

User Manual

pE-300 Series

White Light Fluorescence Illumination System

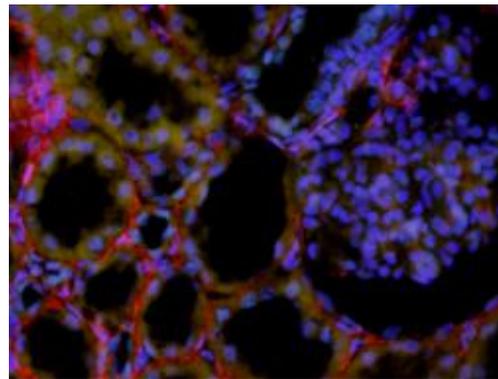
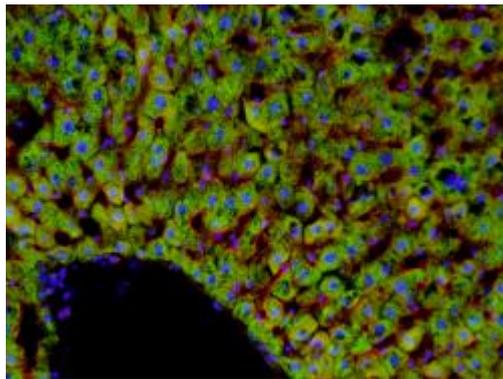
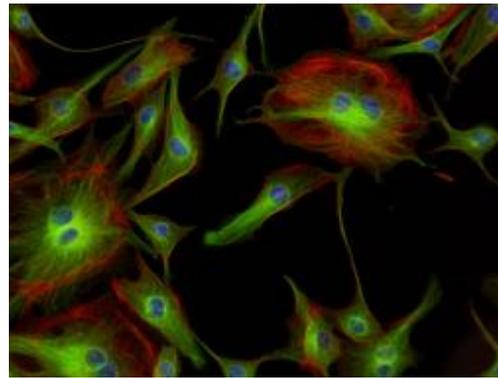
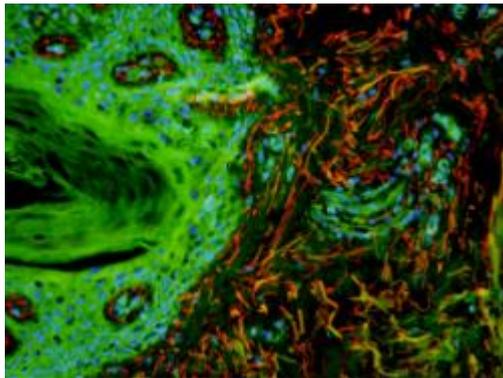
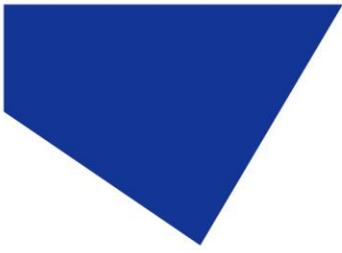




Table of Contents

1.	Introduction	3
2.	Safety Precautions.....	4
3.	pE-300 Series variants	6
4.	Getting Started – System Components.....	8
5.	Installation and setup.....	9
6.	Configuration of LEDs as a White Light Source	11
7.	Operation - Manual Control.....	13
8.	Remote Operation – TTL (pE-300 ^{white} & pE-300 ^{ultra})	17
9.	Remote Operation – USB (pE-300 ^{white} & pE-300 ^{ultra})	23
10.	Optical setup	25
11.	Additional filtering (pE-300 ^{ultra})	27
12.	Settings / Additional Information	29
13.	Routine Care and Maintenance	32
14.	Fitting the pE-300 Series Illumination System to a different microscope	33
15.	Product specifications	34
16.	Product options and order codes.....	35
17.	Warranty and Repairs	35
18.	Compliance and Environmental	35
19.	Contact Details	36
20.	Appendix 1	37



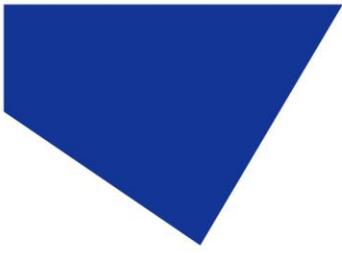
1. Introduction

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 Series 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



2. 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.

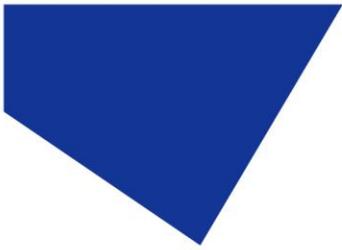
If for any reason the Light Source is to be operated when not attached to a microscope, all personnel should wear eye shielding and clothing to protect the exposed skin.

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.



2.6.

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:

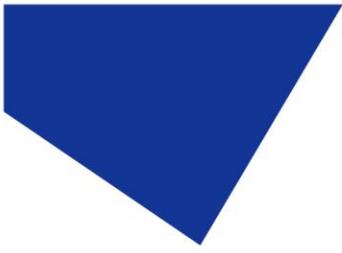
EN/IEC 61010-1:2010	Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory use.
EN62471:2008	Photo-biological Safety of Lamps and Lamp Systems/Guidance on manufacturing requirements relating to non-laser optical radiation safety. Risk Group 3.

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.



3. 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.

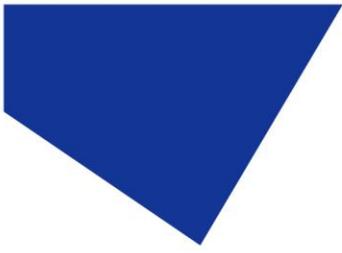




3.3. pE-300^{ultra}

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). One method of TTL control that is unique to the pE-300^{ultra} is the ability to control a multi-channel sequence using a single TTL signal via the Sequence Runner feature. The pE-300^{ultra} also allows additional excitation filters to be placed in the light path of each of the three channels.



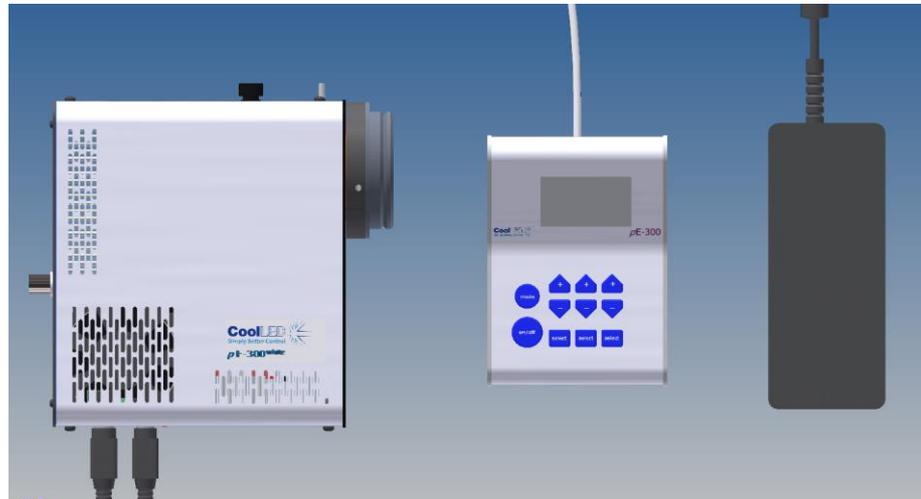


4. Getting Started – System Components

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).

