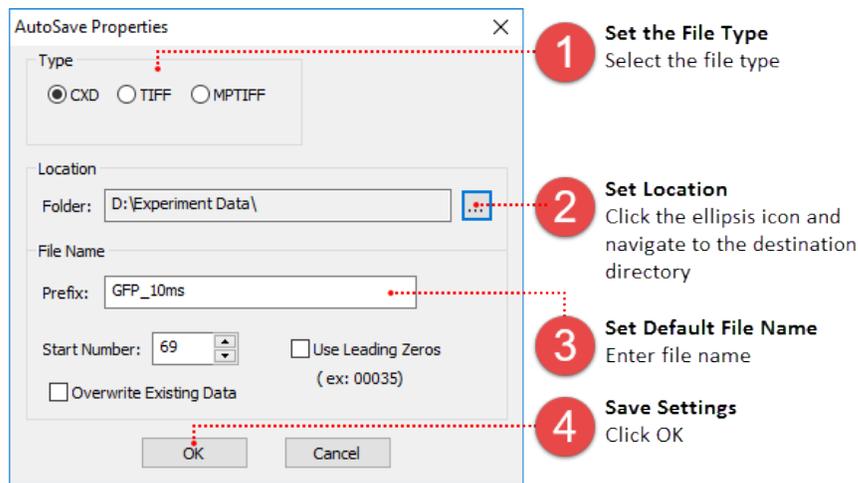
End Frame 2556

End Time 0.0 sec

Type "u", "m", "s", "t" to change Units
u=microsec, m=millisec, s=sec, t=min

How to Use AutoSave

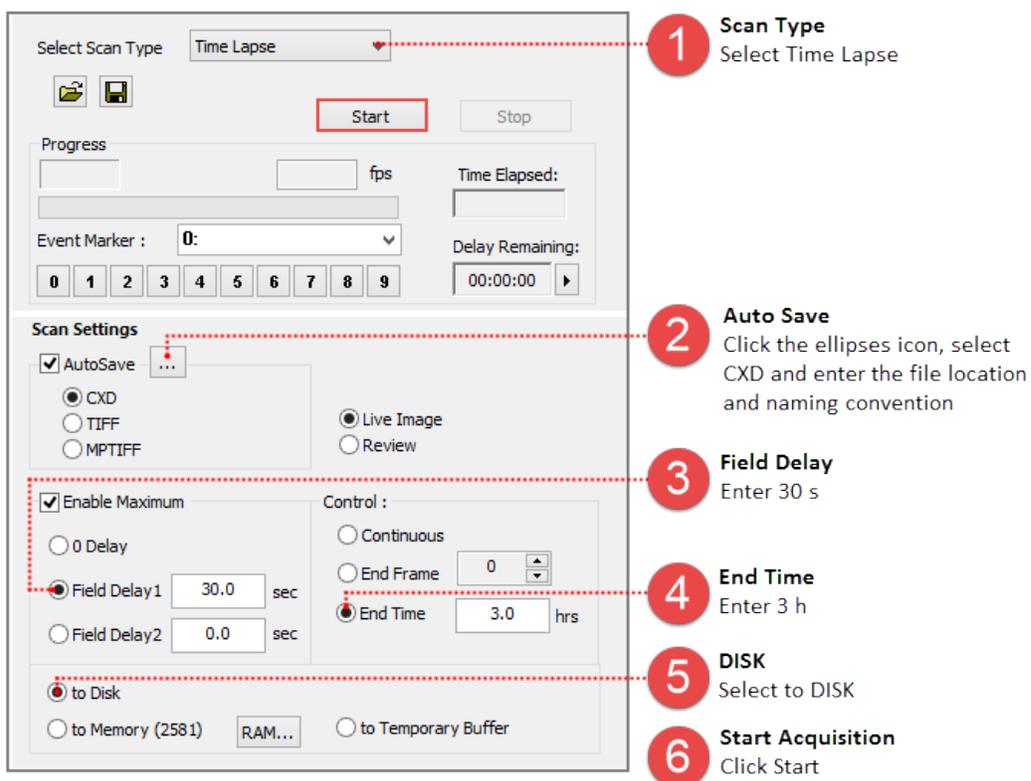
In the AutoSave Properties dialog, the user can determine how and where to store the acquired data. Image data can be saved as a CXD, TIFF or MPTIFF. The example below provides a description of the Auto Save Properties dialog.



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

Setup a Time Lapse - Save to Disk

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



Setup a Time Lapse - Save to the Temporary Buffer

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

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

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

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

Setup a Time Lapse - Save to Memory

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

1 Scan Type
Select Time Lapse

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

3 Field Delay
Select 0 Delay

4 Continuous
Select Continuous

5 Memory
Select to Memory

6 Start Acquisition
Click Start

High Speed Streaming

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

Note: Acquisition rates will vary based on the PC configuration, for information about the computer requirements, please see the [PC Recommendations for ORCA®-Fusion](#).

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

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

AutoSave/AutoConvert
Define how streamed data is handled

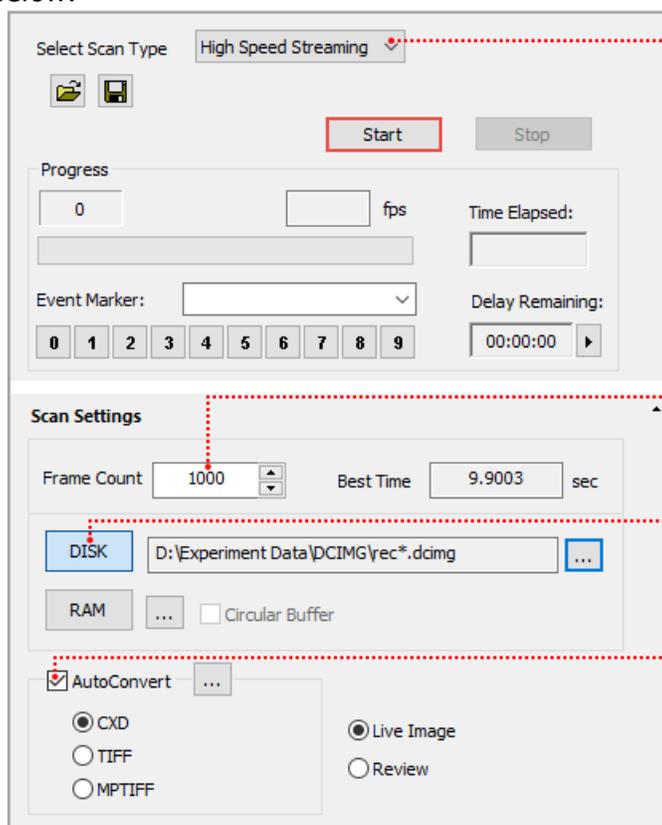
DCIMG Location
Set a file location for streaming data to DISK

Display
Select a live display or to review acquired images

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

Steps for Streaming to Disk

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



The screenshot shows the software interface for streaming to disk. It includes a 'Select Scan Type' dropdown menu set to 'High Speed Streaming', a 'Start' button, and a 'Stop' button. Below these are progress indicators for 'Progress' (0 fps), 'Time Elapsed', 'Event Marker', and 'Delay Remaining'. The 'Scan Settings' section is expanded, showing 'Frame Count' set to 1000 and 'Best Time' at 9.9003 sec. Under 'Stream Type', 'DISK' is selected with the file path 'D:\Experiment Data\DCIMG\rec*.dcimg'. The 'AutoConvert' checkbox is checked, and 'CXD' is selected as the file type. Other options include 'RAM', 'Circular Buffer', 'Live Image', 'TIFF', 'MPTIFF', and 'Review'.

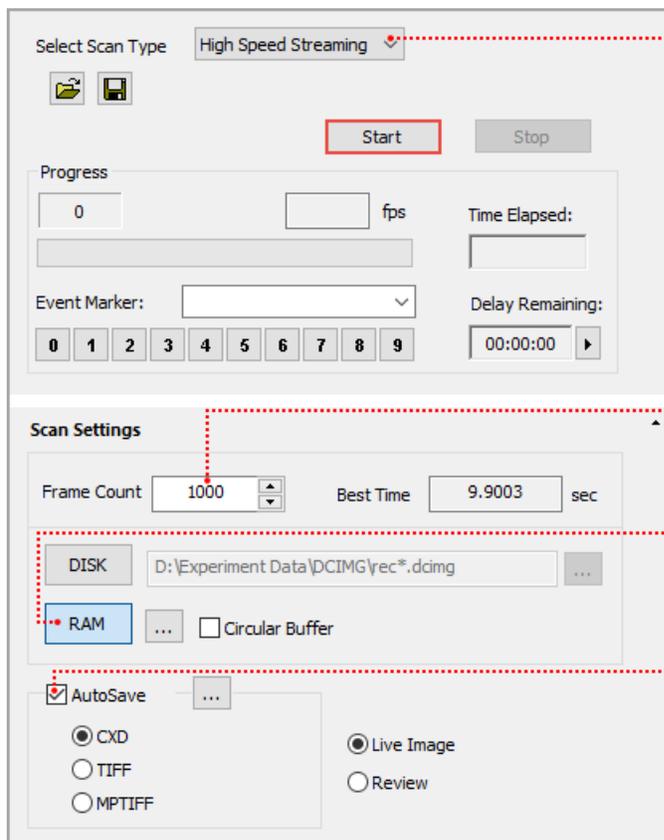
- 1 Select Scan Type**
Select High Speed Streaming
- 2 Enter Frame Count**
Enter the number of images to acquire
- 3 Select Stream Type**
Select DISK
- 4 Auto Convert File Type**
Enable AutoConvert and select file type
- 5 Start Streaming**
Click Start

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

Steps for Streaming to RAM

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

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



The screenshot shows the software interface for streaming to RAM. The interface is divided into several sections: 'Select Scan Type', 'Progress', 'Event Marker', 'Scan Settings', and 'AutoSave'. The 'Scan Settings' section is highlighted with a red dashed box. Five numbered steps are overlaid on the interface:

