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Programmable LEDs Benefit Cell Viability

Modern Light Sources Enhance Sample Lifetime and Save Costs

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In live-cell fluorescence microscopy, maintaining a specimen's viability is essential to experimental efficiency and reliability.

A major cause of cell damage is the system's excitation light, which leads to phototoxicity and photobleaching [1]. Using programmable LED light sources offers multiple ways of controlling light intensity and timing, making it possible to decrease these phenomena significantly.

Reducing Phototoxicity and Photobleaching

There are several ways to reduce phototoxicity and photobleaching: minimize the required concentration of fluorophore; improve the sensitivity of the detector; add antioxidants; or remove certain chemicals from the imaging media [2]. These techniques can be expensive, alter the physiological conditions or decrease the image quality.

An alternative is to limit the excitation light hitting the sample. LEDs can do this while remaining relatively inexpensive and durable [3]. Here, we investigate the characteristics of LEDs that enable the extension of cell viability.

TTL/Software Control of Camera Exposure and Light Switching

TTL triggering is often used to synchronize illumination with a detector (e.g. camera). By making the light source a slave, an output signal from the detector can trigger light only during data acquisition. Traditional light sources can use TTL triggers but need a warm-up period to reach peak power and a cool-down time after turn off, requiring a mechanical shutter to be placed between the light source and sample. This is a potential source of noise and shuttering speed is limited by its inertia. LEDs turn on and off in microseconds, making direct TTL triggering highly efficient.

Some LED systems offer illumination at multiple wavelengths with TTL triggering enabling the light to switch between wavelengths and vary intensities (see advanced built-in triggering controls). Computer controlled synchronizing of advanced protocols with illumination enhances cell viability and lead to more reliable data.

Advanced Built-In Triggering Controls

Some LED systems, have added triggering techniques directly to their hardware.

Some LED systems have TTL triggering and inline excitation filter holders and with high-performance multi-band filter sets can, provide microsecond switching without the need for a filter wheel. Independent control of the intensity for each stain ensures only essential light hits the sample.

More advanced systems feature an internal function generator capable of creating sine, pulse duration and ramp protocols for each channel (fig. 1). These protocols can limit the sample's exposure to light and enable the cells to 'recover' between illumination periods (see pulsing and rest-phase) [5].

Pulsing and Rest-Phase

Engineering the dosage of excitation light by modulating the intensity of illumination over time (e.g. through pulsing or strobing) reduces phototoxicity by limiting the number of photons hitting the specimen and giving cells and fluorophores time to relax to baseline excitation levels between illuminations.

This reduces levels of photobleaching when performing laser scanning fluorescence microscopy and is also applicable to widefield fluorescence microscopy [5].

Using pulsed light and pauses in illumination exploits the way that fluorophores work. In one-photon fluorescence microscopy, the fluorophore absorbs a single photon of a particular wavelength. After the non-radiative loss of some energy, the fluorophore emits the absorbed energy as a single photon with slightly less energy than the absorbed photon. The extra energy added to the fluorophore when in the 'triplet phase', from an additional photon means the fluorophore has enough energy to react with oxygen and produce reactive oxygen species (ROS). Pulsing reduces the probability of two photons exciting the fluorophore simultaneously, limiting the amount of cell damage [5]. The instant on and off and controllability of LEDs make them an ideal light source for pulsing.

Using a pulsed light protocol and allowing for dark-state relaxation can also lead to an increased fluorescent signal. Experiments have shown 5-25 fold increases in fluorescent yield for certain dyes [6].

Discrete Wavelengths

Many fluorophores are excited by relatively discrete wavelengths. Using a white light source means bombarding the sample with light that will not produce the desired image. For this reason, fluorescence microscopes require high-quality filters to limit the spectrum of light hitting the sample to the desired wavelength(s).

When visualizing a different fluorophore(s) these filters need to be exchanged. This exchange can be a limiting factor in imaging speed requiring the use of a filter wheel.

LED selection limits emission to a thin band of wavelengths (fig. 2). While filters are still required they can be of a lower specification and cost. Multi-channel bandpass replace the filter wheel, speeding up channel switching.

Lower energy wavelengths in the red and far-red spectra are known to be less damaging to organic material [1]. Longer wavelengths, especially infrared, penetrate further into biological samples to enable fluorescence imaging at greater depths [7]. By using LEDs with thin bands of longer wavelengths and red-shifted fluorophores, users can reduce phototoxicity.

UV light is highly damaging to living cells and causes significant DNA damage. Most white light sources emit UV radiation, and filters are

required to limit the damage caused. LEDs that produce little or no UV light, can be very beneficial to cell viability.

Further Benefits of LEDs (Cost and Sustainability)

While the initial outlay for an advanced LED system may appear high, the lifespan of modern LED chips (generally >25,000 hours) and energy efficiency [3] makes them more cost-effective over their lifetime. Light produced by an LED also remains stable over an extended period unlike mercury or metal halide bulbs which degrade over time (fig. 3).