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



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



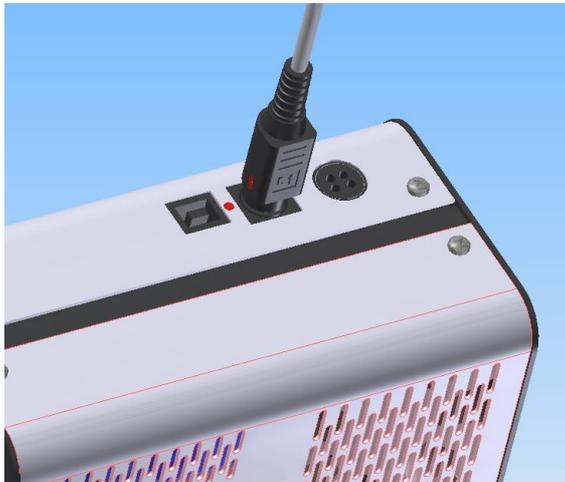
5. Installation and setup

5.1.

Carefully unpack the components from the shipping cartons.

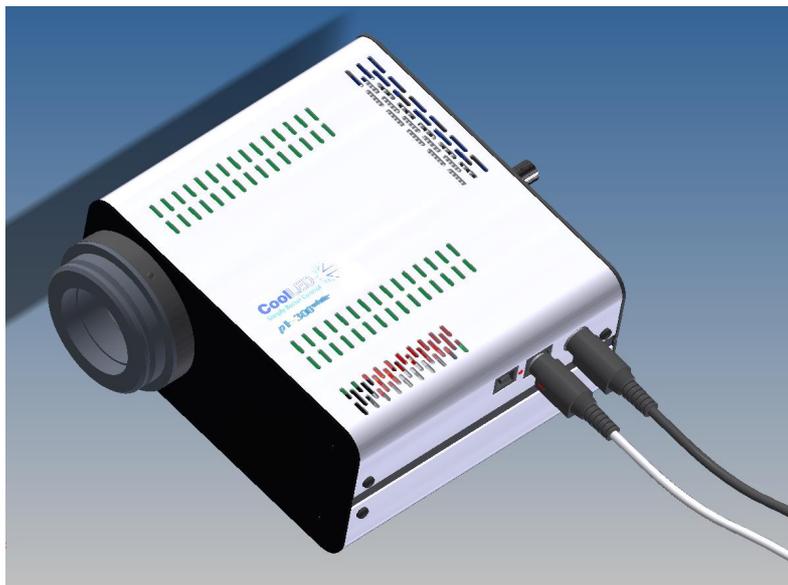
5.2.

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



5.3.

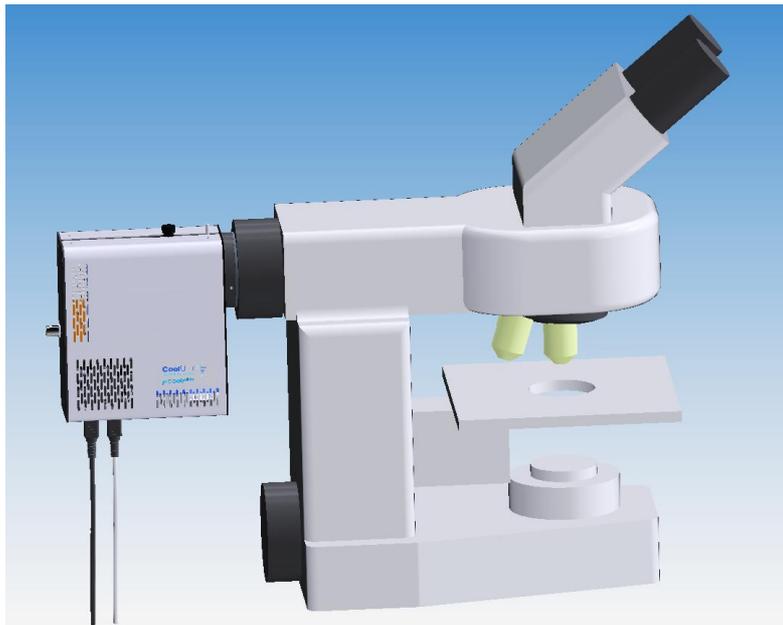
Connect the power connector from the DC power supply as shown. Ensure that the DC power supply is the one supplied with the product. Using non-CoolLED power supplies may damage the Light Source and will invalidate the warranty. At this stage do not connect the mains power lead to the DC power supply.





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 200 mm 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.

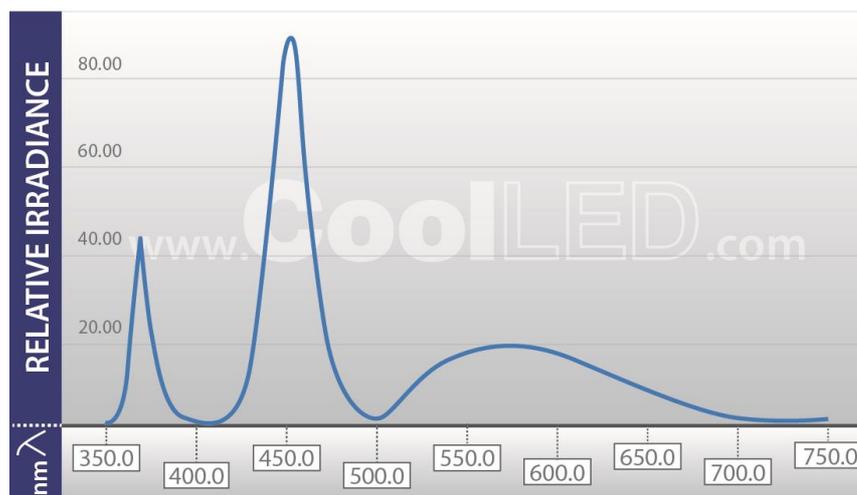


6. Configuration of LEDs as a White Light Source

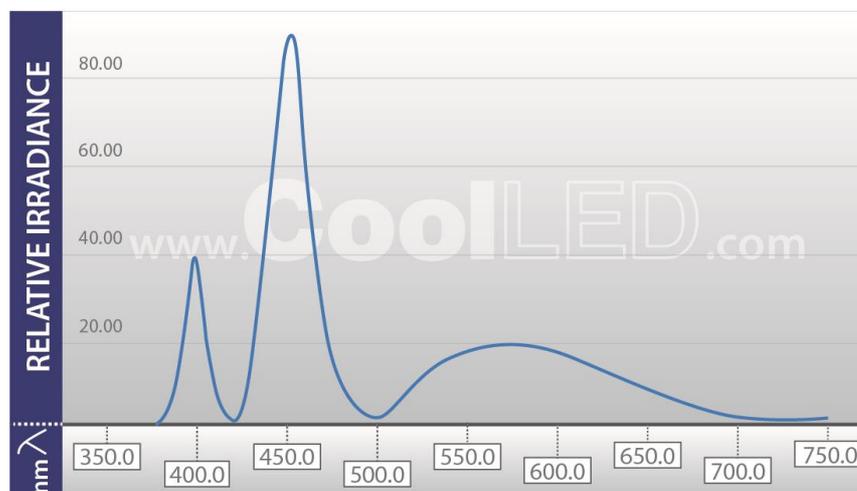
6.1.

