Synchronization and Triggering with the ORCA-Flash4.0 Scientific CMOS Camera

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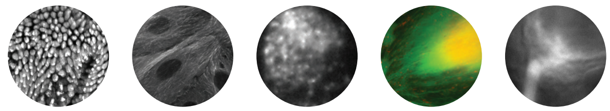


Introduction

CMOS cameras have changed what is possible when imaging fast microscopic events. Speeds that were not realistic in the very recent past are now routinely attainable. These new speeds arise in part because there is a fundamental difference between the sensor architecture of CMOS and CCDs which leads to a difference in how the pixels are "read out." On its own the camera can achieve maximum speeds without the need for triggering. But when the camera is used on a microscope, the need for coordinated and precise timing of the entire system becomes apparent quickly. Maximum speed performance is only possible when synchronization and triggering are appropriately implemented, and understanding how this new technology communicates with other essential parts of the imaging hardware is the first step in achieving this goal.

Users are always asking us how fast they can run their sCMOS cameras. It is a difficult question to answer. Not because it is difficult to do but because there are many user-specific variables to consider. In this paper we will discuss several common microscopy situations and how to configure our ORCA-Flash4.0 camera to get the best image quality and fastest frame rates possible.

Basic CCD Readout Process

First we have to consider how image sensor readout works and then examine how CCD and CMOS sensors differ in the way they read out an image. Every image sensor is composed of pixels, discrete regions of the sensor that collect photoelectrons during an exposure. The readout of an image sensor converts each pixel's photoelectrons to a voltage and then to a digital number (i.e., grey level) that when mapped to a digital file results in the image we see on our computer monitor. For a CCD, this readout process is achieved serially. The data from each pixel is passed through a single readout amplifier which converts electrons to a voltage, then through an A/D converter and finally is transferred as digital data to the computer through the interface cable and frame grabber. In the case of interline CCDs (historically the most common CCD used in microscopy) a pixel structure with a duplicate electron charge storage region enables the reading of one image during the exposure of the next. A frame is exposed, the charge from that frame is moved directly to the storage region, readout begins and at the same time the next exposure is started. The exposure time for all pixels starts and ends concurrently and this is typically referred to as global exposure or "snap shot" acquisition.

For the short integration times used in high speed imaging the frame rates are limited by the total readout time ($T_{readout}$). The maximum frame rate possible using this scheme is calculated as 1/total readout time. So for a camera with a readout time of 10 ms, the frame rate is 100 frames per second. Readout time should not be confused with exposure time ($T_{exposure}$), which is the time when the sensor is actively collecting photons. When $T_{exposure} > T_{readout}$, then frame rates are 1/ $T_{exposure}$. Another way to think about this is if your exposure time is 100 ms on a camera that has a 10 ms readout time, your frame rate is limited by the exposure time, 100 ms in this case. As you'll see below, there are triggering methods that depend on whether $T_{exposure}$ is less than or greater than $T_{readout}$.

CMOS Readout Process

For active pixel CMOS image sensors, each pixel has its own amplifier and readout circuitry. This structure permits parallel readout of many pixels at the same time (multiplexing), often grouped in columns. But unlike CCDs or even some other CMOS, the ORCA-Flash4.0 sensor does not have an on-chip region to store charge so pixels must be read out immediately after the exposure. To manage this, there is a rolling wave of line by line readout, with each successive line shifted in time. This readout process is designated “rolling shutter.”

Rolling Shutter

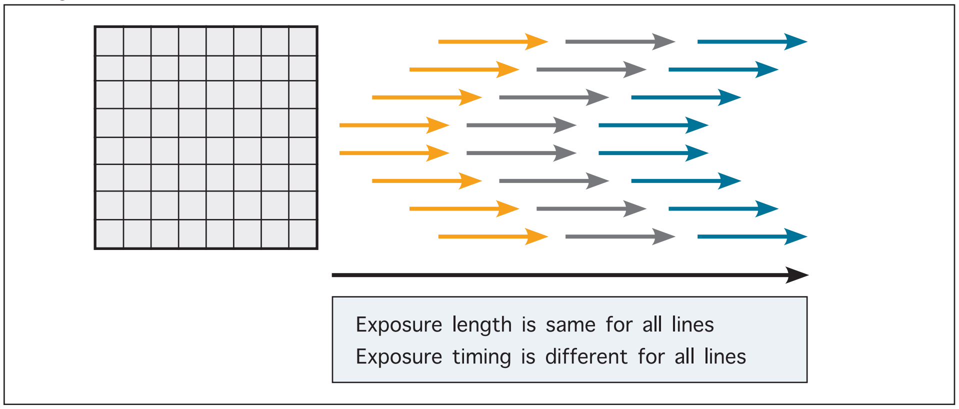
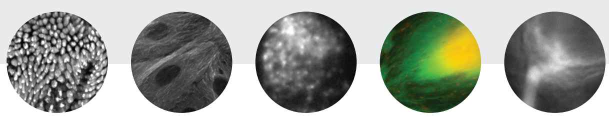