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

DATA REDUCTION

Reduce the amount of image data by using the LUT, a customizable Look Up Table that allows the user to specify the range for 12 or 8-bit output. 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	Scan Mode	CoaXPress	USB 3.0		
		16-bit	8-bit	12-bit	16-bit
2304 x 2304	Fast	89.1	63.3	42.2	31.6
	Standard	23.2		23.2	
	Ultra-quiet	5.42		5.42	
2048 x 2048	Fast	100	80.1	53.4	40.0
	Standard	26.1		26.1	
	Ultra-quiet	6.1		6.1	
256 x 256	Fast	799		799	
	Standard	208		208	
	Ultra-quiet	48.6		48.6	

Note: In order to achieve a reduced file size using 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

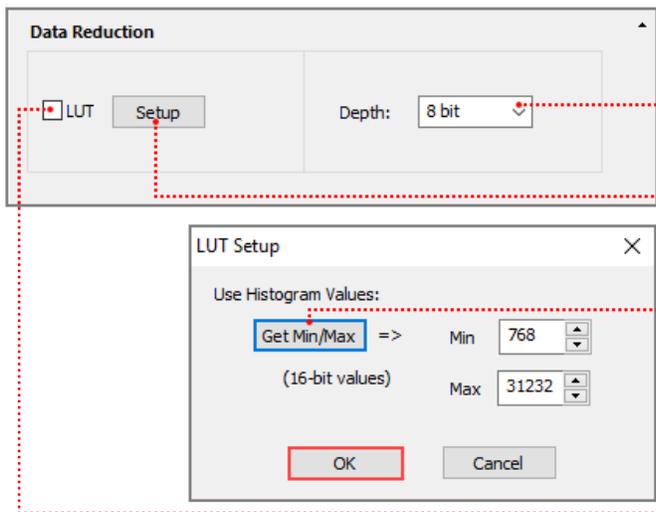
Configure the capture settings and follow the instructions below.

The image shows two software windows. The top window is titled 'Data Reduction' and has a 'LUT' checkbox checked and a 'Setup' button next to it. The 'Depth' dropdown menu is set to '12 bit'. The bottom window is titled 'LUT Setup' and has a 'Get Min/Max' button highlighted in blue. Below this button are 'Min' and 'Max' input fields with values '944' and '31936' respectively. At the bottom of the 'LUT Setup' window are 'OK' and 'Cancel' buttons.

- 1 Set Bits Per Channel**
Select 12-bit
- 2 Setup LUT**
Click LUT Setup button
- 3 Set Min/Max Values**
Click Get Min/Max button or enter the values manually and click OK
- 4 Enable LUT**
Select LUT

How to Setup LUT Range 8-bit

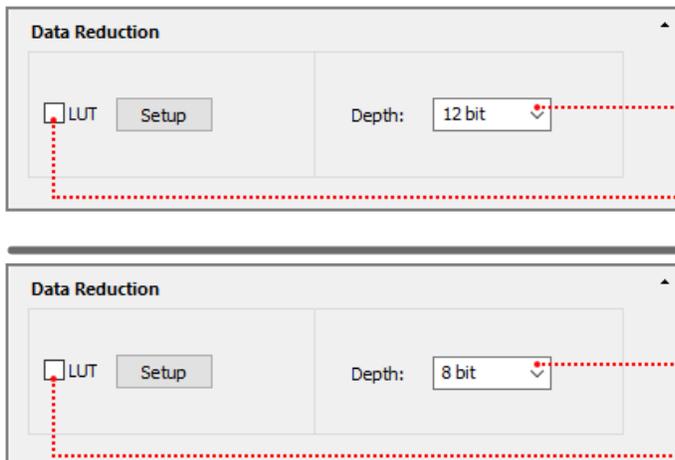
Configure the capture settings and follow the instructions below.



- 1 Set Image Depth**
Select 8-bit
- 2 Setup LUT**
Click LUT Setup button
- 3 Set Min/Max Values**
Click Get Min/Max button or enter the values manually and click OK
- 4 Enable LUT**
Select LUT

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

Configure the capture settings and follow the instructions below.



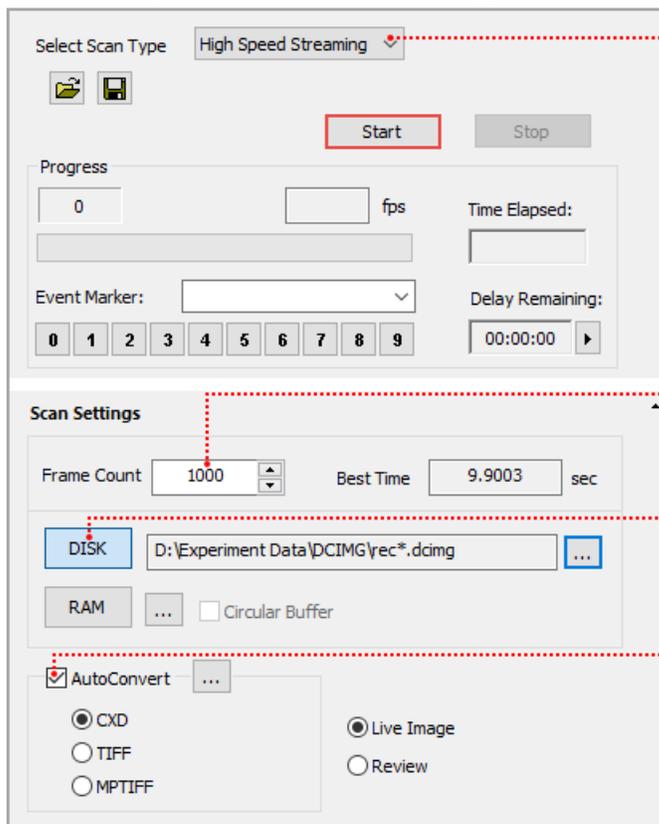
- 1 Set Bits Per Channel**
Enter 12-bit
- 2 No LUT**
Disable LUT (uncheck)

- 1 Set Image Depth**
Select 8-bit
- 2 No LUT**
Disable LUT (uncheck)

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.



1 Select Scan Type
Select High Speed Streaming

2 Enter Frame Count
Enter the number of images to acquire

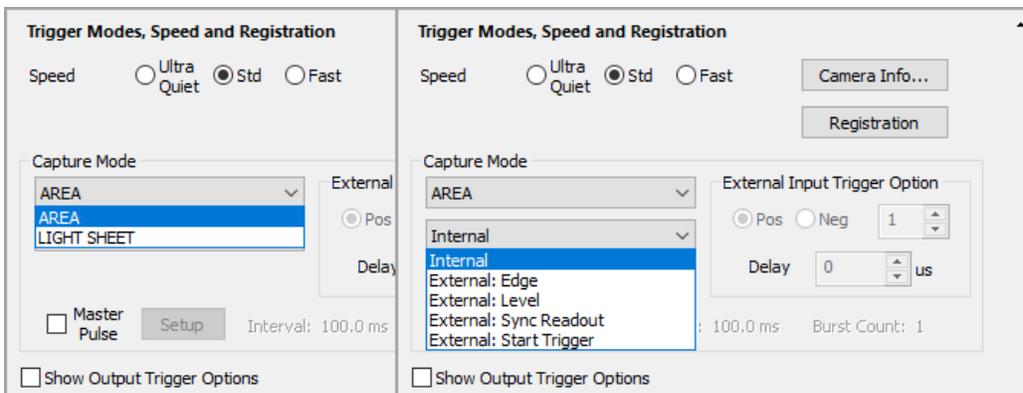
3 Select Stream Type
Select DISK

4 Auto Convert File Type
Enable AutoConvert and select file type

5 Start Streaming
Click Start

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 "**LightSheet Mode**" on page 35.



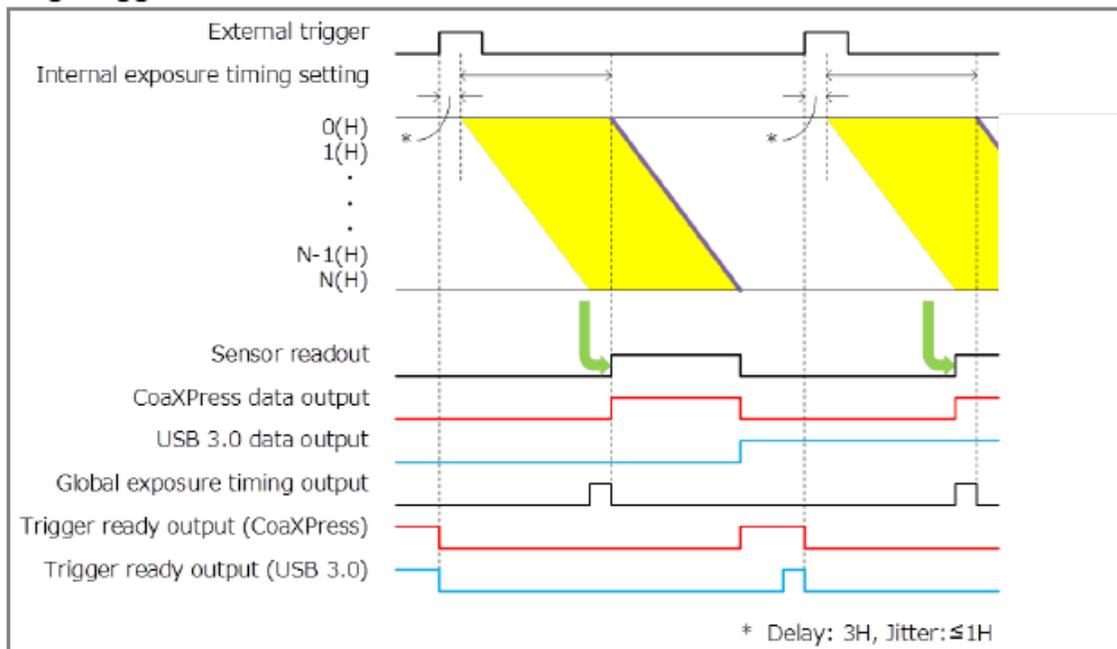
External Trigger Delay Function

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 μ s to 10 s (10 μ s 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 HCIImage.