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.

pE-300 Series SB Spectrum



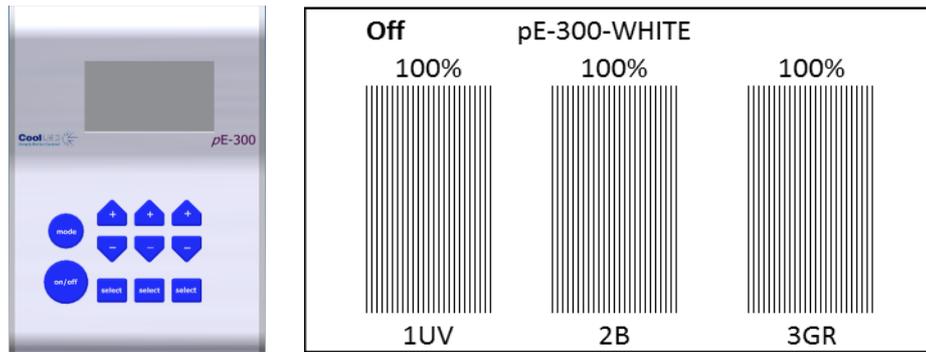
pE-300 Series MB Spectrum





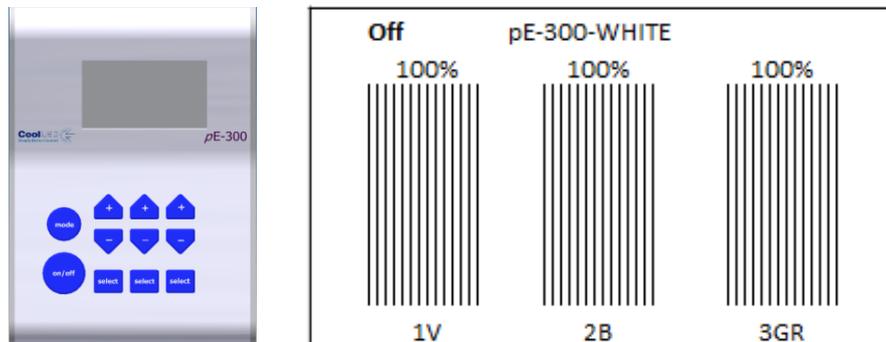
6.2.

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).



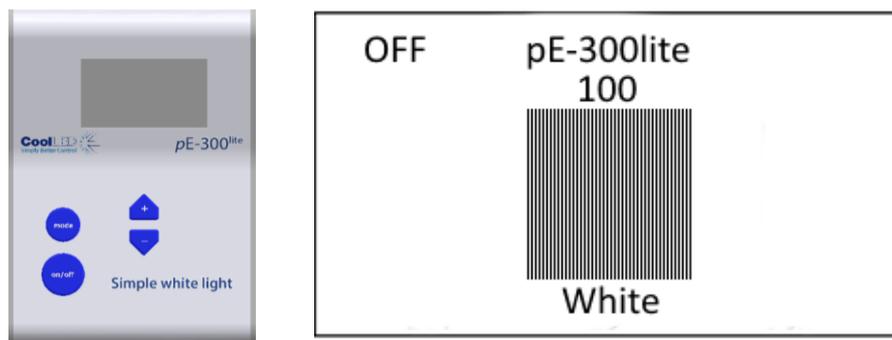
6.3.

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 [Appendix 1](#) 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}

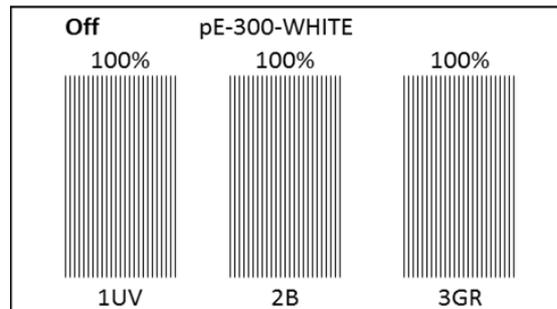
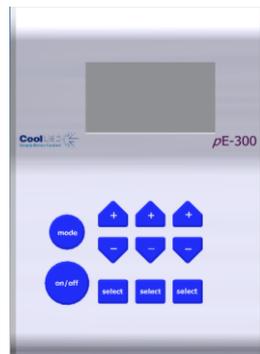
7.1.1.

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.

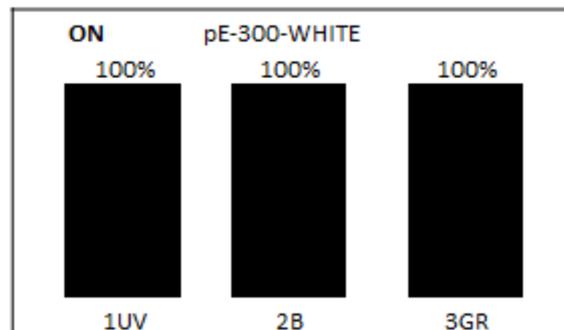
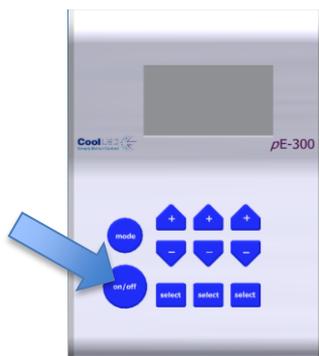
7.1.2.

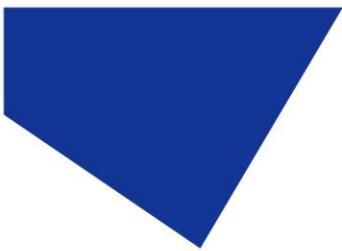
At start-up the Light Source will revert to the same settings that were set when it was last powered down. New Light Sources are supplied with the settings as shown.



7.1.3.

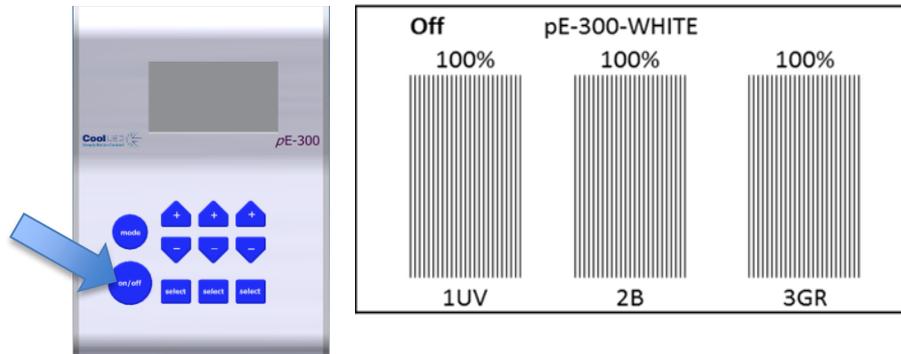
To switch on LEDs press 'on/off' once.





7.1.4.

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



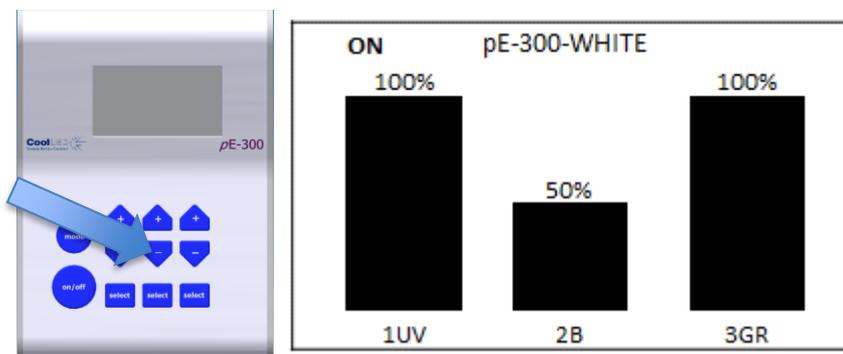
7.1.5.

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 [Appendix 1](#)).

7.1.6.