Figure 10-2 Readout timing of Rolling Shutter

Figure 10-2 shows the line by line temporal shift of rolling shutter CMOS readout. A common misconception is that this temporal shift routinely causes significant image distortion. This is not the case, and in fact the high speed nature of rolling shutter CMOS can be used to prevent the more common problem associated with moving samples, namely, image blur. This topic is discussed in detail in other publications.[2]



Understanding the temporal shift of rolling shutter is not difficult. As a starting point we should be clear that each pixel has an equal exposure time. However, the start and end of the exposure for each sequential line is delayed relative to the previous line. In the case of the ORCA-Flash4.0 this delay is about 10 microseconds[1] and over a region of 1000 lines adds up to only 10 milliseconds. For stationary, slow moving, or even fast moving objects in a localized region this effect will not be measurable or significant. For the few experiments in which a delay between lines matters there are two ways of achieving snap-shot acquisition on a CMOS sensor depending on the sensor design: global shutter readout mode or global exposure synchronization. Global shutter readout mode is enabled by the sensor architecture which requires each pixel to have an additional transistor. This pixel design results in a lower quantum efficiency of the sensor, increases the readout noise by a factor of about 1.6 and increases the dark current. Furthermore it is necessary to acquire a reference frame for each image frame, thereby reducing the frame rate by a factor of two. The ORCA-Flash4.0 does not use this type of sensor architecture.

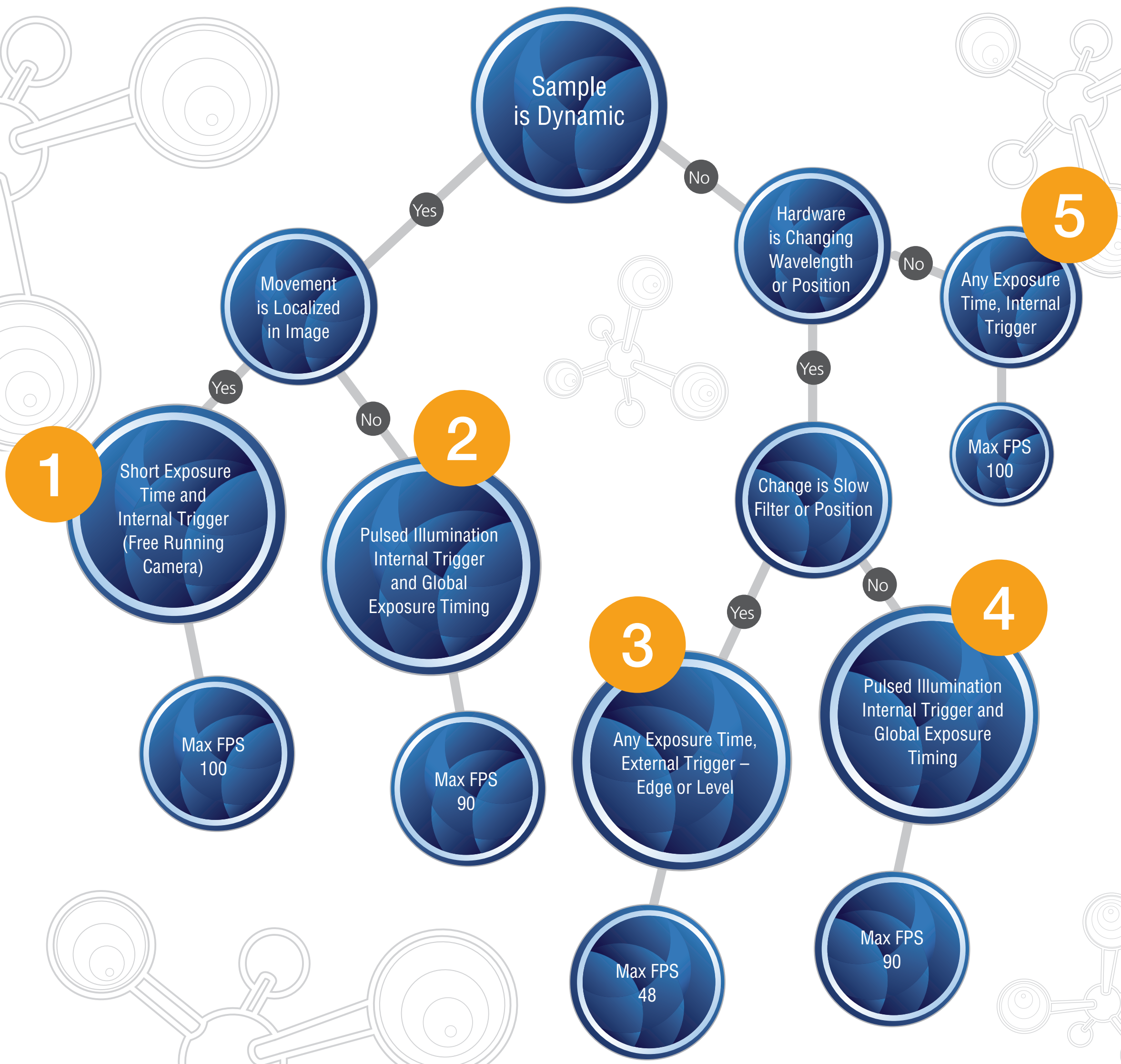
Global exposure synchronization is a method that uses the rolling shutter readout mode with a slightly expanded exposure time, resulting in a slice of time during which all the lines of the sensor are being exposed simultaneously, and in this way emulates a global shutter CMOS. The key to using this method is a pulsed light source or fast external shutter to control the light source. The illumination is turned on only during the time when all of the lines are exposing and is turned off during the rolling shutter readout. The advantages of using this method are the ability to maintain the low readout noise, low dark current, high quantum efficiency (QE), and the high frame rates of rolling shutter.[3] Global exposure synchronization is the method used in the ORCA-Flash4.0.