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 HCIImage. 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.

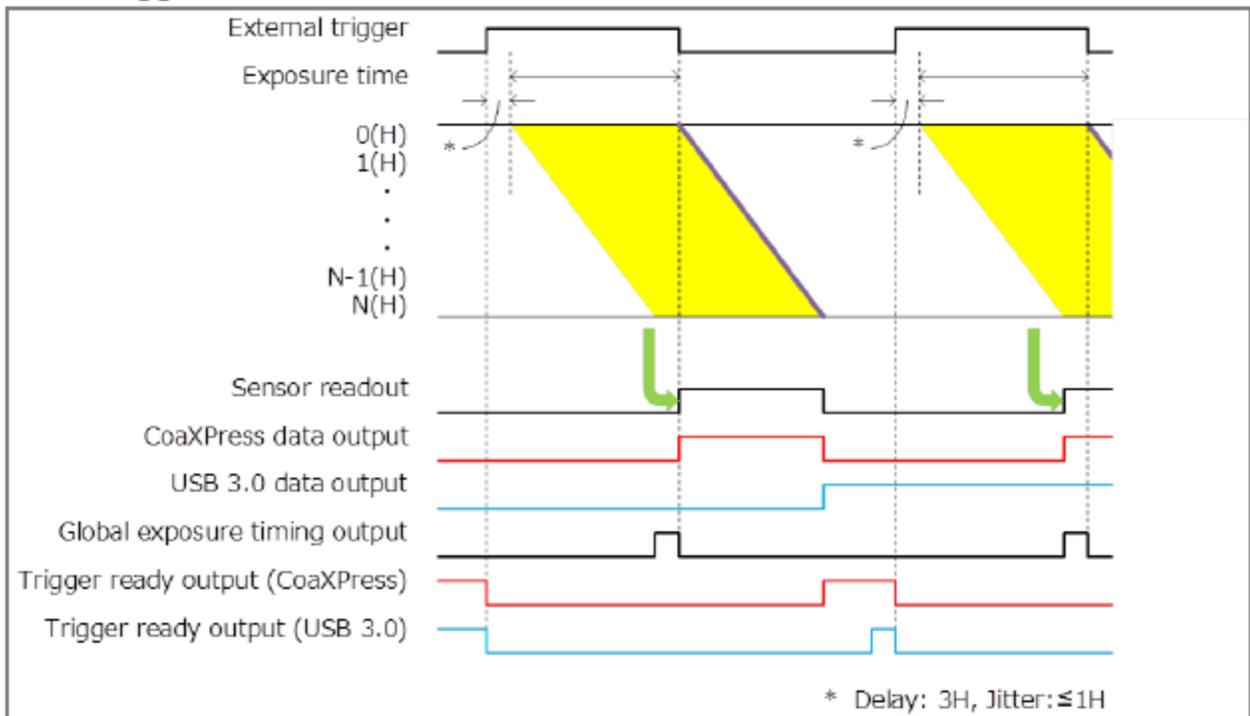
1 Set Capture Mode
Select External Edge Trigger

2 Trigger Delay
Enter optional trigger delay

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 determined 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.

1 Set Capture Mode
Select External Level Trigger

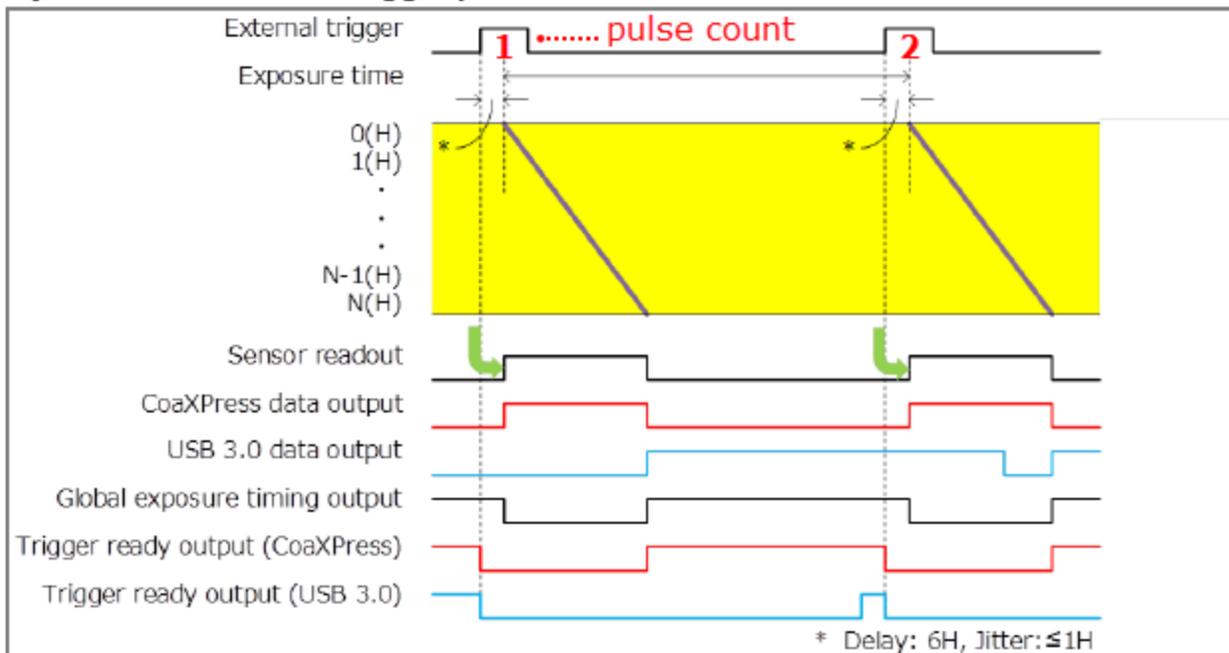
2 Trigger Delay
Enter optional trigger delay

Synchronous Readout Trigger

(1) Normal operation (pulse count 1)

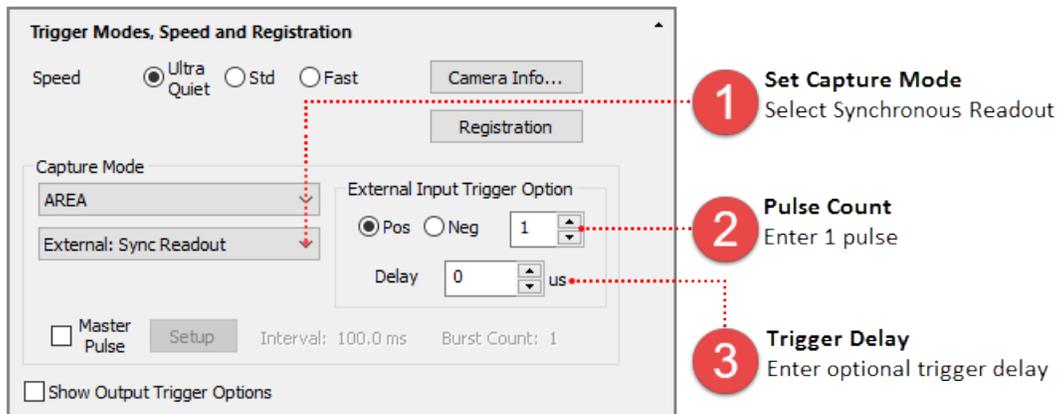
The synchronous readout trigger mode is used for continuous imaging when it is necessary to control the exposure start timing of each frame from an outside source and also when it is necessary to secure as long exposure time as possible. In the synchronous readout trigger mode, the camera ends each exposure,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