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

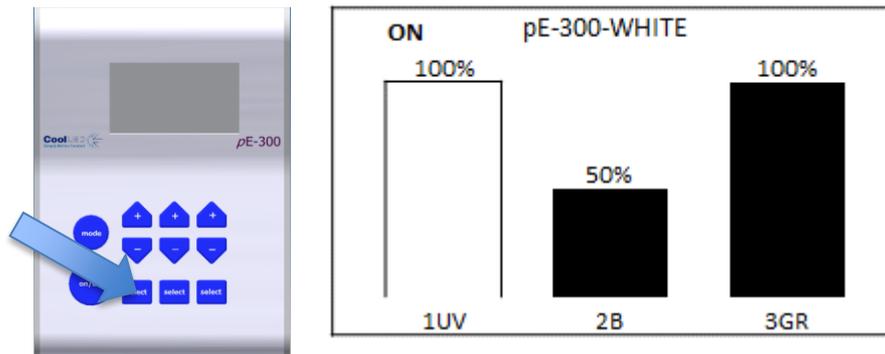




7.1.7.

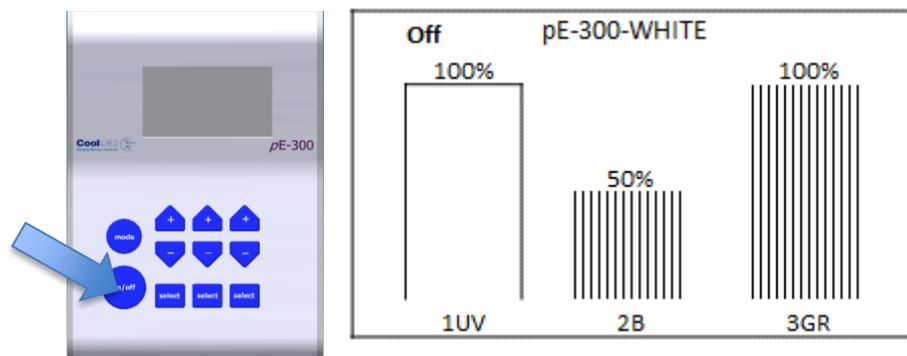
Individual bands can be switched off (de-selected) by pressing the 'select' button. Light is then only generated where it is required to excite the stains in use. This has many attractive benefits with improvements in contrast, cell viability and savings in energy.

Switching off UV will help to reduce damage to cells through photo-bleaching.



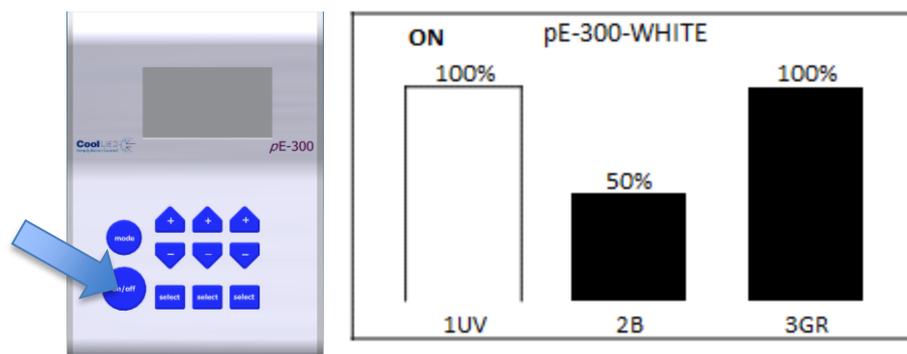
7.1.8.

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



7.1.9.

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

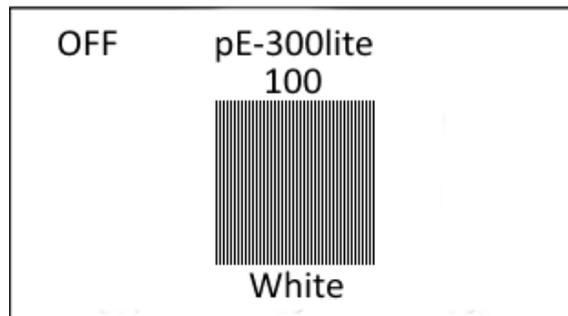
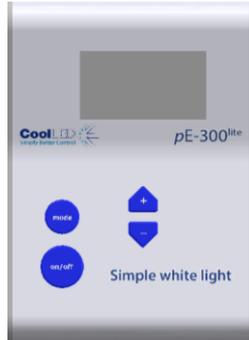




7.2. pE-300^{lite}

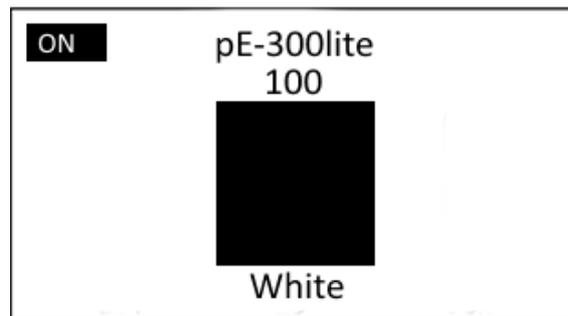
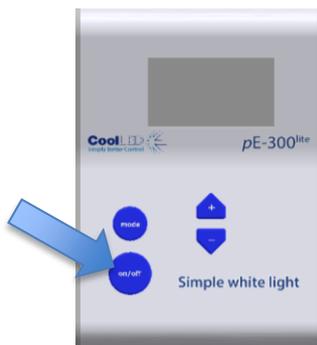
7.2.1.

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



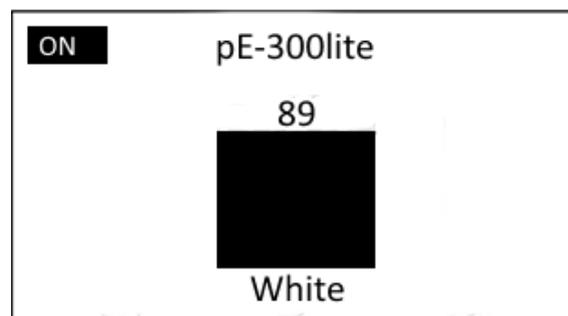
7.2.2.

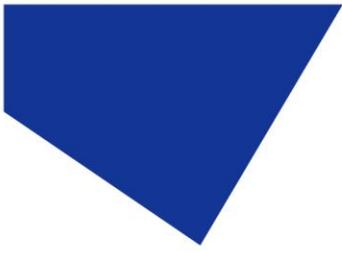
To switch on the LEDs press the “on/off” button once.



7.2.3.

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





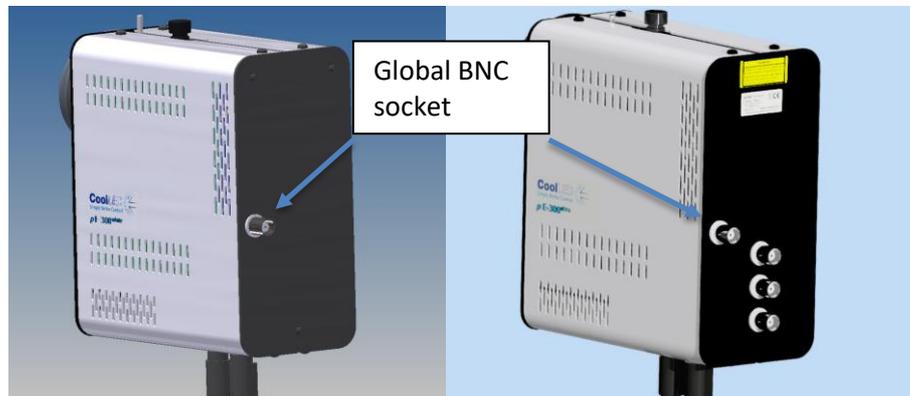
8. Remote Operation – TTL (pE-300^{white} & pE-300^{ultra})

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.



