

IMPROVED MULTI-STAIN TESTING WITH THE pE-300^{white}

The pE-300^{white} broad spectrum illumination system has an additional capability which allows the user to combine single and multi-stain testing to achieve superior results. This is not possible with other broad spectrum illuminators.

While the pE-300^{white} delivers a wide spectral output covering most filter sets, its intuitive manual control pod allows the user to select and adjust the intensity in three key wavebands of the excitation spectrum. Working with these three bands, selected and set at 100% intensity, the system can be operated as a broad-spectrum 'white' illumination system, replacing an existing mercury or metal halide lamp. In this mode, working procedures and filter set selections remain unchanged.

The three waveband control feature of the pE-300-White increases the practical uses of multiband filter sets from providing only multi-coloured images to allowing single fluorophore viewing as well.

Increased contrast, less background

By simply selecting, or switching off, 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 the high signal-to-noise ratio.

Improved balance between stains

In addition, the three waveband control of the pE-300^{white} allows the user to vary the intensity of illumination of individual fluorescent stains on a multi stained sample. An optimal balance can be achieved which prevents brighter stains from over powering or masking weaker ones when viewed through the eyepieces.

By using the pE-300^{white} in conjunction with a multiband filter set, switching between filter cubes is no longer required. The user can select which single or combination of stain emissions is being viewed solely through the illumination system.

Better multi-colour images from a monochrome camera

Although capturing multi-colour images using multiband filters and a conventional broadband white light source is practical using a colour camera, the ability to balance the colours is not possible. More importantly, monochrome cameras tend to be more common in microscopy labs due to their lower cost and superior resolution than a similar (pixel number) colour camera.

As a result, most multi coloured images are constructed by overlaying a series of sequential single colour images generated using single band filters. This sequential single band filter approach does provide images with high signal to noise ratio. However, the physical movement between filter cubes can introduce two new problems: time delays and vibration.

When the individual images are overlaid, pixel shift occurs in the combined image if there are any differences in the optical path through each single band filter cube resulting in that image being misaligned with respect to any other. This cannot happen when the pE-300^{white} with only one multiband

filter set is used. The user can achieve both full simultaneous multicolour imaging as well as sequential imaging without the need for any moving parts.

The three controllable spectral regions of the pE-300-White are:

1. UV/Violet - For UV and Violet excited fluorophores such as DAPI, Hoechst and Calcofluor White etc.
2. Blue - For Blue excited fluorophores such as GFP, FITC, Auramine etc.
3. Green/Red - For green and red excited fluorophores such as Cy3, TRITC, TxRed and mCherry as well as Cy5.

Users of triple multiband filter sets should be aware that DAPI is typically excited at a longer Violet wavelength (400nm) than the common UV band (365nm). To match these triple multiband filters sets, the pE-300^{white} in multiband ("MB") configuration should be specified.

The following is a list of available triple multiband pass sets that can be used with the pE-300^{white} with violet, blue and GYR excitation.

Chroma:

<http://www.chroma.com/product/complete-filter-sets/widefield-microscopy/triple--69000--ET-DAPI-FITC-TRITC>

<http://www.chroma.com/product/complete-filter-sets/widefield-microscopy/triple--69002--ET-DAPI-FITC-Texas-Red>

<http://www.chroma.com/product/complete-filter-sets/widefield-microscopy/triple--69010--ET-DAPI-FITC-Cy3>

Semrock:

<http://www.semrock.com/SetDetails.aspx?id=2738>

<http://www.semrock.com/SetDetails.aspx?id=2757>

<http://www.semrock.com/SetDetails.aspx?id=2710>