What Is Triggering and Why Are There So Many Modes?

Triggering is the mechanism by which the camera, software and hardware communicate to get all the components synchronized for an exposure or series of exposures. For any camera, whether it is a CCD, sCMOS, or EM-CCD the triggering method must be considered to achieve maximum frame rates and to coordinate the movement of shutters, filter wheels, stages and z-motors. Thankfully, almost all of the triggering detail is handled behind the scenes in your imaging software.

For all triggering schemes it is necessary to decide if the camera or some other piece of equipment controls the timing. When the camera is the master (controls the timing), it outputs a signal and can be used in two unique modes: free running or global exposure synchronization. When the camera is a slave, it receives an external input signal and can be used in one of three modes: edge, level or start trigger. The fundamental decision point is always, "Does the illumination change in a time shorter than readout?" Each triggering mode is described briefly below and the details of the modes can be found in the camera manual. Every triggering mode has different capabilities allowing for optimal synchronization of microscope-related hardware and tailors the overall image capture protocol to the requirements of both the sample and the expected analysis.

Decision Tree to Visualize Five Types of Time Sensitive Experiments Using the ORCA-Flash4.0

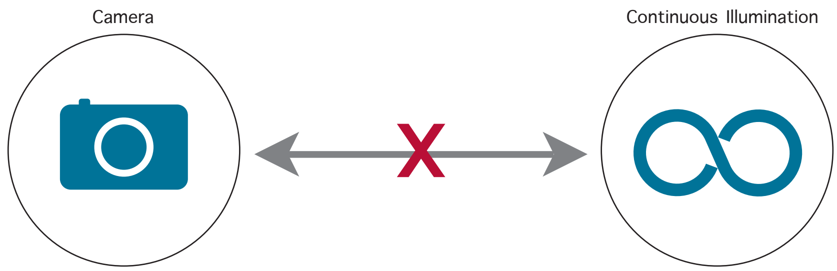


Maximum frame rates are shown for full resolution (2048 x 2048) images on an ORCA-Flash4.0 V2 using the Camera Link data transfer interface and a 1 ms exposure. In experiment 3, a hardware change of 10 ms was used. In experiments 2 and 4, a minimum Global Exposure Timing setting of 10 microseconds is possible which would result in a maximum rate of 99.9 FPS.

Triggering Scenarios: From an Application Point of View

1 Time Sensitive Example → Sample Is Moving → Movement Is Localized

When we think of beating cilia we think “fast.” The analysis of cilia motion is an example of an experiment that benefits from the fast speeds of CMOS camera technology. While the cilia themselves move very fast, they are attached to a cell which is effectively localized. The position of each individual cilium changes from one frame to another but the cell that it is attached to remains in a relatively fixed position across all frames. The exposure time is kept short to stop the beating motion and often a region of interest is used in order to increase the frame rate. There is no need for triggering because the illumination is continuous and the exposure time is short, on the order of 1 ms or less.



No communication between the camera and illumination source. Both devices are operating independently.