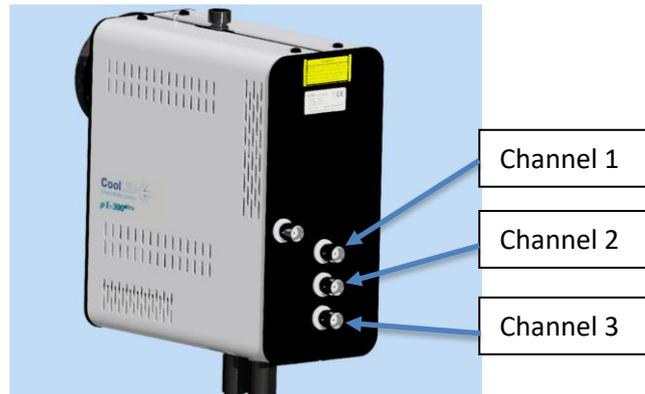
The TTL signal controls the on/off function of the Light Source. A TTL 'high' will cause the LEDs to be on, independent of the state of the on/off button. Only those bands which have been manually selected on the Control Pod (shown by a shaded intensity bar on the Control Pod display) will be switched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



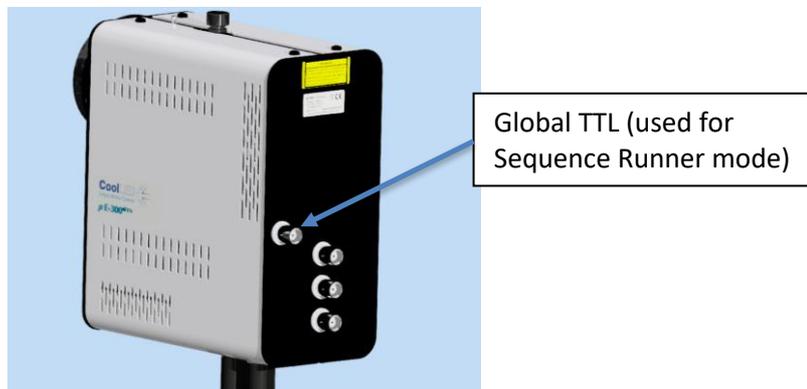
8.2.2.

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.

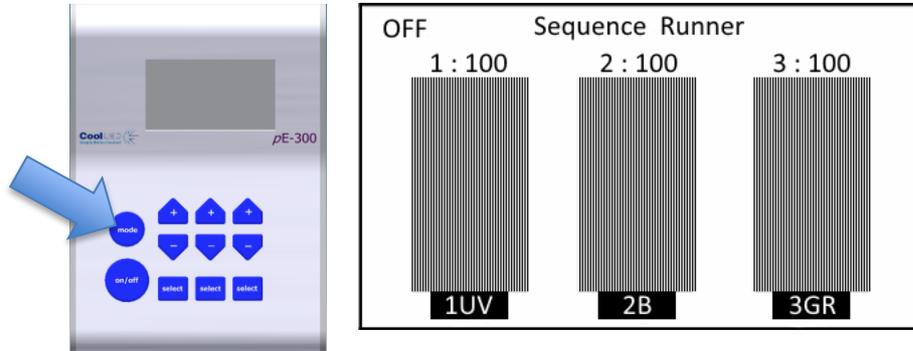
The pE-300^{ultra} allows the Illumination System to be controlled using the Sequence Runner mode. The Sequence Runner allows sequential triggering of multiple channels using a single TTL signal attached to the global BNC socket.



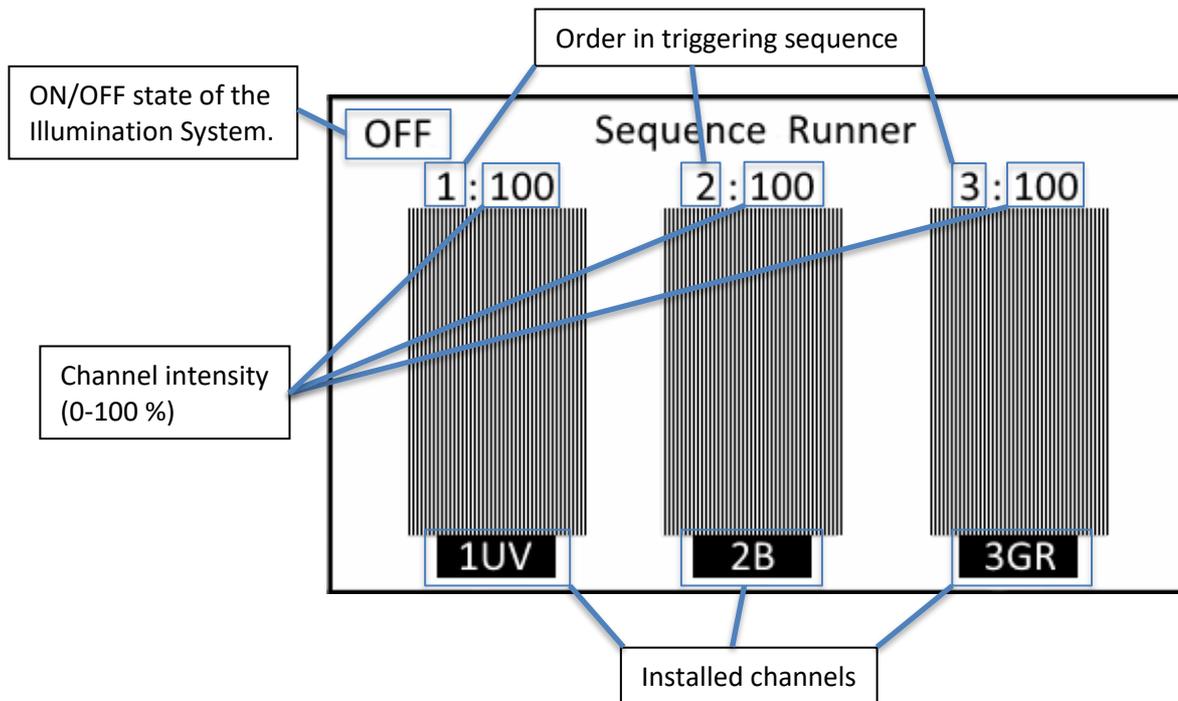


8.3.2.

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



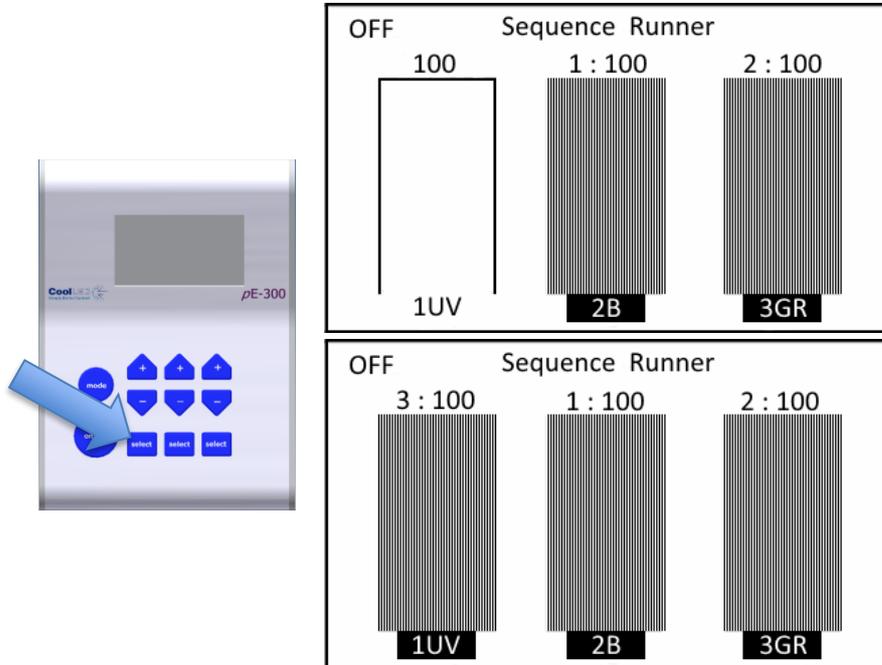
8.3.3.





