



User Manual

pE-300 Series

White Light Fluorescence Illumination System





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1.

Introduction

CoolLED's pE-300 Series illumination systems are designed to offer broad spectrum LED illumination for general use in fluorescence microscopy applications. The pE-300 can be fitted directly to the microscope as a better and safer alternative to high pressure mercury or metal halide illuminators. Spectral coverage is from the UV (DAPI excitation) to the Red region (Cy5 excitation). It will excite common fluorophores used in hospital and research environments.

With a comprehensive range of microscope adaptors, the pE-300 Series can be fitted to most current and older microscopes. The result is a safe, convenient illumination system which will last for many years without any additional operating costs.

This User Manual should give you all the information required to install and operate your new illumination system.

Additional information can be found on our website at www.coolled.com





Safety Precautions

While LEDs are a much safer illumination system than the mercury and metal halide lamps that they replace in microscopy applications, precautions should still be taken with this product.

When operating or maintaining this product, please observe the following safety precautions at all times. Failure to do so may result in personal injury or damage to other items.

Please ensure that only the power supply and cord supplied are used with this equipment.

The AC cord supplied with this light source must only be used with the equipment supplied.

2.1.

UV light may be emitted from this product depending on the version/wavelength selected. Avoid eye and skin exposure. Never look directly into the light output beam from the Light Source or accessories. The emissions could damage the cornea and retina of the eye if the light is observed directly.

2.2.

Always ensure that the Light Source is securely attached to the microscope (either directly or with a light guide and collimator, depending on the version) prior to turning on the power. This will minimise the risk of injury and damage.

2.3.

If for any reason the Light Source is to be operated when not attached to a microscope, all personnel should wear eye shielding and clothing to protect the exposed skin.

2.4.

Disconnecting the mains supply is achieved by unplugging the power cord from the power supply block or the Light Source. Only plug in the power cable, once the Light Source is attached to the microscope.

2.5.

There are no serviceable parts within the Light Source. Removing any of the screws and covers will result in the safety of the Light Source being impaired. The DC power supply unit should be inspected periodically throughout the lifetime of the system.





Any electronic equipment connected to this product must comply with the requirements of EN/IEC 60950.

2.7.

To clean the exterior of the Light Source, use a slightly dampened cloth with a simple water/detergent solution only. Avoid the optical surfaces and lenses. Cleaning of optics should only be carried out using optical wipes and fluids. Please note that the DC power supply unit should be isolated prior to cleaning.

2.8.

This product conforms to the requirements of the Safety Standards as follows:

Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory use.	
Photo-biological Safety of Lamps and Lamp Systems/Guidance on manufacturing	
requirements relating to non-laser optical	
radiation safety. Risk Group 3.	

RISK GROUP 3

WARNING UV emitted from this product. Avoid eye and skin exposure to unshielded product.

WARNING Possibly hazardous optical radiation emitted from this product. Do not look at operating lamp. Eye injury may result.

CAUTION IR emitted from this product. Avoid eye exposure. Use appropriate shielding or eye protection

All warnings may not be applicable depending on the version/wavelength being used.

2.9. EMC compliance

This product is tested to the requirements of standard IEC/EN 61326-1 concerning electromagnetic compatibility. This is a Class B product.





pE-300 Series variants

All versions in the pE-300 Series offer intense, broad spectrum LED illumination. With spectral coverage from the UV (DAPI excitation) to the Red region (Cy5 excitation) they are suitable for imaging most common fluorescent stains. Light sources are available for either direct attachment to the microscope or via liquid light guide delivery.

3.1. pE-300^{white}

The pE-300^{white} allows individual control of the three channels in the Illumination System. This can be controlled by the manual Control Pod, USB or a single global TTL.



3.2. pE-300^{lite}

The pE-300^{lite} is the simplest Illumination System in the pE-300 Series. This is controlled by the manual Control Pod, allowing global intensity control of the illumination system.







3.3. pE-300^{ultra}

The pE-300^{ultra} allows the greatest level of control in the pE-300 Series. The Illumination System allows control of the three channels using the Manual Control Pod, USB or by the four TTL inputs (one TTL input for each channel, as well as a global TTL). The pE-300^{ultra} also allows additional excitation filters to be placed in the light path of each of the three channels.







4.

