Benefits of Synchronising LED Illumination Systems to a Camera

CoolLED

Lynsey Burton, Field Sales Manager at CoolLED

LED based illumination systems are increasingly replacing broad spectrum mercury, xenon and metal halide light sources owing to their numerous experimental, practical and environmental benefits. These highly controllable solid-state devices emit light at discrete wavelength ranges, provide intensity adjustment control, rapid on/off switching and wavelength selection.

Automated microscopy systems typically control the LED illumination system through software, via USB connection, however there are some experimental settings where the greater precision and faster speeds offered by electronic TTL triggering may be preferred. One of the easiest methods for TTL trigger control, and one which provides many of the benefits of TTL triggering, is to simply connect the LED illumination system to the camera. The majority of scientific cameras provide access to the “fire” TTL signal, produced by the sensor during the photon detection phase of the exposure cycle. By connecting the fire signal to an LED illumination system, the camera can be used as a precise timing generator to rapidly modulate illumination with microsecond precision. Additionally, some LED systems can use the fire signal to cycle through the LED selection to provide a simple solution for high speed multi-wavelength imaging without complex software.

Synchronising the LED system with the camera precisely restricts the illumination period to the time during which the camera is detecting usable photons. Software can introduce delays in the illumination switching times, meaning specimens may be exposed to light for longer than is necessary potentially resulting in phototoxicity and photobleaching. To what extent synchronising the illumination to the camera will minimise photodamage is dependent on the nature of the sample, the sensitivity of the camera and the duration of the experiment, but will prove significant for the majority of live cell imaging applications.

Precise control of the illumination can also help to remove image artefacts. One example of this is rolling shutter artefacts which can be seen with sCMOS cameras. Traditionally sensors used in cameras operate in global shutter mode, meaning all pixels start and end their exposure at exactly the same time. sCMOS sensors however typically operate with rolling shutters, meaning the photon signal in each row of pixels is not captured at exactly the same time but instead is acquired by scanning across the detection area very rapidly row-by-row. Whilst this can produce identical results in most cases it can cause image distortions when detecting rapidly moving objects over a large field of view. The easiest approach to eliminate rolling shutter artefacts is to use “simulated global shutter”, where the LED illumination system is precisely modulated so that the specimen is only illuminated when all pixels are exposing simultaneously i.e. the global phase of the exposure. sCMOS cameras provide a fire TTL signal for the complete exposure and for the global phase of the exposure which allows users to evaluate rolling shutter mode and simulated global shutter mode. Rolling shutter may also generate cross talk between captured images, for example when the camera is streaming images and the excitation wavelength or a Z position changes. Using simulated global shutter mode to strobe the light will serve to remove image cross talk.

The pE-300^{ultra} Illumination System from CoolLED is equipped with an additional function known as “sequence runner”. Sequence runner enables the LED illumination system to cycle through the wavelength section on receipt of a fire signal from the camera. Meaning that on the first fire signal received from the camera, generated during the first exposure, the system will switch on LED channel 1. On receipt of the second fire signal the system will switch on LED channel 2 and so on. This provides a simple high speed multi wavelength imaging system using a single multiband filter cube which negates the need for complex software or filter wheels.

For further information please contact CoolLED (www.coolled.com)

Biotechniques 65: 364 (December 2018) doi: 10.2144/btn-2018-2012