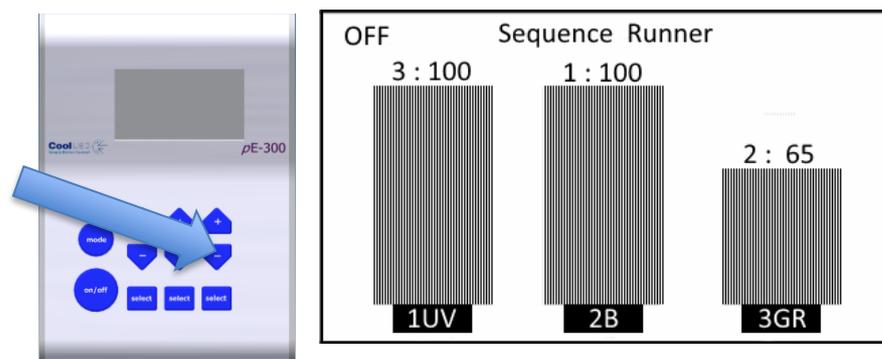
8.3.4.

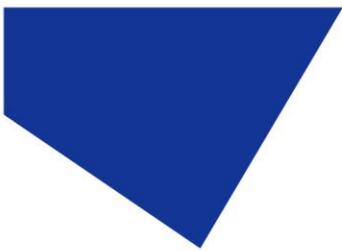
Pressing the channel select button allows you to either deselect a channel or change the order that it triggers in the sequence.



8.3.5.

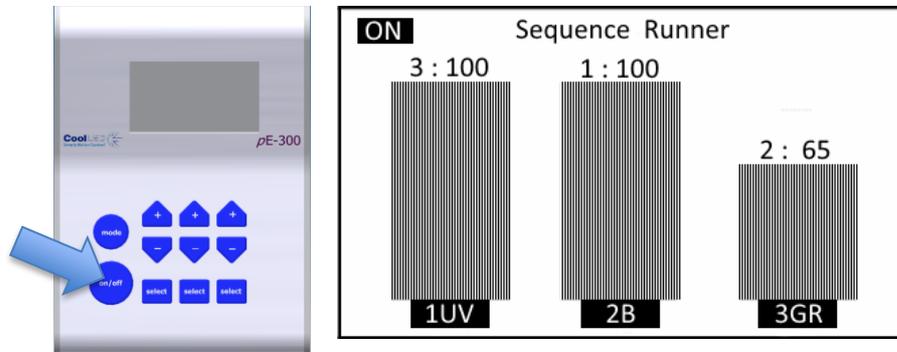
Pressing the + & - buttons on the Control Pod will allow the light intensity of the corresponding channel to be increased or decreased.





8.3.6.

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



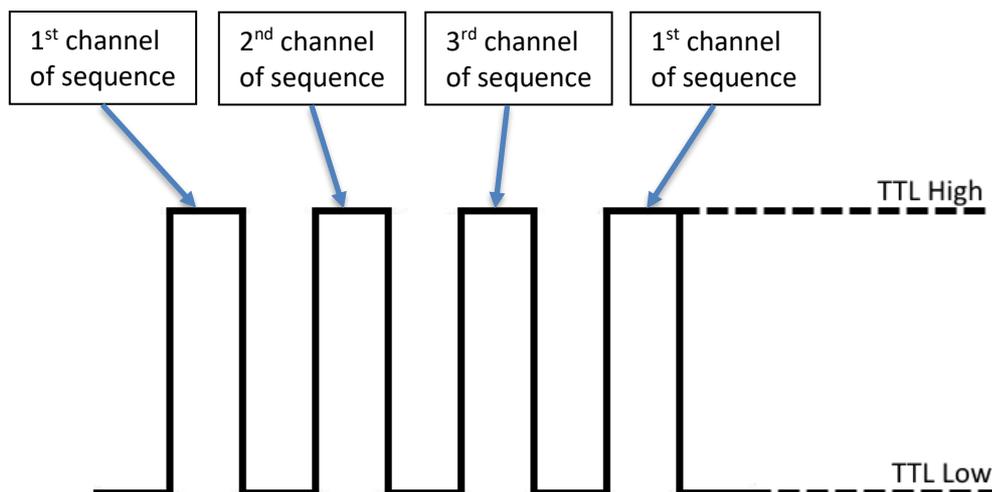
In this example channel 2 will pulse at 100 % intensity, channel 3 will pulse at 65 % and then channel 1 will pulse at 100 %. This Sequence will continue until the ON/OFF button is pressed again to stop the sequence.

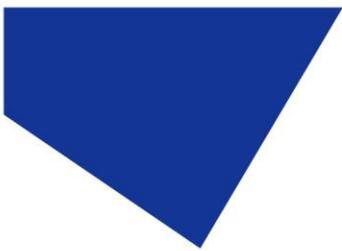
8.3.7.

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.

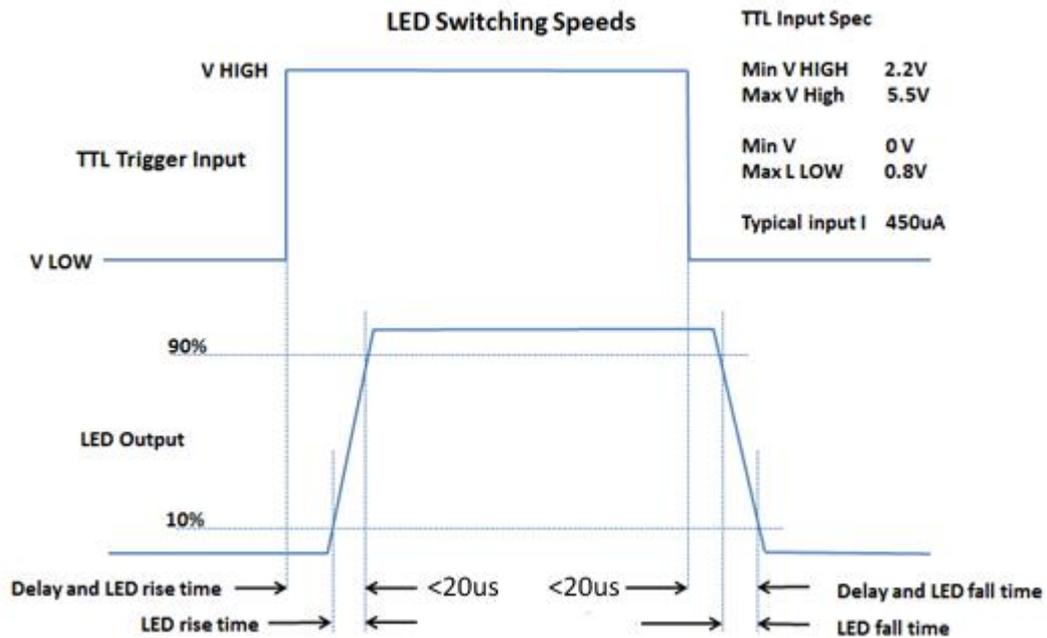




8.4. TTL triggering information

8.4.1.