Getting Started – System Components

A typical CoolLED pE-300 Series Illumination System is supplied with the following components:

- 1. LED Light Source.
- 2. Manual Control Pod.
- 3. Microscope adaptor for specific microscope model (Direct fit only).
- 4. DC Power Supply Type GST120A12-R7B.
- 5. IEC Power Cable (not shown).
- 6. User Guide (not shown).



The image shows a typical direct fit pE-300^{white}system.

If any components are missing or appear damaged, please contact CoolLED immediately.





Installation and setup

5.1.

Carefully unpack the components from the shipping cartons.

5.2.

Insert the Control Pod cable into the LED Light Source using the red dots as a guide for orientation of the plug.



5.3.

Connect the power connector from the DC power supply as shown. Ensure that the DC power supply is the one supplied with the product. Using non-CoolLED power supplies may damage the Light Source and will invalidate the warranty. At this stage do not connect the mains power lead to the DC power supply.







5.4.

Attach the LED Light Source to the epi-fluorescence port on your microscope. Your pE-300 Series Light Source will have been supplied with a compatible fitting for the microscope you specified at order (if direct fit version). Attach the Light Source ensuring that it is secure and true/flush with the microscope.



5.5.

Ensure that there is free airflow around the LED Light Source so that the cooling system is not impaired. A gap of 200mm on either side is sufficient. The diagram shows the Light Source in the preferred orientation. However it may be set with the cables at the top or at either side.

5.6.

With the LED Light Source now attached to the microscope it is safe to connect the mains power. Connect the mains lead supplied to a convenient socket, plug in the IEC connector into the DC power supply and switch the power on at the socket.



6.1.



Configuration of LEDs as a White Light Source

Conventional 'white' illumination systems used for fluorescence microscopy (e.g. mercury lamps) have a single element which emits light in a series of peaks across the spectrum, giving the effect of white light. LEDs are different in that a single LED element will emit light in a particular colour. To create a white illumination system, LEDs of different wavelengths have to be combined together. Using a pumped phosphor, a broader peak covering green, yellow and red emissions can also be created. In the pE-300 Series, LEDs emitting in the UV and blue regions are combined with a pumped phosphor to create a white illumination system covering all the commonly used fluorescence stains.



Enhanced pE-300 Series products are from July 2017 onwards.

DOC-005 Iss 14





6.2.

The pE-300^{white} and pE-300^{ultra} have independent circuits giving the user control of the three main peaks of emissions. On the standard configuration, these are referred to as 1UV, 2B (blue) and 3GR (green, yellow, red).



6.3.

There is also a variant of the pE-300^{white} and pE-300^{ultra} which has been configured for use with multi-band filter sets where the first peak has been shifted from the UV region (1UV) to the violet (1V). See <u>Appendix 1</u> for more information.





6.4.

The pE-300^{lite} also allows the choice between an SB and an MB configuration to suit your filter sets. The Control Pod however displays only one 'White' intensity control bar on the display. This will allow global intensity control of all installed LEDs at the same rate.







7. Operation - Manual Control

7.1. pE-300^{white} & pE-300^{ultra}

7.1.1.

Manual Control Pod Operation on/off.

The pE-300^{white} and pE-300^{ultra} are easily controlled from the manual Control Pod. LEDs are switched on and off by pressing the 'on/off' button.

7.1.2.

At start-up the Light Source will revert to the same settings that were set when it was last powered down. New Light Sources are supplied with the settings as shown.



7.1.3.

To switch on LEDs press 'on/off' once.







7.1.4.

To switch off the LEDs, press the 'on/off' once again.



7.1.5.

Intensity Control.

The Control Pod enables the user to control the intensity of the LEDs that are exciting different stains. This helps to balance the emissions so that one stain does not dominate another. This feature is very useful in multi-band work (see application note in <u>Appendix 1</u>).

7.1.6.

Reduce intensity of one channel by pressing the down intensity button.







7.1.7.

Individual bands can be switched off (de-selected) by pressing the 'select' button. Light is then only generated where it is required to excite the stains in use. This has many attractive benefits with improvements in contrast, cell viability and savings in energy.

Switching off UV will help to reduce damage to cells through photobleaching.



7.1.8.

Switch off selected channels by pressing the 'on/off' button.



7.1.9.

Switch selected channels back on by pressing 'on/off' button again.









7.2. pE-300^{lite}

7.2.1.

At start-up the Light Source will revert to the same settings that were set when it was last powered down.



7.2.2.

To switch on the LEDs press the "on/off" button once.



7.2.3.