1 Set Capture Mode
Select Synchronous Readout

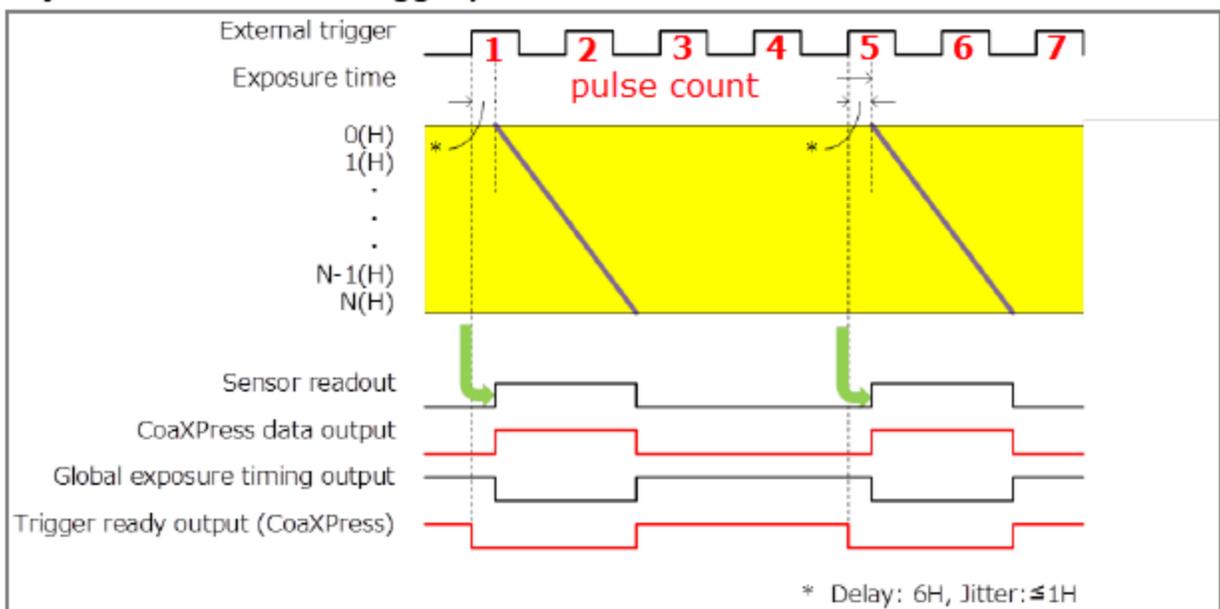
2 Pulse Count
Enter 1 pulse

3 Trigger Delay
Enter optional trigger delay

(2) Pulse count

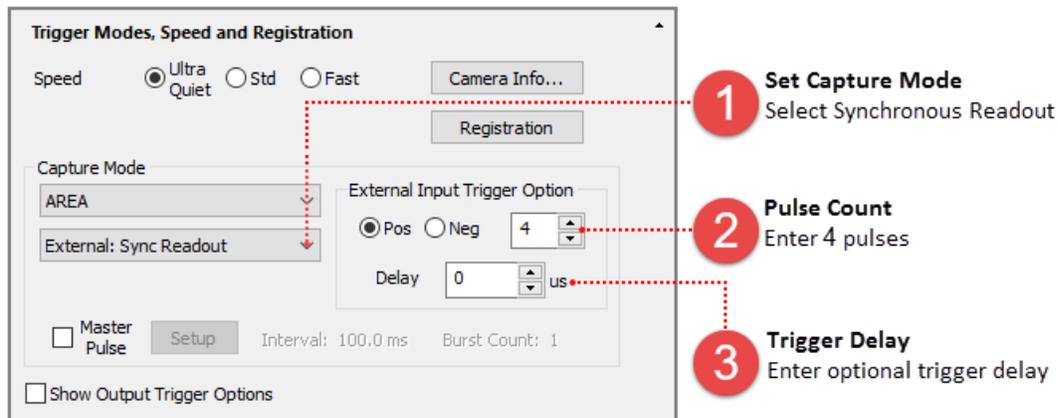
Useful for synchronizing the camera with a spinning disk confocal, the exposure time is determined by a specified number of timing pulses or pulse count. For example, the pulse count is set to 4. The first pulse starts exposing the first frame, the fifth pulse stops the exposure and starts the readout of the first frame and at the same time starts exposing the second frame. The eighth 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 4



Setup Synchronous Readout Trigger (pulse count 4)

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 5 pulses are required to capture a single image.



1 Set Capture Mode
Select Synchronous Readout

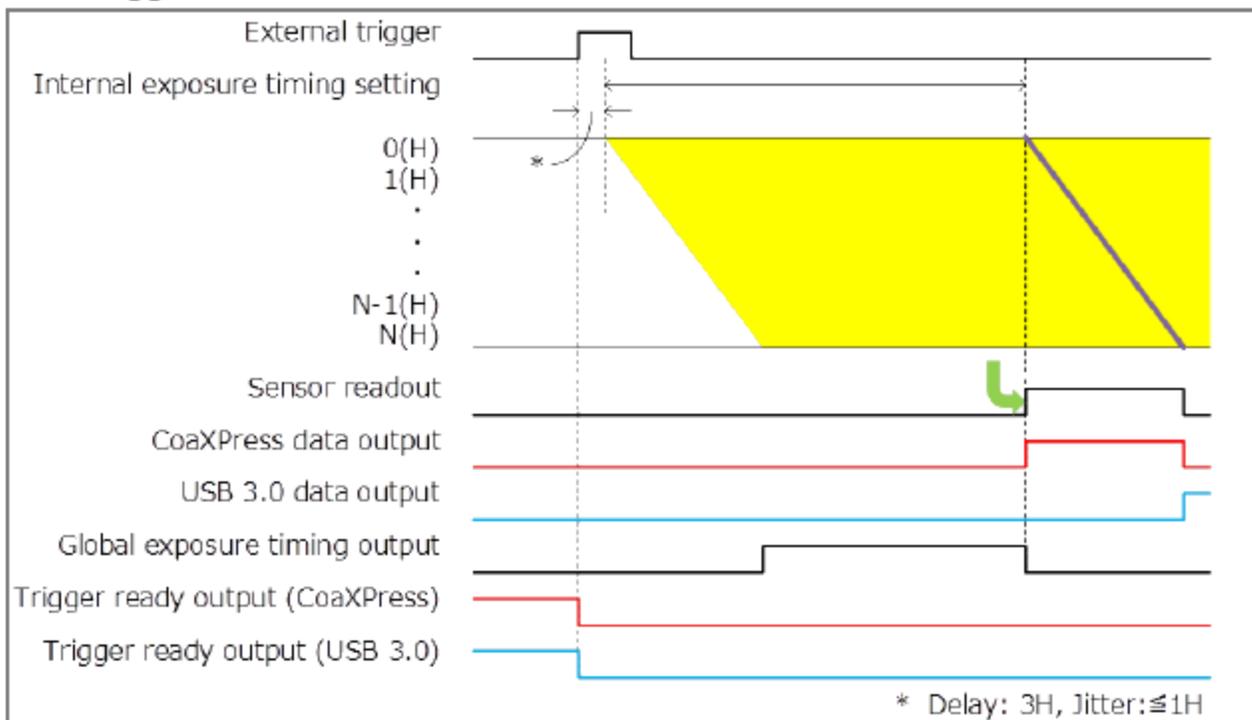
2 Pulse Count
Enter 4 pulses

3 Trigger Delay
Enter optional trigger delay

External Start Trigger

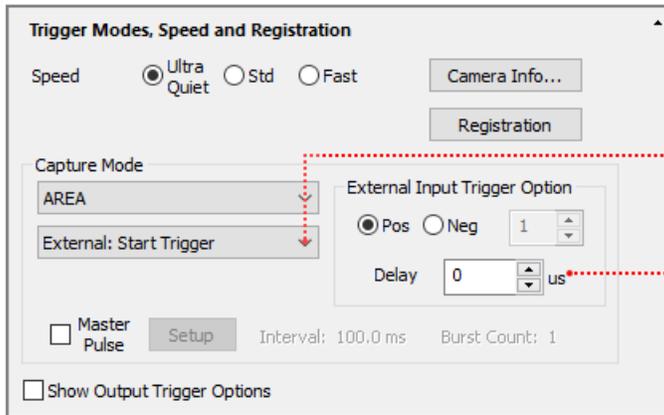
External start trigger mode utilizes a single trigger pulse to start acquiring images. An external signal triggers the start of exposure and then the camera acquires images at max frame rate in internal "free running" mode. This mode is only available when acquiring a single channel.

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.



1 Set Capture Mode
Select External Start Trigger

2 Trigger Delay
Enter optional trigger delay

MASTER PULSE

The ORCA[®]-Fusion 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	Edge	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.

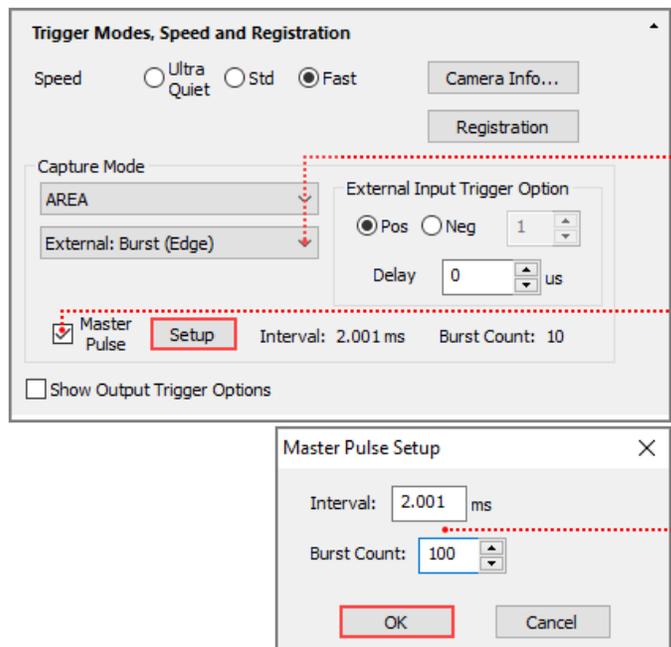
The image shows two screenshots from a software interface. The top screenshot is the 'Trigger Modes, Speed and Registration' panel. It has three radio buttons for Speed: 'Ultra Quiet', 'Std', and 'Fast' (selected). There are buttons for 'Camera Info...' and 'Registration'. Under 'Capture Mode', there are two dropdown menus: the first is set to 'AREA' and the second is set to 'Continuous (Edge)'. To the right, 'External Input Trigger Option' has radio buttons for 'Pos' (selected) and 'Neg', a numeric field set to '1', and a 'Delay' field set to '0' with a unit of 'us'. At the bottom, 'Master Pulse' is checked, and a 'Setup' button is highlighted. The 'Interval' is set to '10.0 ms' and 'Burst Count' is '10'. A checkbox for 'Show Output Trigger Options' is unchecked.

The bottom screenshot is the 'Master Pulse Setup' dialog box. It has an 'Interval' field set to '10.0' with a unit of 'ms' and a 'Burst Count' field set to '10'. There are 'OK' and 'Cancel' buttons at the bottom.

- 1 Set Capture Mode**
Select Internal
- 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

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.



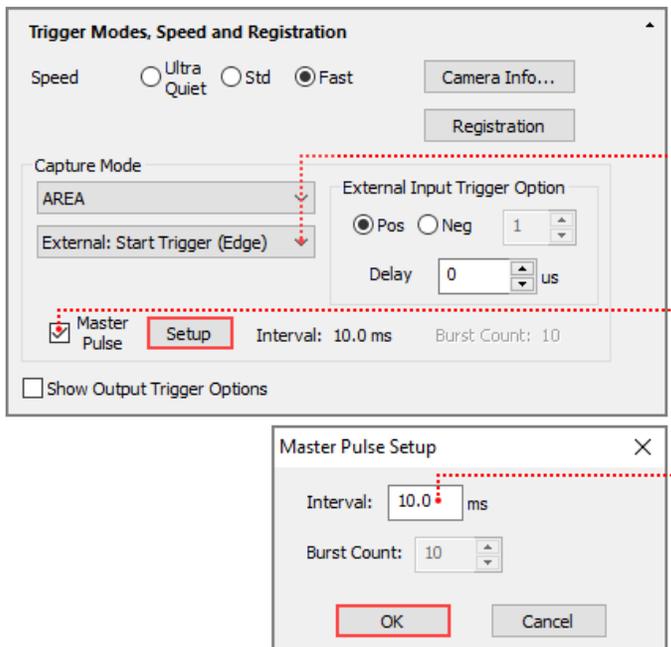
1 Set Capture Mode
Select External Edge Trigger

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.

