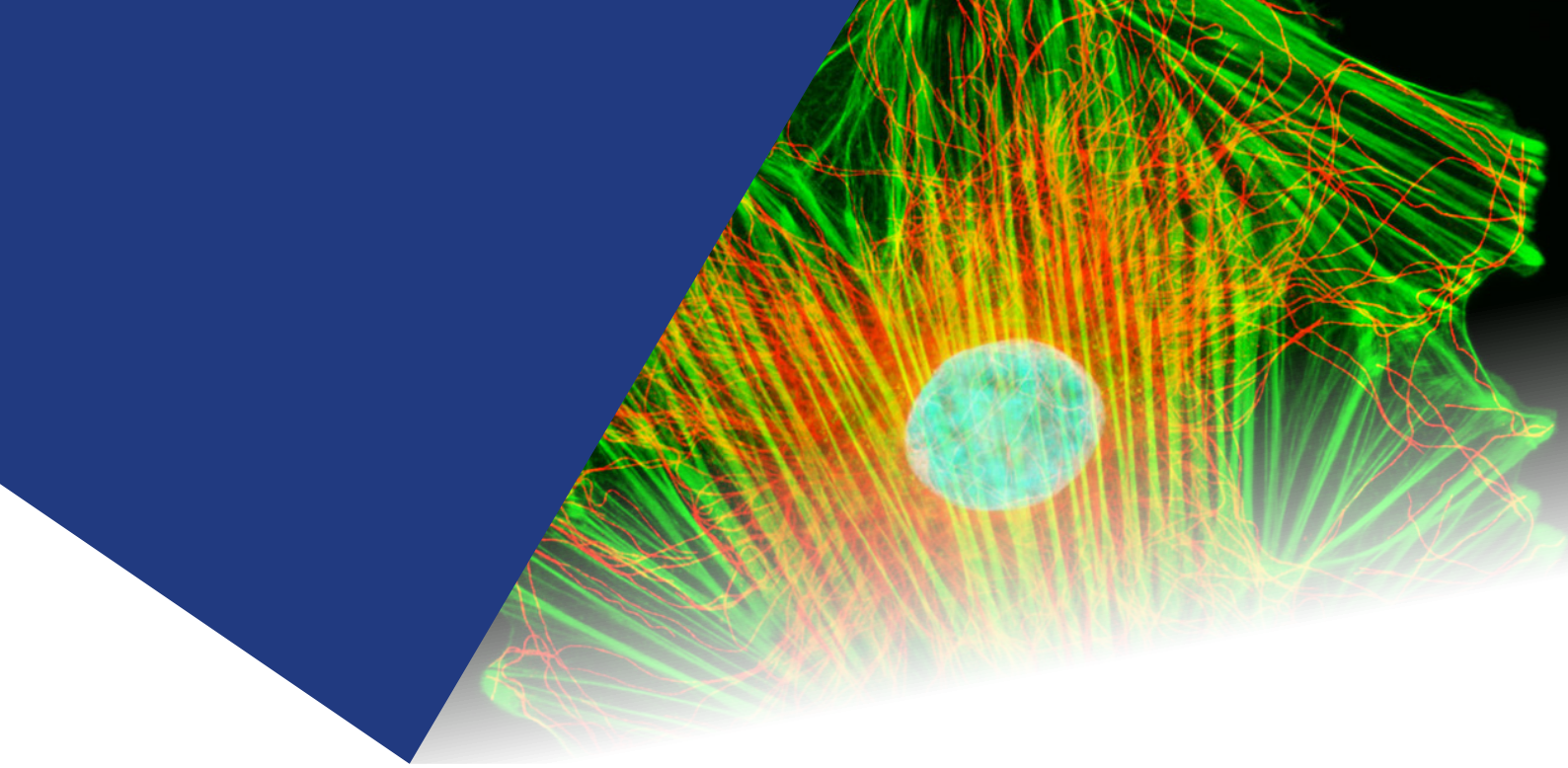


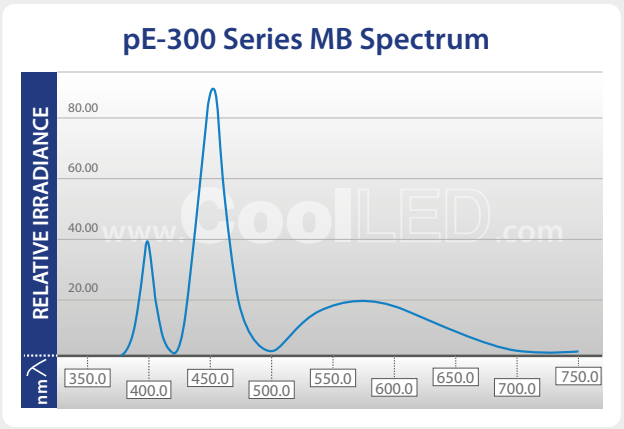
Improved multiplexing with the CoolLED pE-300^{white} and pE-300^{ultra} Illumination Systems

How multi-wavelength LED illumination systems featuring individual channel control enhance both multiplexing and single-colour imaging

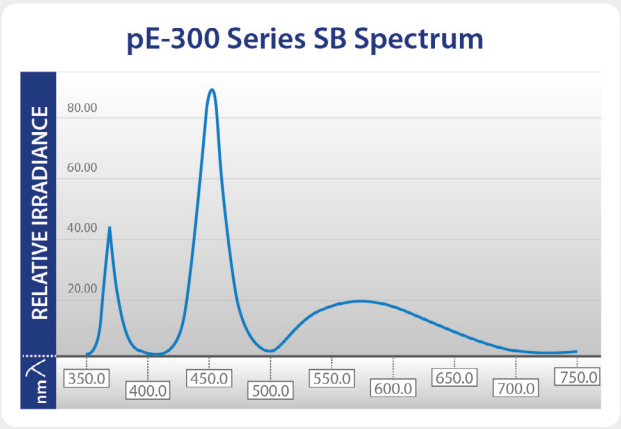
A variety of multi-channel LED illumination systems are now available for widefield microscopy, and one valuable feature to look out for is the ability to control individual LED channels. This is possible with the CoolLED pE-300^{white} and pE-300^{ultra} which are broad spectrum Illumination Systems well suited to the vast majority of fluorescence samples, including both single and multi-fluorophore experiments.