To control the intensity of the light output, use the "+" and "-"buttons to increase or decrease in 1% steps.







Remote Operation – TTL (pE-300^{white} & pE-300^{ultra})

The pE-300^{white} and the pE-300^{ultra} can both be controlled remotely via a TTL signal.

8.1. Global triggering (pE-300^{white} & pE-300^{ultra})

8.1.1.

The pE-300^{white} and the pE-300^{ultra} both have a BNC socket on the rear of the Light Source which allows global control of the Illumination System.



The TTL signal controls the on/off function of the Light Source. A TTL 'high' will cause the LEDs to be on, independent of the state of the on/off button. Only those bands which have been manually selected on the Control Pod (shown by a shaded intensity bar on the Control Pod display) will be switched by the TTL signal. The intensities of the selected bands are manually set on the Control Pod.





8.2. Individual channel triggering (pE-300^{ultra})

8.2.1.

In addition to the global TTL control available, the pE-300^{ultra} also has three additional BNC sockets that allow individual TTL channel control of the Illumination System.



8.2.2.

The TTL signal controls the on/off function of the Light Source. A TTL 'high' will cause the LEDs to be on. The channel controls shall trigger the corresponding channel regardless of its on/off state or whether it has been selected using the Control Pod. The intensities of the selected bands are manually set on the Control Pod.

8.3. Sequence Runner (pE-300^{ultra})

8.3.1.

The pE-300^{ultra} allows the Illumination System to be controlled using the Sequence Runner mode. The Sequence Runner allows sequential triggering of multiple channels using a single TTL signal attached to the global BNC socket.







8.3.2.

The Sequence Runner mode is accessed by a short duration press of the mode button on the Control Pod.











8.3.4.

Pressing the channel select button allows you to either deselect a channel or change the order that it triggers in the sequence.



8.3.5.

Pressing the + & - buttons on the Control Pod will allow the light intensity of the corresponding channel to be increased or decreased.







8.3.6.

The sequence will not begin until the ON/OFF button on the Control Pod is pressed.



In this example channel 2 will pulse at 100% intensity, channel 3 will pulse at 65% and then channel 1 will pulse at 100%. This Sequence will continue until the ON/OFF button is pressed again to stop the sequence.

8.3.7.

Whilst a sequence is running the ability to change the triggering order, select or deselect a channel and the mode button are disabled. The BNC sockets responsible for Individual channel triggering are also disabled whilst in Sequence Runner mode to avoid any conflicts.

8.3.8.

An example of the TTL signal is shown below with labels showing the effect on the light output during the sequence.







8.4. TTL triggering information

8.4.1.

The TTL input circuit has been designed to maximise the switching speed of the LEDs to give the user precise control of the excitation light reaching the sample.



This diagram shows the worst case triggering speeds when triggered at 100% intensity. There will be slight differences in speed between channels and at different intensities.

8.4.2.

With fast repetitive switching, the Control Pod display will not be able to respond at the same speed. This can occasionally result in the Control Pod displaying the incorrect ON/OFF state. If this happens, simply press the ON/OFF button to reset the state on the display.



Remote Operation – USB (pE-300^{white} & pE-300^{ultra})

9.1.

For a remote control using software connection between the host computer and the Illumination system, a USB interface is used. The Light Source has a Type'B' connector socket located adjacent to the Control Pod socket.

CoolLED 3 Simply Better Control 7



9.2.

Connect the Light Source to your computer using a USB cable. As with all USB remotely controlled devices, it will be necessary to set up the driver files on your system to allow the pE-300^{white} or pE-300^{ultra} to be recognised.

9.3.

When you first plug your CoolLED system into your PC with the USB cable, Windows will ask for a driver file unless one has already been installed. You should point Windows to the file available from CoolLED.

9.4.

If you do not have the driver file you can download this from the following page on the CoolLED website:

http://www.coolled.com/product-detail/imaging-software/





9.5.

Click on the CoolLED tab near the bottom of the page and you will see the link 'CoolLED pE Driver'. Just click on this link to download then unzip before pointing Windows to this file.

9.6.

Once the CoolLED device has been successfully installed into Windows you should look at the Virtual COM ports assigned by going into Device Manager. Look within Ports (COM & LPT).



In this example the Illumination System has been assigned two COM ports, COM3 and COM4. You may need this information to connect to the Light Source from your software control package. Either COM port may be used for control. Two COM ports have been assigned to allow for diagnostics to take place in parallel with communication and also allow for dual communication should it ever be desired.