Timing Output
SMA connectors on the back of the camera

Show Output Trigger Options
Expand output trigger panel

Polarity
Positive or Negative

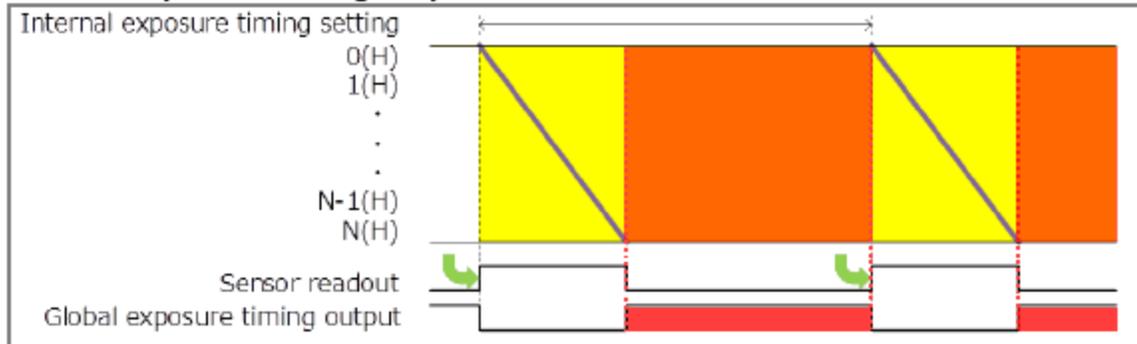
Trigger Output
Three output trigger functions plus a continuous fixed High or Low output

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.

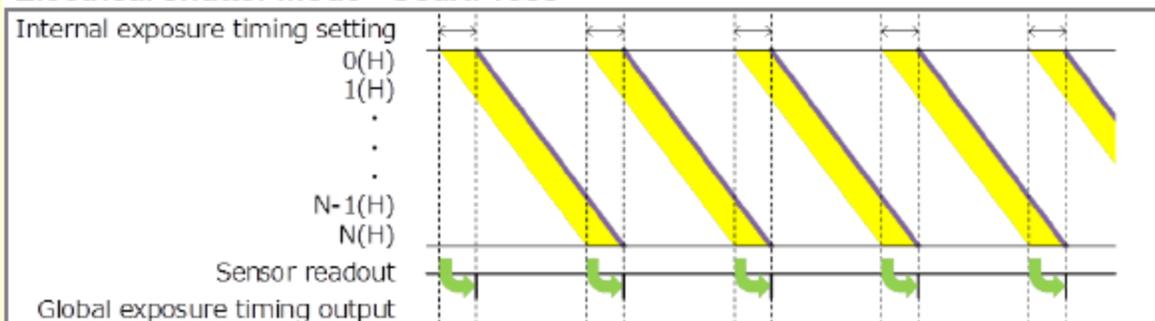
$$\text{Global Exposure Time} = \text{Exposure Time} - \text{Readout Time}$$

Global exposure timing output



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

Electrical shutter mode - CoaXPress



Setup Global Exposure Output Trigger

1 Set Output Port
Select connector 1 from the list

2 Set the Polarity
Select Positive

3 Set Trigger Output
Select Exposure

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 μ s to 10 s, and the range for pulse width is 1 μ s to 10 s (1 μ s steps).

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

1 Timing Output
Select connector 1 from the list

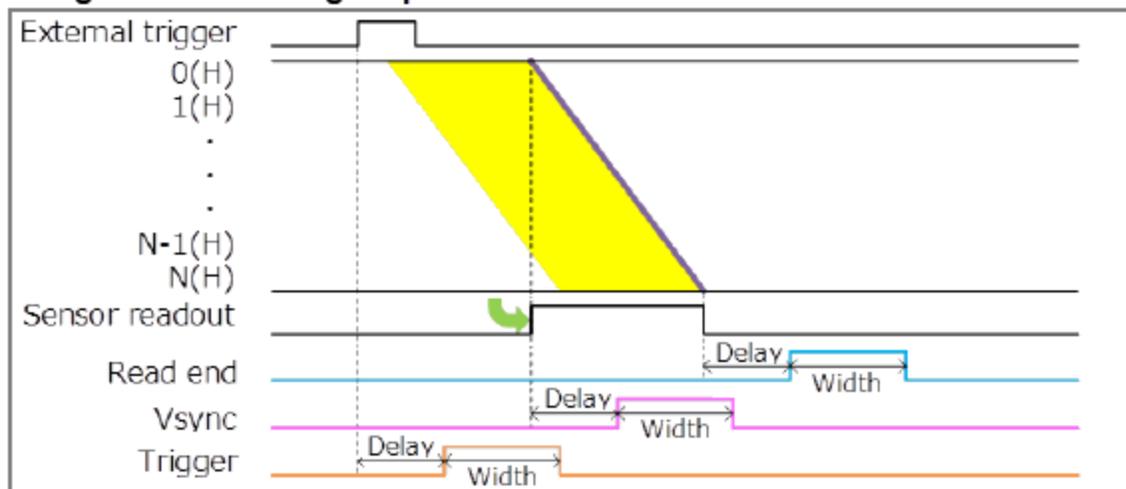
2 Trigger Output
Select Programmable from the list

3 Trigger Options
Configure the:
Pulse Delay
Range 0 μ s to 10 s
Pulse Duration
Range 1 μ s to 10 s
Reference Signal
Determines when the camera outputs a pulse, at the beginning or end of readout

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

- 1 Timing Output**
Select connector 1 from the list
- 2 Trigger Output**
Select Programmable from the list
- 3 Trigger Options**
Configure the:
 - Pulse Delay
Range 0 μ s to 10 s
 - Pulse Duration
Range 1 μ s to 10 s
 - Reference Signal
Determines when the camera outputs a pulse, at the beginning or end of readout

The screenshot shows the software configuration window. On the left, under 'Output Trigger', connector '1' is selected. The 'Kind' is set to 'PROGRAMMABLE'. On the right, the 'Programmable Trigger Option' section shows 'Delay' set to 0 μ s, 'Period' set to 1.0 ms, 'Source' set to 'READOUT END', and 'Pre HSYNC Count' set to 0. Red dotted lines connect the numbered steps to the corresponding UI elements.

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

- 1 Set Output Port**
Select connector 1 from the list
- 2 Set the Polarity**
Select Positive
- 3 Trigger Output**
Select Trigger Ready from the list

The screenshot shows the software configuration window. Under 'Output Trigger', connector '1' is selected. The 'Kind' is set to 'TRIGGER READY'. The 'Pos' radio button is selected under the polarity options. On the right, the 'Programmable Trigger Option' section shows 'Delay' set to 0 μ s, 'Period' set to 1.0 ms, 'Source' set to 'TRIGGER', and 'Pre HSYNC Count' set to 0. Red dotted lines connect the numbered steps to the corresponding UI elements.

GLOBAL RESET

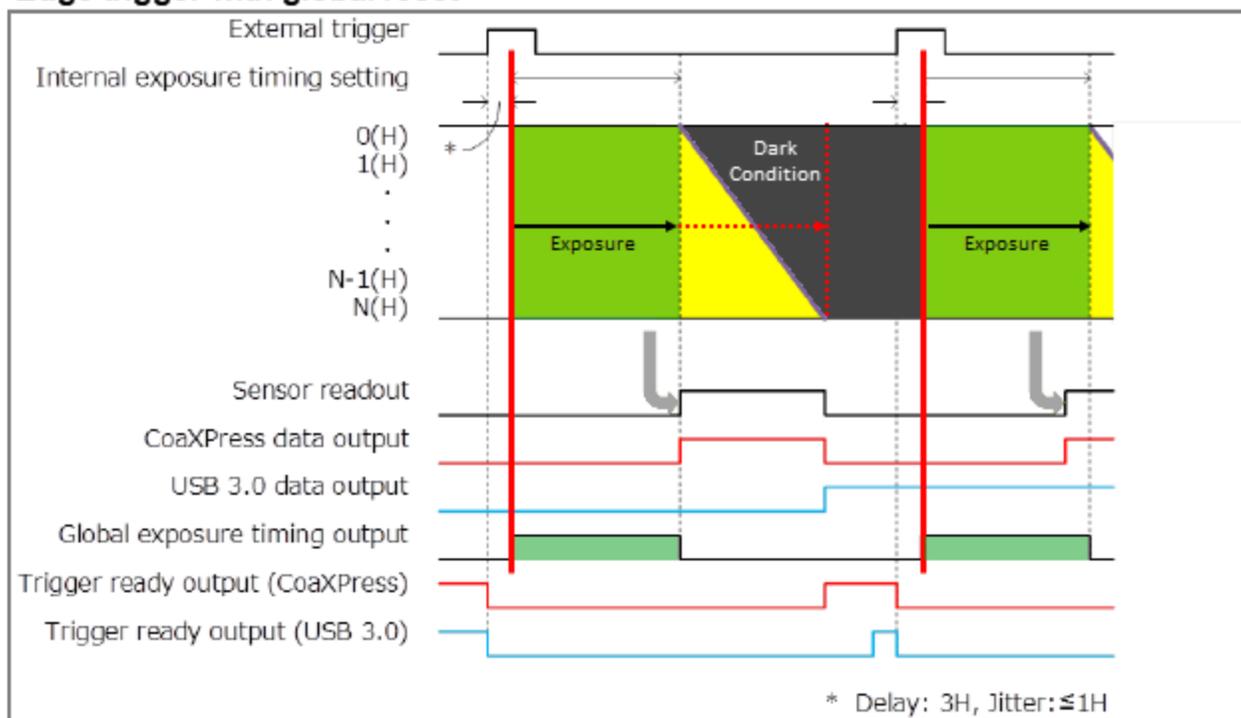
Global reset function is used to reset the electric charge of all of the pixels at the same time, just before acquiring an image. This allows all of the pixels to start exposing at the same time. The first line of the sensor exposes for the exposure time. The subsequent lines expose for the exposure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