Conclusion

The use of programmable LEDs in fluorescence microscopy can limit phototoxicity and photobleaching. The resulting benefits to cell viability will enable living samples to survive longer and act in a manner that closely resembles their normal physiological behaviour. Thus, the researcher can use the same specimen to collect more experimental data, and this data will be more repeatable.

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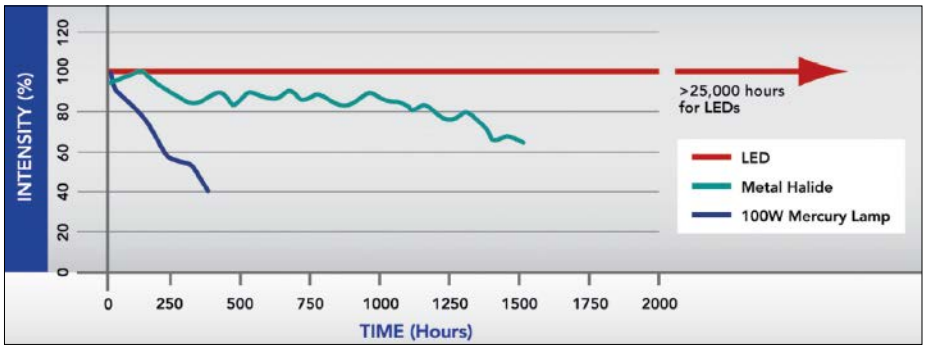

Fig. 1: Example sine wave pulse generated by an advanced LED system

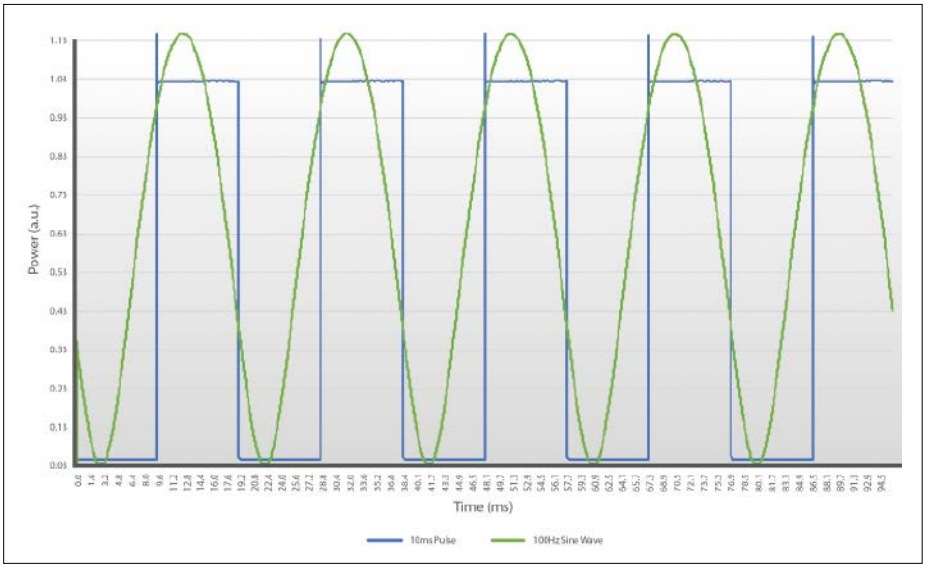

Fig. 2: Normalised power spectra for a wide range of advanced LEDs and a 100W mercury lamp.

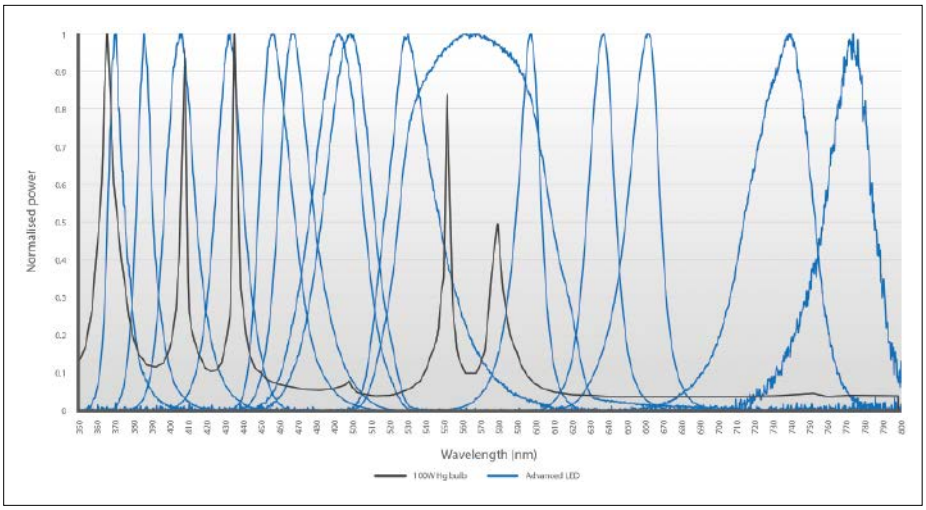

Fig. 3: Comparison of light source intensity over time. The graph shows relative intensity over time of LEDs, metal halide lamps, mercury lamps.