9.7.

The majority of microscopy imaging software systems have integrated the pE-300^{white} and pE-300^{ultra} into their packages. If you are developing your own software, a Software Development Kit (SDK) is available giving the full instruction sets necessary. Contact <u>support@coolled.com</u> and request access to this information.



10. Optical setup

10.1. Direct fit version

10.1.1.

The pE-300 Series has been designed to work on the majority of fluorescence microscopes, both new and old. As would be expected, there is some variation in the optical path and elements within every microscope. In order to accommodate these variations, the pE-300 Series is supplied with a small adjustment which allows the user to optimise the performance of the illumination system when it is first fitted. This is a one-time adjustment. No further adjustment will be required during the life of the product unless changes are made to the microscope or the illumination system is fitted to a different microscope.



10.1.2.

To make the adjustment, set up a typical sample on the microscope that gives an image over the whole field of view. Loosen the thumbscrew and slide the post back and forth until you achieve the maximum brightness with an even field of view. Tighten the thumbscrew to prevent the setting from changing.





10.2. Liquid light guide version

10.2.1.

Fully insert light guide as shown and tighten up grub screw to ensure end of light guide is prevented from sliding out.



10.2.2.

Do not bend the liquid light guides through sharp corners. It is recommended to ensure a minimum bend radius of 75mm. Ensure that the Light Source sits upright on a flat surface and keep the clearance of 200mm on both sides to ensure adequate airflow for the cooling system.

10.2.3.

The pE-300 Series Illumination Systems with liquid light guide output are provided with a 'cradle' to ensure that they remain in a stable position during operation, as shown in the image below.







10.2.4.

The use of a liquid light guide will be attractive for use in electrophysiology as this allows the light source to be placed outside the Faraday cage to reduce vibration and electrical noise close up to the samples. The pE-Universal Collimator is available for these applications. See <u>Product options</u> and order codes for details.



10.2.5.

When using this collimator, it is important to set up the optics correctly to optimise the performance of the Illumination System. Full setup instructions are given in the separate User Manual for the pE-Universal Collimator.

11. Additional filtering (pE-300^{ultra})

11.1.

The pE-300^{ultra} allows additional filtering of excitation light through the use of three excitation filter holder slides (one in the optical path of each of the three channels).







11.2.

The excitation filter holder slides accept a standard 25mm diameter filter and is secured in place with a ball ended grub screw.



11.3.

Due to the shape of the excitation filter holder slide, it can only be fitted into the corresponding channel in one orientation.

11.4.

To install excitation filters in the optimal orientation, the direction of light through the Light Source should be observed. This is shown in the image below with arrows.





12. Settings / Additional Information

12.1. Display Backlight and Contrast settings



The Control Pod display settings can be adjusted to suit the lighting environment that the instrument is being operated in. To make adjustments, press and hold the 'mode' button for 3 seconds.

12.1.1. pE-300^{white} & pE-300^{ultra}

Use the first column up/down buttons to adjust the backlight to the desired level.





Use the second column up/down buttons to adjust the display contrast as required.





To return to the main screen, either press and hold the mode button for 3 seconds again or wait 10 seconds for the screen to automatically return.





12.1.2. pE-300^{lite}



Use the "+" and "-" buttons to increase and decrease the backlight intensity. To return to the main screen, either press and hold the mode button for 3 seconds again or wait 10 seconds for the screen to automatically return.



OFF	Settings	
	55	
	Back	





12.2. System Information

To interrogate the product on its hardware and firmware revisions, press and hold the 'mode' button for 3 seconds. Once the display settings screen appears as in 9.1, release the 'mode' button and then give it a second short duration press. The following display will appear.



Off	Info
Model:	pE-300-Wh
Serial:	
Firmware:	1.0.10
Hardware:	1
Pod Ver:	1.0.6
Pod H/W:	3

To return to the main screen, either press and hold the Mode button for 3 seconds or wait for 10 seconds for the screen to automatically return.





12.3. LED Usage

The system automatically records the total time that the LEDs are actually on. To retrieve this information, repeat the process in 9.2 above except give two short duration presses of the 'mode' button rather than a single one. The following screen will appear:

	Off	Info 2	
	UV:		0.5h
	BLU:		1.2h
Соо!с. рЕ-300	GYR:		0.8h
on/off select select			
	pE-300 ^{white} & pE-300	Jultra	
	OFF	Info 2	2
Cool D 😤 pE-300 ^{lite}	LED Usage	:	12.2h
Single Deter Carlot 1/5.			
Simple white light			
	No. 10. X		

pE-300^{lite}

To return to the main screen, either press and hold the 'mode' button for 3 seconds or wait for 10 seconds for the screen to automatically return.