Trigger Modes, Speed and Registration

Speed Ultra Quiet Std Fast

Capture Mode

AREA
External: Edge

External Input Trigger Option
 Pos Neg 1
Delay 0 us

Master Pulse Interval: 10.0 ms Burst Count: 1

Show Output Trigger Options

Output Trigger

1 Output Trigger
 Pos Neg
Kind EXPOSURE

Programmable Trigger Option
Delay 0 us
Period 1.0 ms
Source READOUT END
Pre HSYNC Count 0

Advanced Camera Properties

DCAM Properties	
Name	Value
TRIGGER GLOBAL EXPOSURE	GLOBAL RESET <input type="button" value="v"/>
TRIGGER CONNECTOR	BNC
INTERNAL TRIGGER HANDLING	SHORTER EXPOSURE <input type="button" value="v"/>

1 Capture Mode
Select External Edge Trigger from the list

2 Timing Output
Select connector 1 from the list

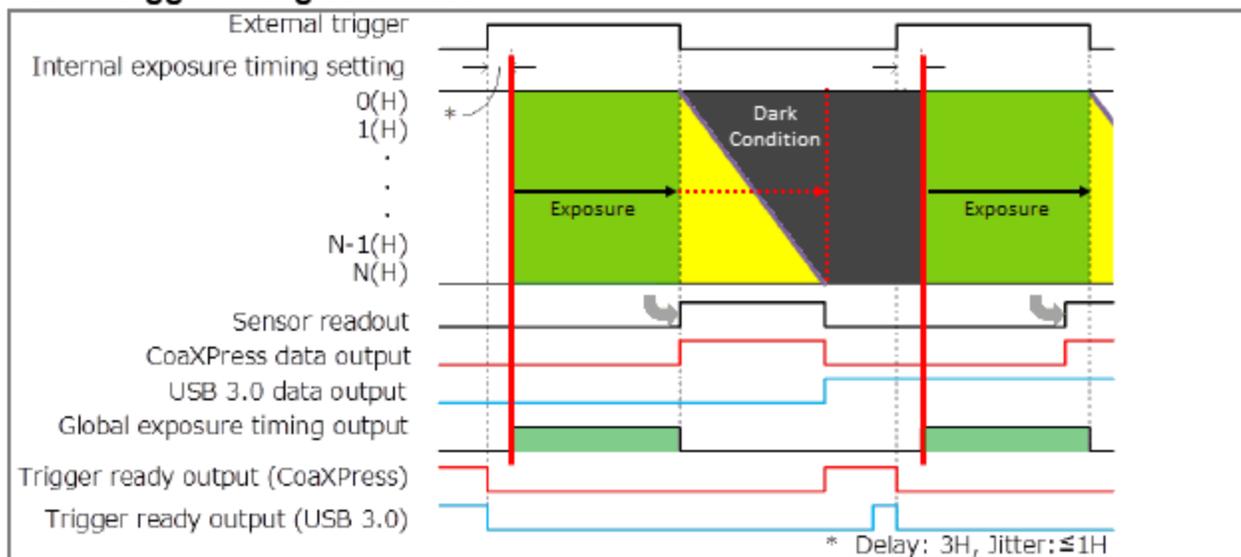
3 Trigger Output
Select Exposure from the list

4 Trigger Global Exposure
Select Global Reset from the list

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 with global reset



Setup Capture Mode for External Level Trigger

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

Trigger Modes, Speed and Registration

Speed Ultra Quiet Std Fast Camera Info...

Registration

Capture Mode
 AREA
 External: Level

External Input Trigger Option
 Pos Neg 1
 Delay 0 us

Master Pulse Setup Interval: 10.0 ms Burst Count: 1

Output Trigger
 1
 Output Trigger
 Pos Neg
 Kind EXPOSURE

Programmable Trigger Option
 Delay 0 us
 Period 1.0 ms
 Source READOUT END
 Pre HSYNC Count 0

Advanced Camera Properties

DCAM Properties	
Name	Value
TRIGGER GLOBAL EXPOSURE	GLOBAL RESET
TRIGGER CONNECTOR	BNC

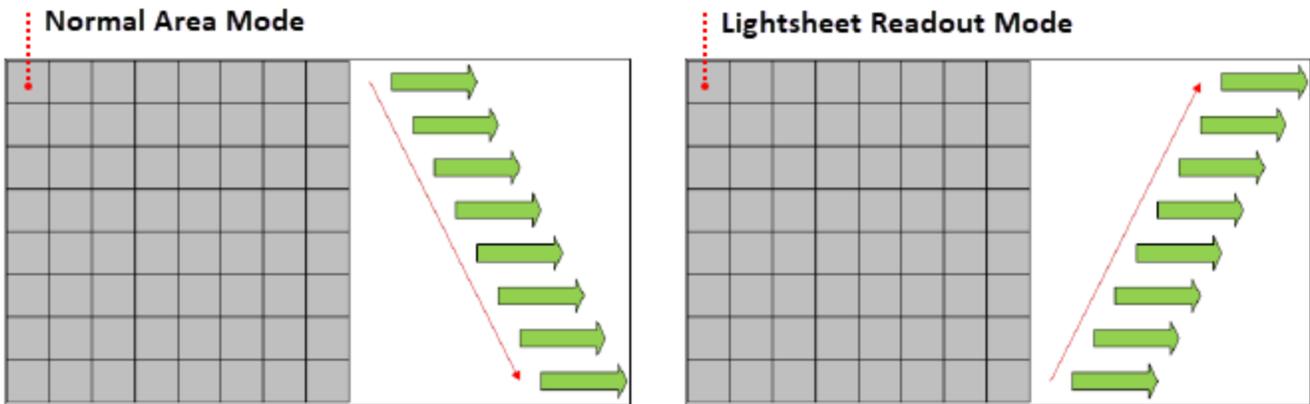
- 1 **Capture Mode**
Select External Level Trigger from the list
- 2 **Timing Output**
Select connector 1 from the list
- 3 **Trigger Output**
Select Exposure from the list
- 4 **Trigger Global Exposure**
Select Global Reset from the list

LIGHTSHEET MODE

Lightsheet 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[®]-Fusion Lightsheet Mode incorporates specific timing features and a unified readout direction allow for this synchronization to occur.

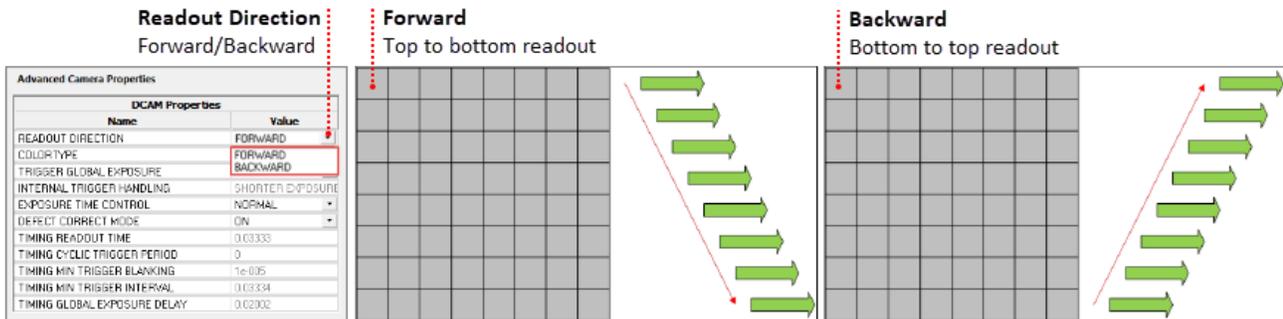
Readout Direction

With normal area mode, the camera readout is from the center line to the top line and to the bottom line simultaneously. With lightsheet 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 lightsheet mode enabled, expand the Advanced Camera Properties panel and under DCAM Properties, select Forward or Backward from Readout Direction list.

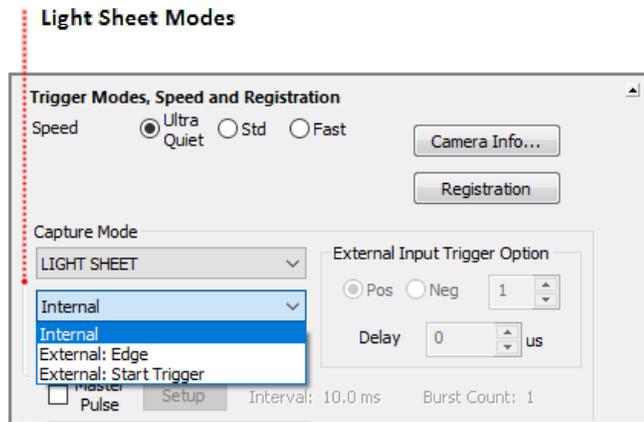


The size and position of the sub-array can be configured according to the table below.