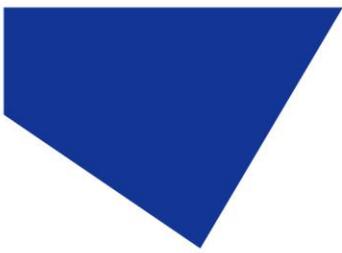
The TTL input circuit has been designed to maximise the switching speed of the LEDs to give the user precise control of the excitation light reaching the sample.



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.

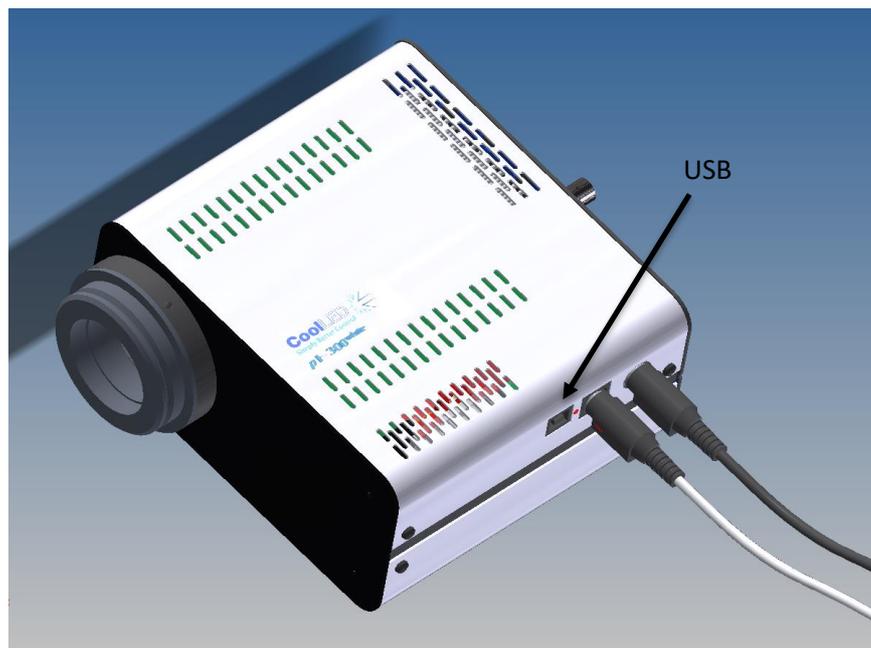
With fast repetitive switching, the Control Pod display will not be able to respond at the same speed. This can occasionally result in the Control Pod displaying the incorrect ON/OFF state. If this happens, simply press the ON/OFF button to reset the state on the display.



9. 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.



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.

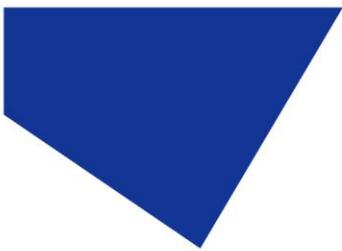
9.3.

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:

<https://www.coolled.com/support/imaging-software/>

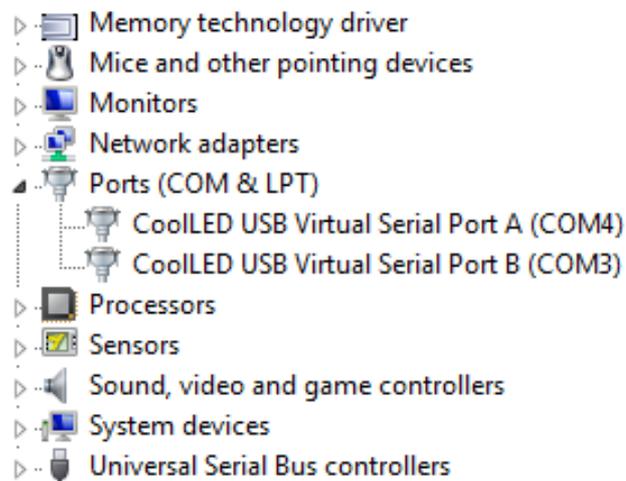


9.5.

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 support@cooled.com and request access to this information.

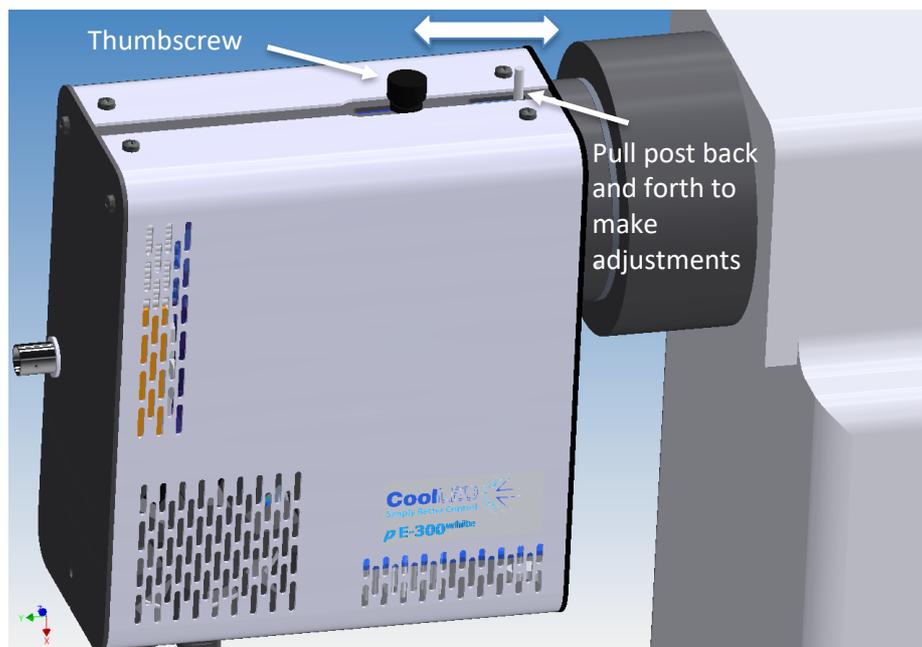


10. Optical setup

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.

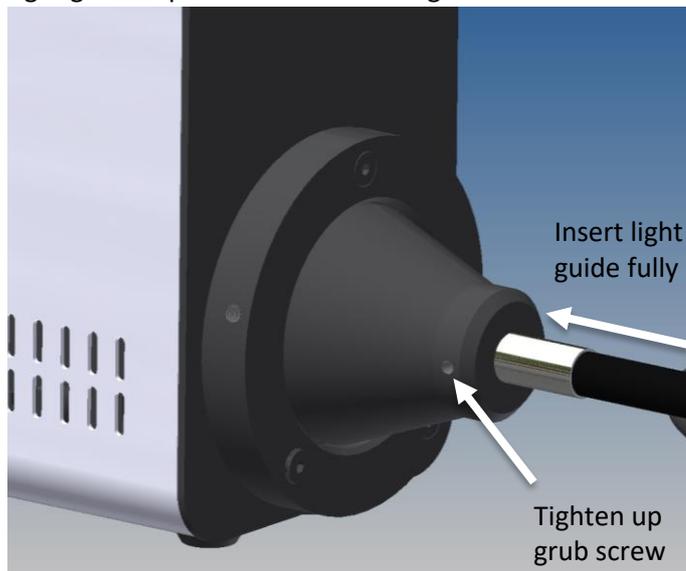
To make the adjustment, set up a typical sample on the microscope that gives an image over the whole field of view. Loosen the thumbscrew and slide the post back and forth until you achieve the maximum brightness with an even field of view. Tighten the thumbscrew to prevent the setting from changing.



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.



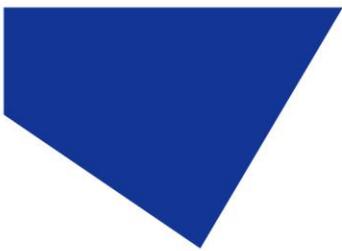
10.2.2.