No Triggering Required:

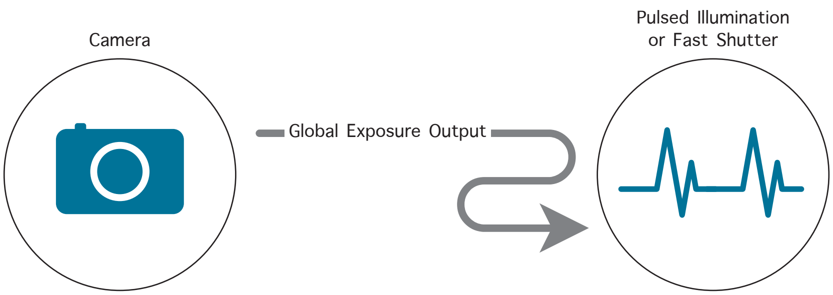
In this example the camera is “free running” using internal triggering, producing images as fast as the exposure and readout time will allow. Images are “pushed” to the data acquisition software at maximum frame rate.

2 Time Sensitive Example → Sample Is Moving → Movement Is ***Not*** Localized

Blood flow experiments present a unique set of challenges that are easily overcome with simple triggering. Unlike the cilia in the previous example these blood cells are not localized. They flow, sometimes very rapidly, through the lumen of a vein or artery and can move across the entire field of view. In order to accurately measure their velocity we need to know their exact locations in subsequent frames. Precise and repeatable exposure times and exposure intervals are required. Using triggering to control the illumination source's pulse duration and repetition rate makes this possible.

For these experiments a global exposure or "snap shot" acquisition is appropriate. With pulsed illumination (or a fast external shutter) and the Global Exposure Synchronization function, every pixel on the sensor can be illuminated simultaneously to produce this snap shot. However, as detailed below, there is one caveat to this scheme which turns out to be easily addressed by using "global exposure timing" and a trigger cable.

At very short exposure times a rolling shutter with pulsed illumination can produce an artifact that presents itself as a "banding" in the image. The problem is that some lines of the sensor might be exposed when the illumination is on and others when it is off. The solution is to invoke the camera's global exposure timing mode in the imaging software and by doing so trigger the illumination with perfect synchronization. The camera exposure is seamlessly extended to be slightly longer than the readout and the illumination occurs when all the lines of the sensor are in integration mode. The maximum frame rate is reduced by just the time required for the illumination pulse width plus a small margin for safety.



The camera sends a trigger signal to the shutter or pulsed illumination.

The Camera Runs the Experiment:

In this example the camera is the master and images are "pushed" to the data acquisition software at maximum frame rate. A Global Exposure Timing Output signal is required to synchronize the pulsed illumination source.

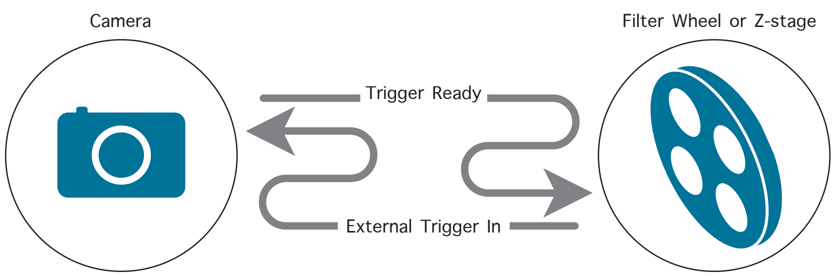
3 Time Sensitive Example → Sample Is Not Moving → Hardware Is Changing Wavelength or Position → Hardware Change Is Slow

Now we'll slow things down a bit. Many experiments have samples which don't move but need to be imaged repeatedly under different conditions. Maybe a filter wheel needs to be moved or a stage needs to go to a different z-position or possibly some combination of these during a time lapse. The combinations seem limitless. But one thing doesn't vary: they all have an inherent need for coordinating hardware movement and image capture. So even for slow experiments triggering can be essential.

Key to these types of experiments is that the camera doesn't capture images while the hardware is still moving. Exposure during hardware movement can degrade the images in many ways including combining signals that should have been discrete in co-localization experiments or causing blur during a z-series acquisition. The most precise way to avoid these kinds of problems is by two-way communication between the microscope hardware and the camera, enabling each component to be "aware" of when the other has completed its task. The devices are connected by trigger cables, and the user invokes "external trigger mode" in their imaging software and starts image acquisition.

A good example of this scheme at work is a time lapse experiment. These are most often run in software control mode with the software as the master and all other hardware, camera included, as slaves. Here's an overview of how one of these experiments might run. The imaging software instructs a filter wheel to move to a user-defined position. The filter wheel does as instructed and then sends a signal to the camera indicating that movement has stopped and it's OK to take an image. The camera starts an image acquisition. The filter wheel remains idle until the camera signals that the image has been captured and the filter wheel is now free to move. The next filter is moved into position and the process repeats.