Their wide spectral output which includes three excitation channels covering the UV, blue and green-yellow-red (GYR) regions (Figure 1) are independently selectable and irradiance is controllable via manual control pod, software or TTL and analogue.

CoolLED
Simply Better Control



MB (400 nm LED)



SB (365 nm LED)

Figure 1: The CoolLED pE-300 Series Illumination Systems include three LEDs: UV-Violet (for fluorophores such as DAPI, Hoechst and Calcofluor White), Blue (for fluorophores such as GFP, FITC, Auramine) and GYR (for fluorophores such as Cy3, TRITC, TxRed, mCherry and Cy5). The MB variant Illumination System is designed for use with multi-band filter sets where DAPI is excited at a longer Violet wavelength (400 nm) than the standard single-band 365 nm.

Working with these three channels selected and set at 100% irradiance, they can replace an existing mercury or metal halide lamp, where working procedures and filter set selections remain unchanged but with the added advantages of:

- Fast and controllable: instant on/off and high temporal resolution with TTL or software control. Irradiance finely controlled to balance brightness with phototoxicity and photobleaching
- Sustainability: mercury-free, low energy consumption and long lifetime
- Consumable-free: no lamps or liquid light guide to replace

Increasing image contrast

The ability to control three channels independently increases the practical uses of multi-band filter sets. With the pE-300^{white} or pE-300^{ultra}, they not only provide multi-coloured images, but also enhance single fluorophore viewing.

By simply selecting or de-selecting regions of the excitation spectrum, single fluorophores can be viewed in isolation or in conjunction with one or two other fluorophores on the same sample. This is possible due to LED emissions being limited in bandwidth, thus delivering practically no energy outside the excitation region of interest. The result is reduced background with a high signal-to-noise ratio, in addition to reduced phototoxicity and photobleaching and therefore the potential for longer time-lapse studies and increased data accuracy.

Improved balance between fluorophores

Individual three channel control also allows the user to vary the illumination irradiance of individual fluorophores on a multi-stained sample. An optimal balance can be achieved which prevents brighter fluorophores from overpowering or masking weaker ones when viewed through the eyepieces. Moreover, with this level of flexibility it is also possible to optimise the irradiance balance to maximise signal whilst minimising photobleaching and phototoxicity in the case of sensitive samples or fluorophores.

Harnessing the speed of light

When it comes to capturing multi-colour images, colour cameras can be used with multi-band filters and a conventional broadband white light source, but this does not allow for colour balancing. Monochrome cameras instead tend to be more common in microscopy labs due to their lower cost and superior resolution. As a result, most multi-coloured images are constructed by overlaying a series of sequential monochrome single colour images generated using single-band filters, which are then coloured in software to match the emission colours. This sequential single-band filter approach provides images with high signal-to-noise ratio. However, the physical movement between filter cubes introduces latency.

This is where individual channel control enables high-speed imaging. By using a multi-band filter set or a Pinkel set in the case of the pE-300^{ultra} with its inline excitation filter holders, switching between filter cubes is no longer required (Figure 2). Combined with TTL triggering enables speeds of 10 µs which not only enables the capture of highly dynamic events in living samples, but once more reduces photobleaching and phototoxicity. For more information on high-speed imaging with LED Illumination Systems, please see our white paper.

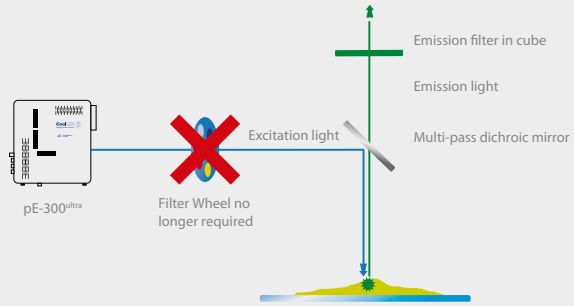


Figure 2: Capturing fast events with an LED Illumination System and Pinkel filter setup. Thanks to individual LED channel switching and inline excitation filters, the CoolLED pE-300^{ultra} with a Pinkel filter configuration (single-band excitation filters and multi-band dichroic and emission filters) overcomes the latency of a filter wheel.

Conclusion

Individual channel control offers many extra advantages for single colour and multiplexing experiments which go beyond enhancing image quality, especially for live cell imaging. High-speed imaging increases temporal resolution of experiments, while samples can be protected from photobleaching and phototoxicity like never before - enabling higher quality images and more accurate data.

Contact us at info@coolled.com, or visit our website to find out more about the pE-300 Series and recommended optical filters.

About CoolLED Illumination Systems

pE-300^{ultra}: Fast, controllable illumination

- Individual control of three channels
- Removable inline excitation filter holders
- Sequence Runner
- TTL and USB control

pE-300^{white}: Simple controllable fluorescence

- Individual control of three channels
- TTL, USB and manual pod control
- Most popular LED Illumination System