13. Routine Care and Maintenance

13.1.

The pE-300 Series Illumination System requires little or no maintenance throughout its life. There are no field serviceable parts so there is no need to remove the covers.

13.2.

Cleaning of the external surfaces can be carried out with a mild soap and water solution used to lightly dampen a lint-free cloth. Ensure that no liquid is allowed to enter the product through vents and panel edges. Avoid optical surfaces.





13.3.

Cleaning of optical surfaces maybe necessary if debris or finger prints accidentally come into contact with the lens during installation. In the first instance remove any loose debris with an air duster (aerosol or rubber blower).

13.4.

Finger prints or other liquid type contaminants should be removed using standard lens cleaning procedures. Do not flood the lens surfaces with fluid as liquid could enter the product and cause damage.

14. Fitting the pE-300 Series Illumination System to a different microscope

14.1.

The pE-300 can be easily fitted to most fluorescence microscopes, both new and old. Every microscope manufacturer has one or a number of methods of attaching the fluorescence light source. CoolLED has designed a comprehensive range of adaptors to match these microscopes.

14.2.

There are a small number of microscopes which require additional optics or special settings internal to the pE-300 Series Light Source. Light Sources for these microscopes will be supplied with a label on the back panel, next to the serial number. These light sources cannot be transferred on to other microscopes without first returning them to CoolLED for internal modifications. Contact info@coolled.com if a light source needs this modification and ensure that the complete Illumination System is returned.

14.3.

The adaptor can be removed and replaced by simply unscrewing a pair of M4 grub screws as shown.







Fit the new adaptor and tighten grub screws.

14.5.

A complete list of adaptors can be found on the CoolLED website using the link: <u>www.coolled.com/product-detail/adaptors-2/</u>

14.6.

The simple optical setup procedure will need to be followed when fitting the pE-300 to a different microscope. See the <u>Optical setup</u> section.

15. Product specifications

15.1.

Power requirements		
110-240Va.c	50/60Hz	1.4A

15.2.

Power consumption	
Standby mode	max 2W
Three bands at 100% (pE-300 ^{lite} at 100%)	max 46W
Two bands at 100%	max 38W
Single band at 100%	max 20W

15.3.

) x 162mm (h)	
40kg	
) x 37mm (h)	
.32kg	
167mm (w) x 67mm (d) x 35mm (h)	
62kg	
40kg) x 37mm (.32kg) x 35mm (62kg	

15.4.

Environmental Operating Conditions Operating 5 – 35°C





16. Product options and order codes

See website (<u>Microscope Illuminators | LED Illumination Systems | CoolLED</u>) for full details of product options and order codes.

17. Warranty and Repairs

Please refer to CoolLED's current Warranty Policy available on our website <u>CoolLED Warranty - CoolLED</u>. Although warranty terms are fixed at the time of ordering according to the terms and conditions of sale in place, the Warranty Policy may be subject to periodic change so please check to avoid confusion.

For any warranty queries or in the event of the product developing a fault, make contact with support@coolled.com for further assistance. You shall be asked to provide your microscope make and model, the product serial number and a brief description of the issue. You shall then be issued with a Support Case to manage your issue.

18. Compliance and Environmental

18.1. WEEE

All qualifying products that are subject to the WEEE Directive and supplied by CoolLED are compliant with the WEEE marking requirements. Such products are marked with the "crossed out wheelie bin" WEEE symbol and in accordance with European Standard EN 50419.

CoolLED Certificate No: WEEE/GB4236XX

18.2. RoHS

Based on information obtained from our component suppliers, this statement certifies that ALL products manufactured and supplied by CoolLED Ltd are in compliance with Directive 2011/65/EU of the European Parliament and of the Council of 8 June 2011 on the restriction of the use of certain hazardous substances in electrical and electronic equipment (also known as "RoHS").

This declaration is correct to the best of CoolLED Ltd knowledge, information and belief at the date of its release.





18.3. CoolLED's Recycling Program

At CoolLED, we recognize the importance of preserving the global environment. We are proud to provide a Recycling Program that enables CoolLED customers and end-users to send back used CoolLED Light Sources for recycling, free of charge.