Size		Position	
Horizontal	Vertical	Horizontal	Vertical
1 pixel	4 lines	1 pixel	4 lines

Lightsheet Capture Modes

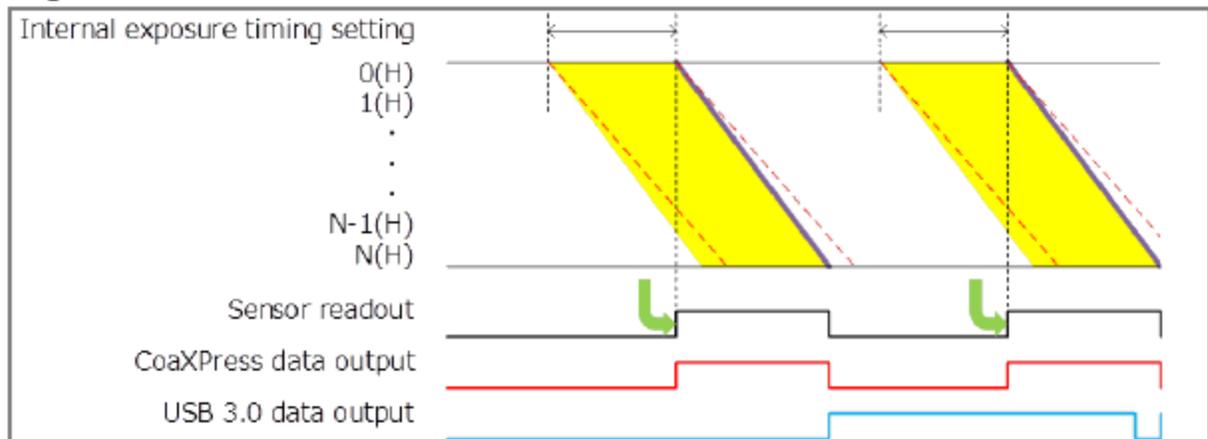
Lightsheet Modes are accessible from the Capture Mode list in the Trigger Modes, Speed and Registration panel shown below. The ORCA[®]-Fusion supports three modes for lightsheet microscopy as described below. Along with each description is a basic set of steps for enabling that particular lightsheet mode in HCImage.



Internal "Free Running" Mode

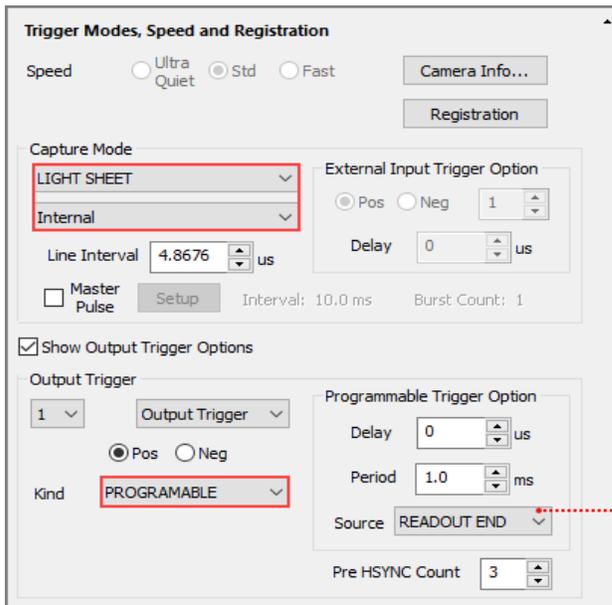
Synchronization is determined by the empirically matching the rate of the sweep of the light sheet and the camera readout to each other. There is no hardware or software triggering involved.

Lightsheet internal mode



How to Setup Light Sheet Internal Mode

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



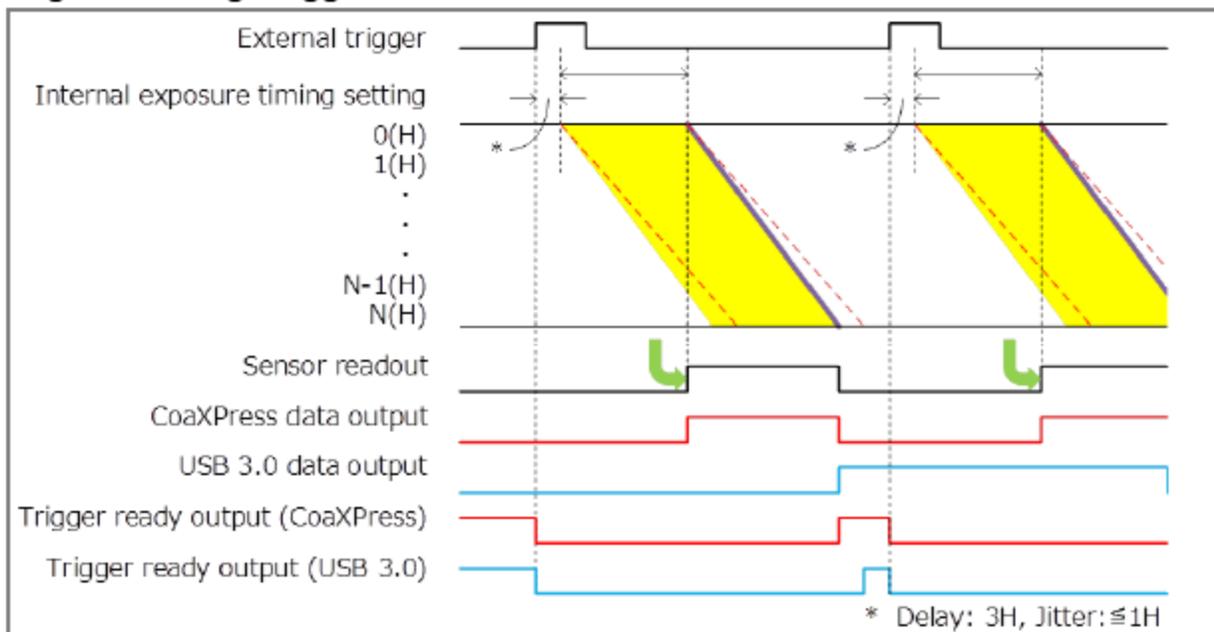
- 1 **Capture Mode**
Select Light Sheet and Internal from the list
- 2 **Timing Output**
Select connector 1 from the list
- 3 **Trigger Output**
Select Programmable from the list
- 4 **Trigger Options**
Select Readout End from the list
Configure the:
Pulse Delay
Range 0 μ s to 10 s
Pulse Duration
Range 1 μ s to 10 s

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 lightsheet synchronization.

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

Lightsheet edge trigger mode



Setup Lightsheet External "Edge" Trigger Mode

Follow the steps in Part 1 below in RED, to enable External Lightsheet 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.

Trigger Modes, Speed and Registration

Speed Ultra Quiet Std Fast

Capture Mode

LIGHT SHEET External: Edge

Line Interval 4.8676 us

Master Pulse Interval: 10.0 ms Burst Count: 1

External Input Trigger Option

Pos Neg 1

Delay 0 us

Output Trigger

1 Output Trigger

Pos Neg

Kind TRIGGER READY

Programmable Trigger Option

Delay 0 us

Period 1.0 ms

Source HSYNC

Pre HSYNC Count 0

Output Trigger

2 Output Trigger

Pos Neg

Kind PROGRAMMABLE

Programmable Trigger Option

Delay 0 us

Period 1.0 ms

Source HSYNC

Pre HSYNC Count 0

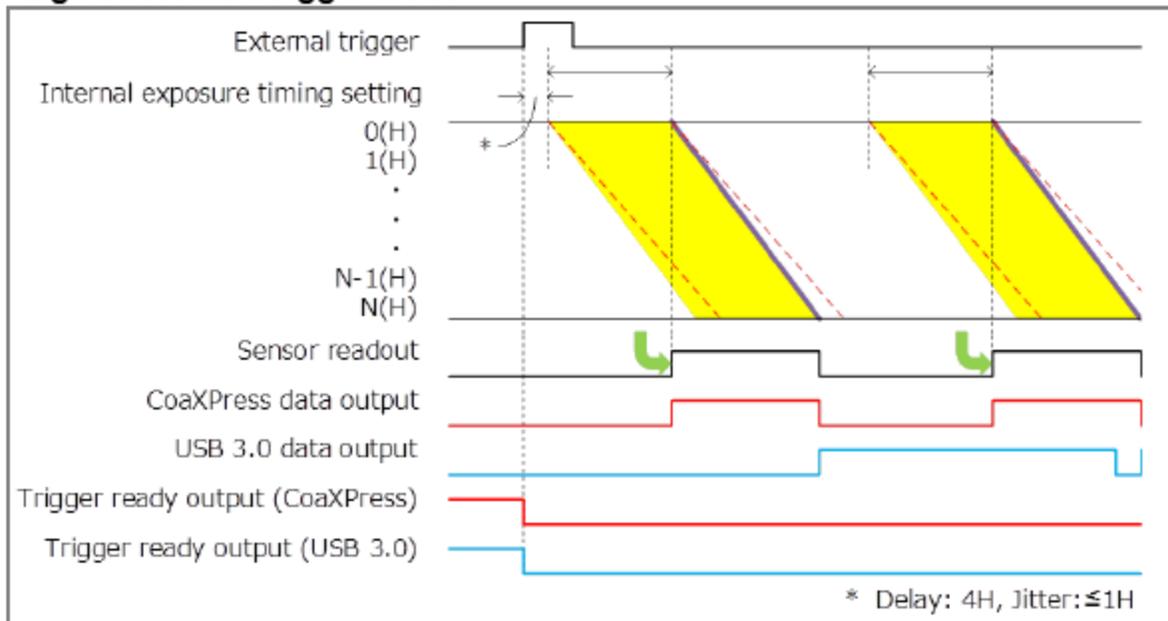
- 1 Capture Mode**
Select External Light Sheet from the list
- 2 Timing Output**
Select connector 1 from the list
- 3 Trigger Output**
Select Trigger Ready from the list
- 1 Timing Output**
Select connector 2 from the list
- 2 Trigger Output**
Select Programmable from the list
- 3 Trigger Options**
Select Hsync from the list
Configure the:
Pulse Delay
Range 0 μ s to 10 s
Pulse Duration
Range 1 μ s 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.