There are several additional ways of configuring these camera/hardware interactions which are detailed in the camera user's manual.

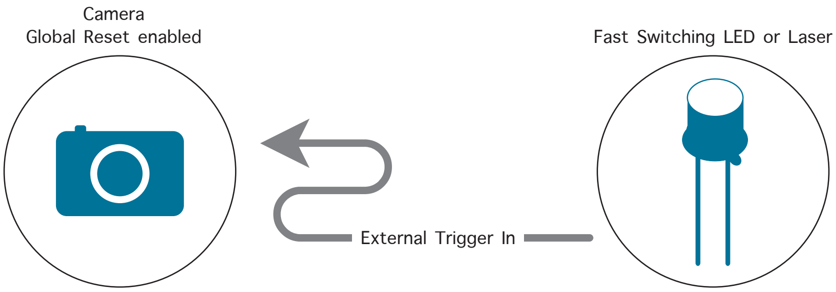


The filter wheel or z-motor tells the camera that it has stopped moving.
The camera takes an image and tells the filter wheel or stage the image is captured and it is OK to move to the next position.

4 Time Sensitive Example → Sample Is Not Moving → Hardware Is Changing Wavelength or Position → Change Is Fast

For an increasing number of fluorescence co-localization experiments the wavelength switching requirements are measured in milliseconds. Fast switching lasers and LED light sources are a great match for these needs and by now you likely realize that triggering and synchronization between the camera and these types of illuminators is critical. Without this communication and with illumination times that have durations which are less than the readout time of the camera it is probable that a subset of the lines in the image would not be exposed. Once again "banding" in the image could be problematic.

Just as illustrated in example two above (sample is moving --> movement is not localized), global exposure timing will create "snap shot" images. Using this scheme, lasers and fast LEDs are integrated with the ORCA-Flash4.0. Such paradigms are becoming increasingly common and global exposure synchronization usually provides the best SNR while maintaining speed and resolution.



The pulsed illuminator triggers the camera to start acquiring an image and shines light on the sample only during the period when all lines on the sensor are available to be exposed simultaneously.

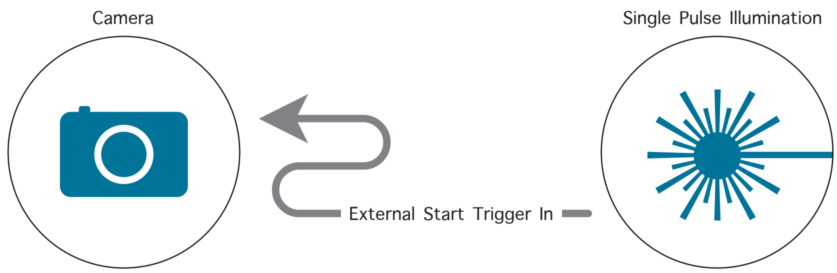
The Light Source Is in Charge:

Unlike in the blood flow experiment above, in this example the light source is the master. Key to this scheme is that global exposure timing is achieved using an external trigger signal from the illuminator to the camera. The global reset function is invoked in software before starting image acquisition. Now the global exposure period of the camera is synchronized to the pulse of the light source, providing a sequence of "snap shot" images.

5 Time Sensitive Example → Sample Is Not Moving → Hardware Is *Not* Changing → Intensity Is Changing

In FRAP (Fluorescence Recovery After Photo-bleaching) experiments there is a bleaching event immediately followed by fast image acquisition to measure the sample intensity as proteins in the cell migrate and fluorescence recovers. The sample is not moving and there is typically no additional hardware movement during the image acquisition period. Because exposure times are kept short the maximum frame rate is limited only by the readout time for the image.

The requirement for triggering is simple: tell the camera to start image acquisition at the instant of the bleaching event. From that point on images are collected until fluorescence recovery is completed. So in this example the camera is triggered by the illumination device via a trigger cable to start the capture sequence.



The illumination device triggers the camera to start image acquisition.

A Special Synchronization Case: Light Sheet Microscopy

Advances in Light Sheet Microscopy (sometimes called Selective Plane Illumination Microscopy or "SPIM") are moving forward quickly. Individual cells, networks of neurons and whole organisms are being visualized and characterized with unprecedented detail. Upright, inverted, even vertical microscopes that utilize illumination techniques including Bessel beams and asynchronous bi-directional illumination are being developed with increasing frequency. Even with their unique design properties, all of these systems have two things in common: sweeping light across the sample and the need to synchronize that movement with image acquisition. Using the ORCA-Flash4.0 V2's "Light Sheet Mode" is Hamamatsu's solution for this need.