Together we can reduce the burden on our environment through responsible disposal and recycling of End-of-Life Light Sources. You can help us by filling in our online contact form and providing us with your contact details and the serial number of the CoolLED Light Source that you wish to return and we will collect it free of charge.

If you are taking delivery of a replacement CoolLED Light Source, why not send the old one back in the packing box of the new one?

18.4. ACT labelling

CoolLED is delighted to announce that it's pE-300 Series has been awarded the ACT Label for sustainability.

Accountability, Consistency, Transpare The Environmental Impact Fac	ency. tor Label
pE-300 Series	
Andover, United Kingdom	
Manufacturing Manufacturing Impact Reduction	10
Renewable Energy Use	No
Responsible Chemical Management	1
Shipping impact	8.8
Product Content	10
	5
Energy Consumption	1.4
Water Consumption	N/A
Lifetime Rating	4
End of Life Packaging	5
Product	1
Environmental Impact Factor	46.2
Label Valid Through	June 2020
My Green Lab Transmission mygreenlab "Energy and water consumption reported at ship values, all other 1-10: 1 indicates the least environment impact and 10 indicat	D.OFG values on a scale of tes the greatest.

For more information on the ACT label please visit the <u>My Green Lab</u> website.





19. Contact Details

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Email	info@coolled.com	
Online	www.coolled.com	



20.

Appendix 1

CoolLED pE-300^{white} & pE-300^{ultra} with Multi-band Filter Sets

New ways of working with multiband filter sets can now be achieved using the pE-300^{white} or the pE-300^{ultra}. These are not possible with other broad spectrum light sources.

While the pE-300^{white} and pE-300^{ultra} deliver 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 bands of the excitation spectrum.

Working with these three bands (selected and set at 100% intensity) the illumination system can be operated as a broad spectrum 'white' light source, replacing an existing mercury or metal halide bulb. In this mode, working procedures and filter set selections remain unchanged.

The three band control feature of the pE-300^{white} & pE-300^{ultra} increases the practical uses of multi-band filter sets from only providing multi-colour images to also allowing single fluorophore viewing.

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 and so adding practically no energy outside the excitation region of interest. The result is reduced background with the greater 'signal-to-noise ratio'.

In addition the three band controls of the pE-300^{white} & pE-300^{ultra} allow the user to vary the brightness of the individual fluorescence stains on a multistained sample so that a balance can be achieved between them. This can prevent brighter stains from over powering or masking weaker ones as viewed through the eyepieces.

By using a pE-300^{white} or pE-300^{ultra} in conjunction with a multi-band filter set, swapping between filter cubes is no longer required. The user can select which single or combination of stain emissions is being viewed solely through the pod controls.

Apart from not having to remember which filter cube is in each position there are additional benefits in having a single multi-band filter set.





Capturing multi-colour images with multi-band filters and conventional broadband white light sources is practical with the use of a colour camera although the ability to balance the colours is not possible. However monochrome cameras tend to be more common in microscopy labs as they are generally cheaper and provide better sensitivity and resolution than a similar pixel numbered colour camera. For this reason a multi coloured image will generally be constructed by taking a series of sequential single colour images through a series of single band filter cubes. This sequential single band filter approach does provide images with great signal to noise. However the movement between filter cubes can introduce problems in the form of time delays and vibration. Misalignment of overlaid images (pixel shift) can also occur when using different single band sets due to the individual dichroics in each cube not being set at exactly the same angle. There are complicated solutions to these issues involving the use of excitation and emission filter wheels.

By contrast the pE-300^{white} or pE-300^{ultra} with a straight forward multiband set 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} & pE-300^{ultra} 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 excited at a longer Violet wavelength (400nm) than the normal UV band (365nm). This is caused by complications for the filter manufacturers in producing a single filter with three pass bands with the lowest one at 365nm.

To match these triple multiband filters sets, the $pE-300^{white}/pE-300^{ultra}$ MB should be used which has the DAPI excitation band positioned in the violet region, rather than the UV.





The following is a list of available triple multiband-pass sets that can be used with the $pE-300^{white}/pE-300^{ultra}$ Violet, Blue and GYR.

Chroma:

https://www.chroma.com/products/sets/69000-et-dapi-fitc-tritc https://www.chroma.com/products/sets/69002-et-dapi-fitc-texas-red https://www.chroma.com/products/sets/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