Why synchronisation with the camera matters

Solid State Light sources in fluorescence imaging – why synchronisation with the camera matters?

There are numerous technical benefits of using fast switching solid-state (LED and laser diode) illuminators in live-cell imaging. These include higher stability, increased lifespan, lack of vibration, lower power consumption and reduced physical size. More of this to follow, but in this note I will focus only on the ability of the light source to be precisely slaved to the output of an imaging camera with the camera clock, auxiliary control card, or, software, acting as the (timing) master. In order for this to work effectively the digital modulation rate of the light source should be at least 10KHz (100usec response), which is an order of magnitude beyond what can be achieved with a traditional source.

Mercury, xenon and metal halide lamps have historically been used as the light source of choice for biological micro-fluorescence measurements. These sources are broad spectrum with very high point intensity, and can be switched on and off and varied in intensity and wavelength using mechanical shutters, filter wheels, or galvanometers in the time domain of several milliseconds or tens of milliseconds.

Scientific CCD, Electron Multiplied CCD or CMOS cameras typically have a suitable ‘fire’ or ‘expose out’ connector and any well-designed LED, diode laser or solid-state phosphor-base microscopy light source should have a digital TTL “shutter” external input. So what are the benefits of directly interfacing the camera with the light source?.

To minimise phototoxicity and photobleaching

Unfortunately for biologists, (most) living cells have not evolved to thrive on being exposed to large dosages of ultraviolet or visible radiation, and neither have genetic indicators or organic dyes. Perversely the laws of physics dictate that the fundamental shot noise, which limits the dynamic range of measurable signal changes, is dictated by the square root of the number of detected photons. This conflicting requirement, to both minimise and maximise light levels, makes it highly desirable to restrict the light exposure of the specimens to the precise intervals when the camera sensor is detecting useful signal. How much this helps to reduce photo damage will depend on the sensor type and the timing of the experiment, but the benefit will be significant in most cases. By simply connecting the camera expose output to the light source external input then this is achieved without making any demands on the software. For solid-state illuminators with different emitters supplying different wavelengths then it is useful to have second digital input to each channel so that the illumination can be controlled by software in parallel with the camera exposure. We provide such additional inputs as standard on our OptoLED and LaserBank illuminators, and can also supply auxiliary boxes to simplify operation with any modullable light source.

To avoid timing artefacts

Depending on the camera architecture it may be necessary to switch off the light during part of its cycle in order to avoid readout artefacts. With conventional, interline, CCDs the charge is transferred almost instantaneously from each photosensitive pixel row to an adjacent masked storage row. This enables the sensor to be rapidly "cleaned", and also minimises the overhead associated with overlap readout modes, so artefacts from continous light exposure do not typically occur. In a frame transfer configuration however, which includes most Electron Multiplied cameras, the charge is shunted as a block across the active area of the sensor to an equivalent storage area once per cycle. If photons are allowed to reach the detecting area during this transfer period then they will be inappropriately read out to the wrong rows. This will manifest itself as streaking across the image in the direction of the read out. This transfer period can last for up to a millisecond or longer which could be a significant portion of the frame integration period. Where this would matter most is if there is a large intrascene dynamic range. This is the characteristic of fluorescence measurements, especially if trying to simultaneously image areas of strong and weak signal (e.g. dentrites and soma or cell body and membrane).

The situation with the sCMOS cameras is somewhat more complicated as these typically run in rolling shutter mode where the readabout of individual rows is not simultaneous. If exposing a rolling shutter camera to a continuous light source then timing artefacts are always a risk, as there is a progressive delay (for up to 10msec) between the acquisition of central and edge rows.

For single channel biological imaging artefacts will probably only be noticeable when recording fast dynamics over a large field of view (as timing discrepancies are a function of how far the pixel rows are displaced from each other). However, if in doubt the safest approach is to modulate the light source so that it is only on when all the rows are being exposed simultaneously, i.e. during the virtual global shutter period. This reduces the acquisition down to below the maximum rates and necessitates a brighter light source as the illumination duty cycle is significantly reduced.

Most sCMOS camera operate at 100Hz at full resolution (10msec exposure). This assumes that the pixels are being continuously read out so there is a negligible "global" shutter period. If the light source is at least 10 times brighter than needed for continuous illumination then increasing the exposure time to 11msec allows equivalent images using 1msec of 10X intensity exposure per frame. This will remove readout artefacts without significantly slowing down acquisition rates.

sCMOS cameras provide digital outputs for both the entire exposure time and for the global period so timing artefacts can be investigated and eliminated (at the expense of the acquisition rates or the need for a brighter light source) if necessary.

To prevent crosstalk between multi-wavelength images

Multi-wavelength or other multichannel image streaming presents an additional challenge for rolling shutter sCMOS sensors. In this case images n and n+1 can be completely different to each other so the non-simultaneous exposure of different rows becomes a serious problem. Using the "Exposure All Rows" or equivalent output of the camera to modulate the light source prevents crosstalk between channels, but for high speed streaming it is also necessary to have a hardware timing signal to progress to the next wavelength on sequential exposures (a software timed signal is unlikely to be fast or accurate enough). Some light

sources include this option, or it can also be achieved with an additional control box; please contact us for advice.

To simplify software and minimise delays caused by other devices

Simply use a camera to directly modulate a solid state light source reduces complexity and potential delays in the software and will serve to maximise the benefits of reduced photon exposure to the sample. If the software or hardware is capable of generating additional signals during the camera exposure time then further speed improvements can be attained by removing the overheads associated with other devices. For example, assuming that a mechanical filter wheel has a transition time of 30msec then by streaming the camera and “shuttering” the light source during transition time for acquisition control can be conducted with a parallel flow rather than with sequential instructions. By using a filter wheel in a continuous spinning mode and/or a piezo in a continuous scanning mode with a modulated light source yet further speed benefits can be achieved by pulsing the light source in synchrony with the movement.

To improve time resolution

Although the camera readout speed will determine the repeat rate between sequential frames, a modulated light source can independently allow much shorter exposures and hence reduce blurring in dynamic recordings. sCMOS cameras allow this functionality directly (using the expose all rows output), but the same benefits can be achieved using an interline, EMCCD other frame transfer camera with a simple timing pulse to reduce the illumination duty cycle to be less than the entire camera exposure time.