In all other readout modes of the ORCA-Flash4.0 V2 the sensor is read from the vertical center line in two directions, simultaneously. When Light Sheet Mode is invoked the sensor is read continuously in one direction, from top to bottom or bottom to top. This facilitates synchronizing the sweep of the light sheet and the readout of the sensor. While synchronization is improved, the trade-off is that frame rates are decreased by roughly 50%. This is a result of the unidirectional nature of the readout. Subarray readout can be applied the same as in normal operation to increase frame rates.

There are three ways to run the camera in Light Sheet Mode: internal (with no triggering between the light sheet and camera), and edge trigger or start trigger, both of which provide synchronization between the light sheet and camera.

With internal or "free running" operation the synchronization is determined by empirically matching the rate of sweep of the light sheet and the camera readout to each other. There is no hardware or software triggering involved.

During "edge trigger" operation, an external device (e.g., from the illumination system of the microscope) sends a signal via a cable to the camera at the start of each image frame. This provides synchronization between the readout of the camera and each subsequent sweep of the light sheet. This method provides the most control over camera/light sheet synchronization.

When using "start trigger" operation, an external device sends a signal via a cable to more precisely time the beginning of image acquisition to the sweep of the light sheet. As in "free running" operation the sweep of the light sheet and the readout of the camera need to have been previously matched empirically.

Basic Trigger Methods: From the Camera's Point of View

The following section is intended to be a general reference for all the types of triggering possible with the ORCA-Flash4.0. The descriptions are not exhaustive but should give a basic idea of when and how they are used. Unlike in the section above, they are not listed by application type but organized by where the timing originates: internally by camera or externally from another device. Each of these two categories can then be compartmentalized into "Exposure Time Shorter or Longer than Readout." More detail is available in the camera manual and Hamamatsu has experts who can assist you in planning a triggering scheme for your application.

Internal Trigger Timing

When using internal trigger timing the camera does not receive any timing signals from external sources. The camera runs at the maximum frame rate allowed which is determined by the readout time or exposure time, whichever is larger. If timing output signals from the camera are used to drive other devices such as shutters or filter changers then the camera is considered to be the "master."

Internal Trigger: Exposure Time Is Longer Than Readout Time

Continuous Illumination

In this case, under continuous illumination, the effect of rolling shutter would be to create a time difference between the start of exposure of the first lines and the start of exposure of the last lines even though all lines have the same elapsed exposure time. As the exposure time increases, the readout time becomes a relatively smaller percentage of the overall cycle and the time difference becomes less significant. Consider a readout time of 10 ms on a 1000-line sensor with an exposure time of 100 ms. Only 10% of the exposure time of the first line does not coincide with the exposure time of the last line, and the percentage difference is as little as 0.01% for adjacent lines. The start of exposure of the first line of the subsequent frame overlaps the end of exposure of the last line of the first frame by the readout time.

Pulsed Illumination

When pulsed illumination is used with internal triggering, the pulsed illumination should be synchronized to the camera via a trigger cable using the "Global Exposure Timing Output" of the camera. This will send a signal to the illumination source that coincides with the time when all the lines are exposing. The sample should be dark between illumination pulses.

Internal Trigger: Exposure Time Is Shorter Than Readout Time

Continuous Illumination with Stationary or Localized Moving Sample

In this case, under continuous illumination, the effect of the rolling shutter would be to create a time difference between the start and end of exposure of the first lines and the last lines, even though all lines have the same elapsed exposure time. If the movement of the sample is localized this effect is minimal. And because you would be comparing the same x-y position from frame to frame (and not from line to line), rolling shutter is not a factor.

Pulsed Illumination

Pulsed illumination is not recommended when using internal trigger and the exposure is shorter than the readout as it can result in an image artifact. If you see horizontal banding in your image, it is worth checking to make sure your illumination and triggering schemes are compatible.

External Trigger Timing

In external trigger timing mode the camera receives timing signals via a trigger cable from external sources. The camera runs at a frame rate determined by the time between trigger inputs, but not less than the exposure time plus the readout time. This mode is implemented by using one of the three options listed below.

External Trigger Input: External Edge

As the voltage sent via trigger cable changes state (e.g., low to high or high to low), the edge of the external trigger input starts the exposure of the first line of the sensor. The rest of the lines start exposure in sequence following the readout timing. The exposure time is set by software in the camera prior to the start of image acquisition.

Edge Trigger with Global Reset

This mode is used with Global Exposure Synchronization. On the rising (or falling, programmable) 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 by software prior to imaging. 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.