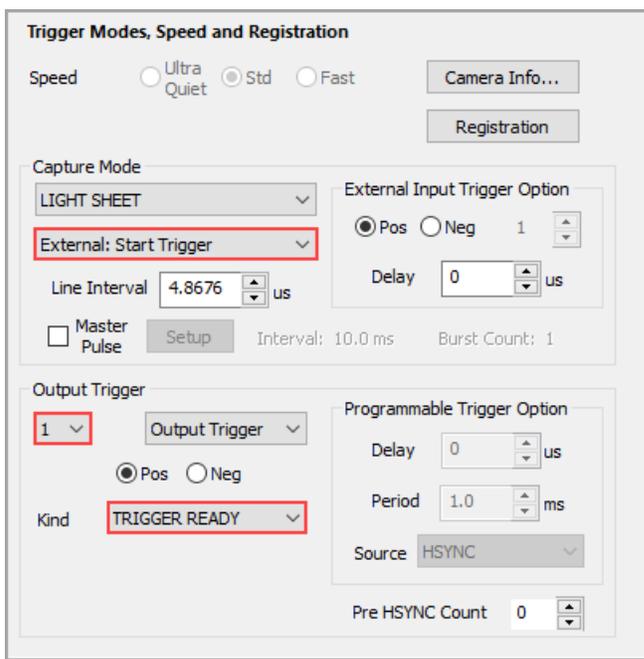
Note: Lightsheet External Start Trigger Mode is only available when acquiring a single channel.

Lightsheet start trigger mode



Setup Lightsheet External Start Trigger Mode

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

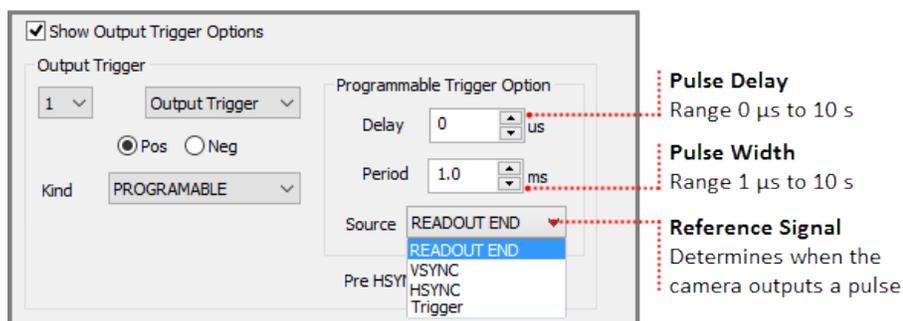


- 1 Capture Mode**
Select External Start Trigger Light Sheet from the list
- 2 Timing Output**
Select connector 1 from the list
- 3 Trigger Output**
Select Trigger Ready from the list

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, Input trigger signal or Hsync. The range of delay is 0 μ s to 10 s, and the range of pulse width is 1 μ s to 10 s (1 μ s steps).

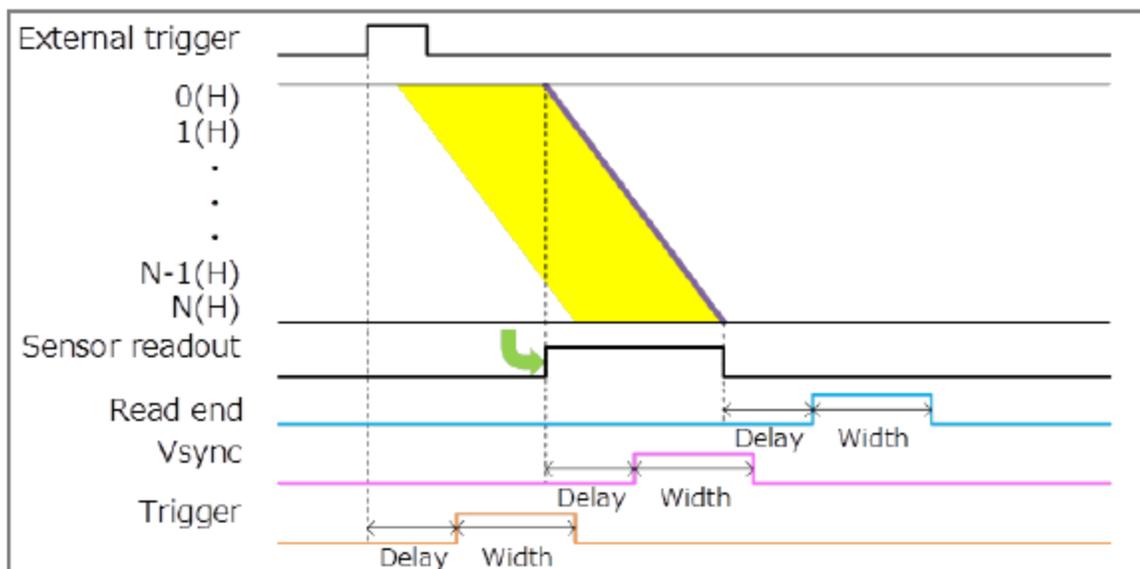
Note: In Lightsheet mode, programmable timing out can output an external trigger every line (Hsync) and frame (Readout End, Vsync, Input trigger signal). The camera can be a master to control a stage and light source.



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

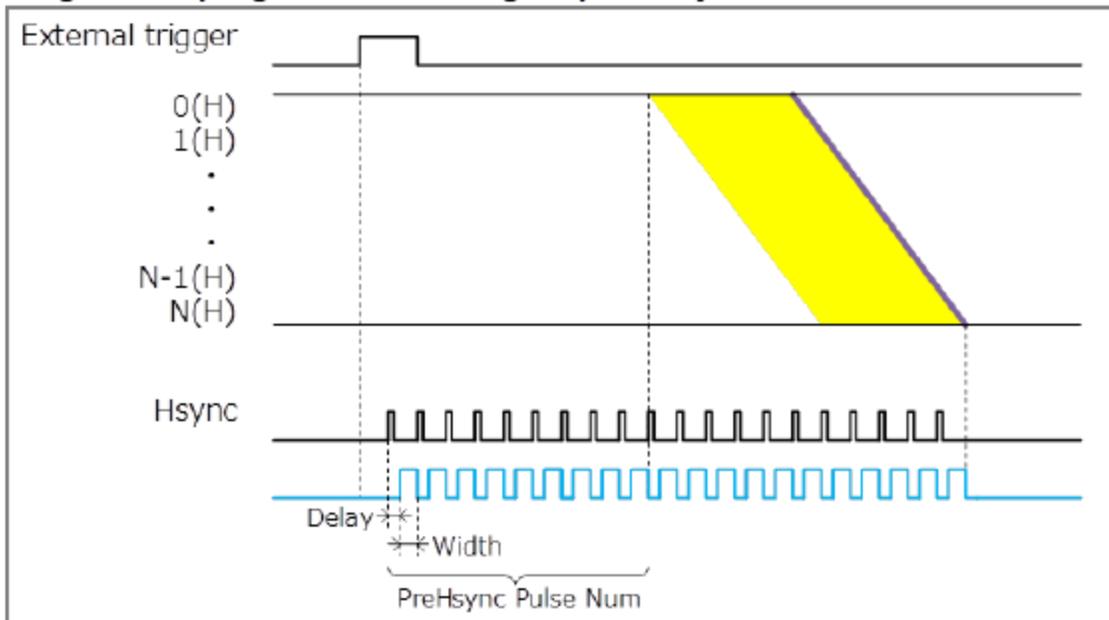
Reference Signal	Output Signal
Readout End	Camera outputs a pulse after certain delay from the end of sensor readout for each frame.
Vsync	Camera outputs a pulse after certain delay from the beginning of readout for each frame.
Trigger	Camera outputs a pulse after a certain delay, from the master pulse.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

Lightsheet programmable timing output



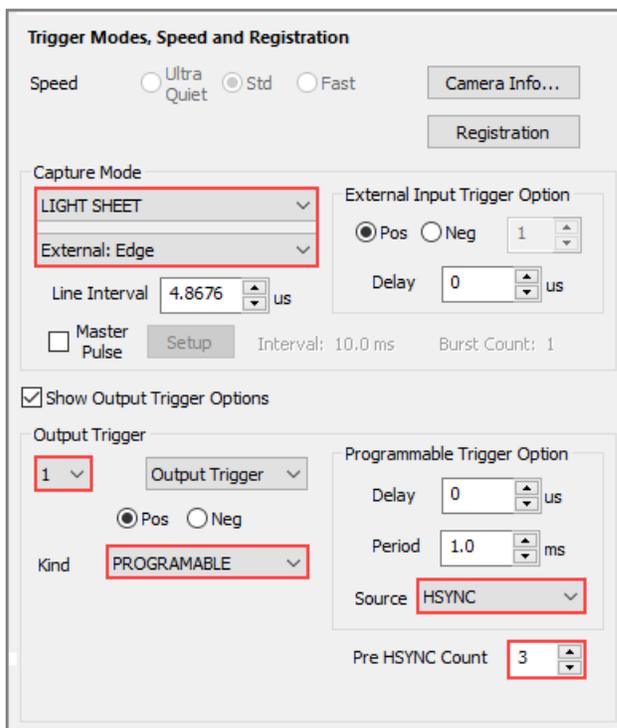
When you choose Hsync for the reference of programmable timing output, the camera can output a number of pulses before the start of exposure. This is called Pre-Hsync.

Lightsheet programmable timing output - Hsync



How to Setup Pre-Hsync Pulses

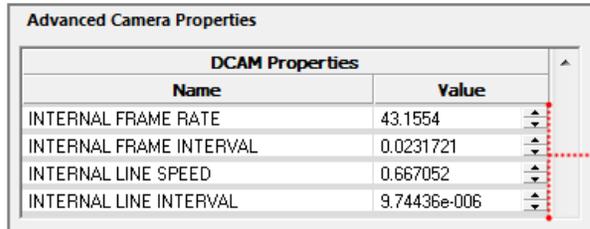
Follow the steps below to enable External Lightsheet Mode and then to have the camera output a number of Pre-Hsync pulses prior to the start of exposure, as well as, send an external trigger for every line.



- 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 μ s to 10 s
Pulse Duration
Range 1 μ s 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 lightsheet 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.



DCAM Properties	
Name	Value
INTERNAL FRAME RATE	43.1554
INTERNAL FRAME INTERVAL	0.0231721
INTERNAL LINE SPEED	0.667052
INTERNAL LINE INTERVAL	9.74436e-006

Internal Timing Settings

For synchronizing the image acquisition with the movement of the light