External Trigger Input: External Level

The changing edge of the external trigger input starts the exposure of the first line of the sensor. The rest of the lines start exposure in sequence following the readout timing. The returning edge of the external trigger input ends the exposure of the first line and starts readout of the sensor. The exposure time is determined by the amount of time the external trigger is high (or low, set by software).

Level Trigger with Global Reset

This mode is used with Global Exposure Synchronization. On the rising (or falling) 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 falling (or rising) edge of the voltage change of the external trigger input. The subsequent lines expose for the first line 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.

External Trigger Input: Start Trigger

The changing edge of the external trigger input starts the exposure of the first line of the sensor. The rest of the lines start exposure in sequence following the readout timing. The camera continues to acquire images in the internal trigger mode until the desired number of frames has been acquired and software puts the camera into another state.

Table 1. Organizes the various modes and shows the max FULL frame rates possible in each mode.

		Exposure Time Shorter than Readout			Exposure Time Longer than Readout (11 ms)		
		Internal Trigger	External Edge	External Level	Internal Trigger	External Edge	External Level
	Frame Rate Maximum	100	90	90	90	48	48
	External Trigger Input	✗	✓	✓	✗	✓	✓
Available Trigger Output	Global Exposure Out	✓	✗	✗	✓	✓	✓
Available Trigger Output	Programmable Timing Out	✗	✓	✓	✓	✓	✓
Available Trigger Output	Trigger Ready Out	✗	✓	✓	✗	✓	✓

Table 1. The chart above shows which trigger signals (both inputs and outputs) are available depending on whether your experiment requires a scheme in which the exposure time is shorter or longer than the readout time. The frame rates shown above are for an ORCA-Flash4.0 V2 using the Camera Link interface. For details on the speeds attainable using the USB 3.0 interface, please refer to the camera datasheet.[4]

Final Comments

The triggering and synchronization possibilities with the ORCA-Flash4.0 are extensive. A wide range of microscopy equipment can be configured to communicate with the ORCA-Flash4.0 to achieve maximum performance tailored to the sample and analysis needs of the experiment. While triggering with this camera is not difficult to achieve, the user does need to do some planning to determine the right triggering scheme for their experiment. In the end, most of the heavy lifting is done by the camera, software and microscope hardware.

We have attempted to show examples and descriptions of the most common triggering scenarios. There are, of course, unique cases that are not covered in this document. The user's manual that accompanies your camera has many details that should prove useful for setting up your system. However, if you need help with any aspect of triggering a Hamamatsu camera, please contact us and we'll be happy to help.

Glossary

CCD — Charge-coupled device (CCD) is a device for the movement of electrical charge, usually from within the device to an area where the charge can be manipulated, for example conversion into a digital value. This is achieved by "shifting" the signals between stages within the device one at a time. CCDs move charge between capacitive bins in the device, with the shift allowing for the transfer of charge between bins.

Dark current — Currents that are thermally generated when the imager is in the dark. These currents are normally generated in the bulk silicon of the detector. The dark current scales with operating temperature and exposure time. The dark current is reduced by approximately half for each 7°C of cooling. Normally, the camera noise and dark noise are mixed together when reading the camera output in the dark. Therefore, you must back calculate to dark current by using the overall camera noise and the read noise values.

Data streaming mode — A mode in which the computer acquisition program is set to receive the image data from the camera in one continuous flow. The length of the flow is set prior to acquisition in the software by either the number of images to acquire or the total amount of time to acquire.

Data transfer interface — The protocol and hardware for transferring the image data from the camera to the computer. The speed of the data interface (in Mbytes/second) can be a limiting factor in transferring image data for computer storage. Camera Link is a parallel interface capable of transferring multiple lines of data to the computer. USB is a serial interface that transfers packages of data to the computer. For the ORCA-Flash4.0 at full resolution the maximum speed using a Camera Link interface is 100 frames per second, continuous. The same camera using the USB interface can achieve 30 frames per second continuously at full resolution.

EM-CCD (Electron Multiplier CCD) — A CCD camera that achieves on-chip multiplication gain by impact ionization. Because of the noise associated with this multiplication process the technology is primarily useful for very low light samples with no background.[5]

External trigger input — A hardware input to the camera where a signal to start imaging is sent from an external source via a trigger cable.

Global exposure — The period of time when all of the pixels in the image are accumulating data. For a CCD, this period of time is equal to the exposure time setting. For a CMOS camera with rolling shutter, this period of time is equal to the time between when the last line of the image starts exposure and the first line of the image ends exposure.

Global exposure synchronization — A technique where the global exposure output signal is used to precisely time the on/off of an external illumination source.

Global exposure timing output — The global exposure timing output is only available when the exposure time setting is longer than the readout time. Under this condition, the first line, and all subsequent lines in the image, are still exposing when the last line starts exposing. This overlap region, called the global exposure time, has duration equal to the exposure time setting minus the readout time. The global exposure timing output has a rising (or falling) edge which coincides with the start of exposure on the last line and a falling (or rising) edge which coincides with the end of exposure of the first line.

Global shutter readout mode — The CMOS equivalent to CCD snap-shot mode, where all pixels are exposed for the same amount of time at the same time. Global shutter readout mode requires each pixel to have an additional transistor. This pixel design and its operation lowers the quantum efficiency (QE) and increases dark current.

Master — This is the device in which the timing signals are created to control or synchronize external devices.

Multiplexing — The process of combining several input data lines into one output line. This allows for simultaneous transmission of multiple signal data.

Programmable timing output — This output signal has user-defined pulse width and timing delay referenced to the end of readout of the last line of the sensor. When used with exposures longer than the readout time, this output signal can be used to trigger a pulsed illumination delayed and centered to the global exposure timing region of the image frame.

Pulsed illumination — Illumination that is controlled to be active for a period of time and inactive for a period of time. Typically, the active period is much smaller than the inactive period.

Quantum efficiency (QE) — The ratio of incoming photons converted into photoelectrons inside of the detector. It is usually represented in percentage and varies with wavelength. For example, a QE of 70% means that 70 of every 100 incoming photons are converted to photoelectrons. Many factors of pixel design determine the QE.

Read noise — Noise induced by the readout electronics, typically dominated by the noise on the floating diffusion amplifier and/or the analog-to-digital converter. It typically increases with clocking speed or frame readout speed. This noise is the result of the statistical variation or "error" that occurs when the amplifier attempts to reset itself to zero before the next image.

Region of interest — A two-dimensional area of the image which contains the sample or event of significance.

Rolling shutter — Operation of CMOS sensors referring to the line by line exposure and readout of the CMOS pixel array versus the all-at-once exposure followed by readout of CCD sensors, often called "snap shot" mode. In CMOS rolling shutter operation, there is a small temporal delay between the beginning of photon acquisition for the nth line of pixels versus the n+1th line, and so on. This delay is most likely to be an issue when the exposure time is less than the readout time and the imaged object is moving or changing rapidly.

sCMOS — Scientific complementary metal oxide semiconductor (sCMOS) image sensor. Unlike standard CMOS sensors, the pixel size is appropriate for microscopy and the data output is suitable for quantification. In CMOS sensors each pixel has its own amplifier and readout circuitry. The design of the amplifier circuitry in the sCMOS sensor results in high speed operation with low noise.

Snap shot — An image in which all pixels have been exposed over the exact same time period.

Software control mode – A mode where the software sends the appropriate commands to the camera to acquire images and other components to activate or change state. In this state the camera and the other components are slaves and the software is the master.

Start trigger mode — An external trigger input mode where the input signal from an external device precisely starts the image acquisition of a stream of images. The camera is in a waiting state prior to the signal and operates on internal trigger timing after the input signal.

Subarray — A setting in the camera where a subset of pixels are selected to define the image size by the number of horizontal and vertical pixels being scanned. For a CMOS camera, the readout rate is determined by the number of horizontal lines being scanned. Therefore, using a subarray with less vertical lines will decrease the readout time and increase the maximum frame rate. Sometimes referred to as "region of interest" or ROI.

Trigger cable — A cable that carries electronic timing signals between the camera and other components such as lamps, shutters and positioning equipment.

Trigger ready — An output signal from the camera to signify when the camera has completed acquiring the previous image and is available to accept the next external input trigger to acquire a new image. In the ORCA-Flash4.0 this signal is available only when using an external trigger input signal.

Trigger ready timing output — This is an output signal available from the camera when the camera is in one of the external trigger input modes. This signal changes from low to high (or high to low, selected by software) at the end of the frame readout, signaling that the camera can now accept a new input trigger. The trigger ready timing output changes to low (or high) again when the next trigger signal is received.

[1] The actual line time differential is 9.7 μs, but for the sake of easy conceptualization, we round this up to 10 in this discussion. The time delay depends upon the readout mode. For "low noise" readout mode is 30 μs.

[2] For a great discussion of distortion and rolling shutter, check out Shalin Mehta's blog: http://blog.mshalin.com/2012/08/global-exposure-with-scientific-cmos.html

[3] For short global exposure times (less than the readout time), the frame rate will be reduced by less than the factor of two introduced by global shutter readout.

[4] http://www.hamamatsu.com/resources/pdf/sys/e_flash4.pdf

[5] http://www.hamamatsu.com/resources/pdf/sys/e_flash4_whitepaper.pdf

To contact Hamamatsu Technical Support in the US call 908-231-0960.