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

10.2.3.

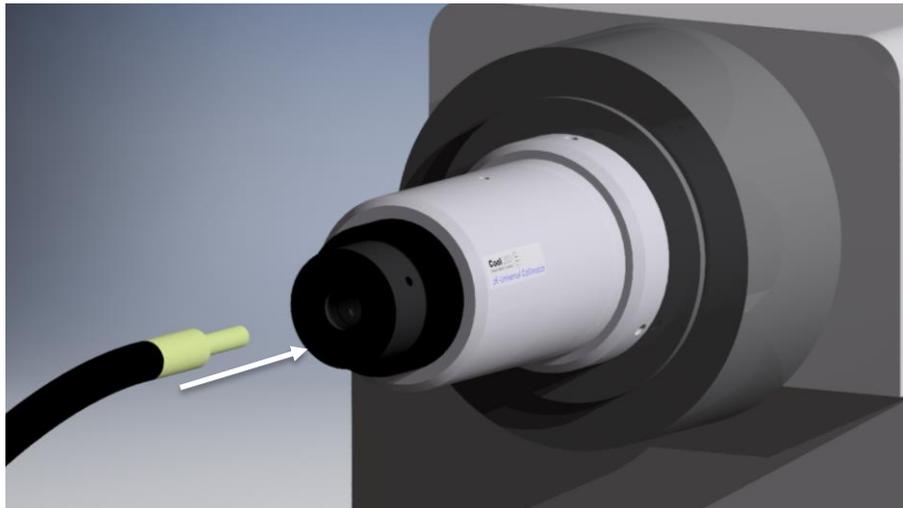
The pE-300 Series Illumination Systems with liquid light guide output are provided with a 'cradle' to ensure that they remain in a stable position during operation, as shown in the image below.





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 [Product options and order codes](#) for details.



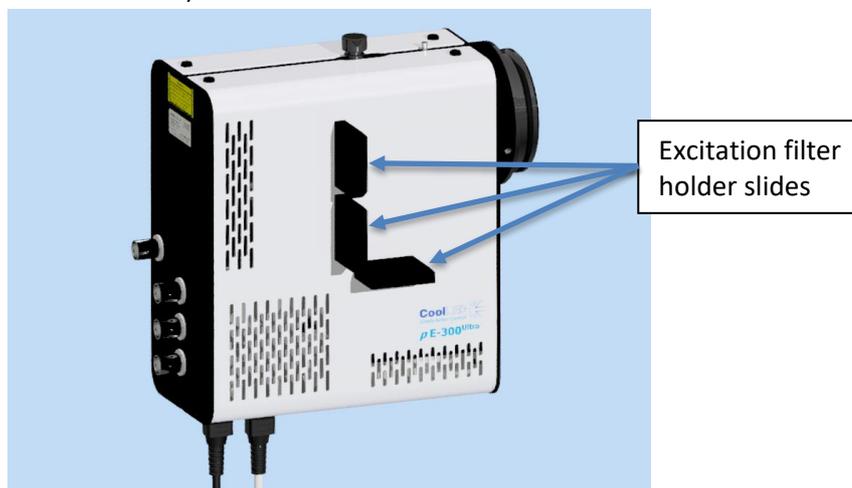
10.2.5.

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.

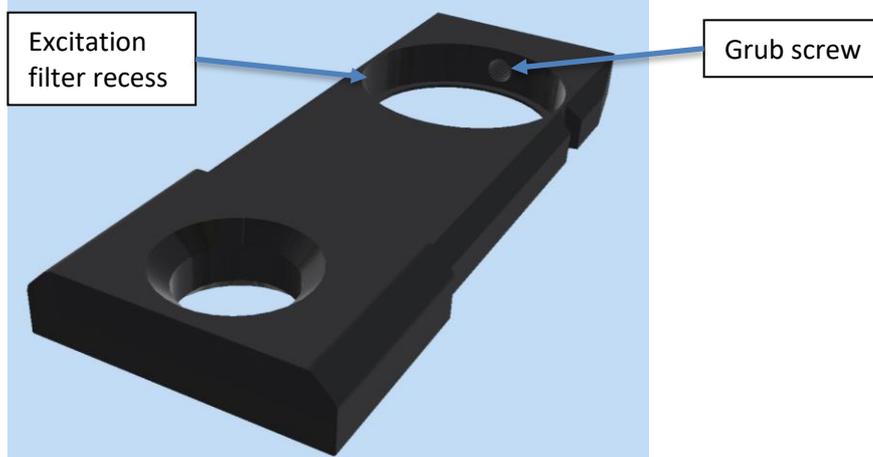
The pE-300^{ultra} allows additional filtering of excitation light through the use of three excitation filter holder slides (one in the optical path of each of the three channels).





11.2.

The excitation filter holder slides accept a standard 25 mm 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.

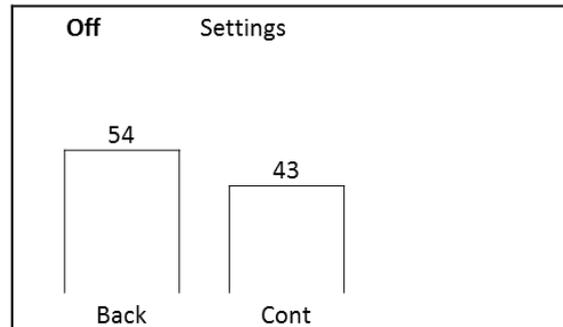
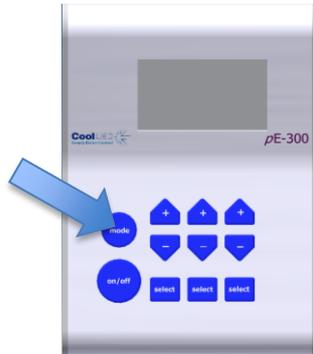
To install excitation filters in the optimal orientation, the direction of light through the Light Source should be observed. This is shown in the image below with arrows.





12. Settings / Additional Information

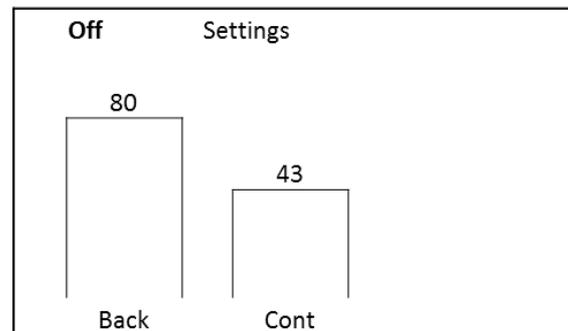
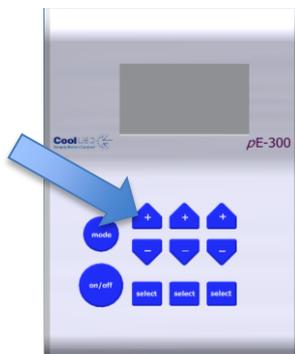
12.1. Display Backlight and Contrast settings



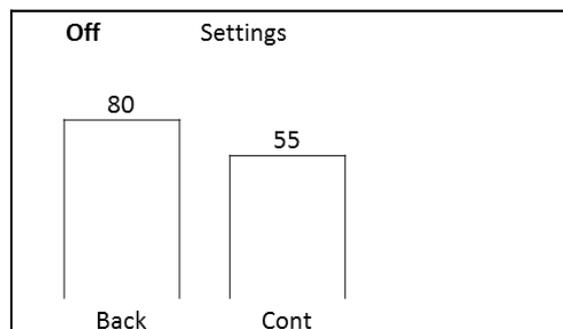
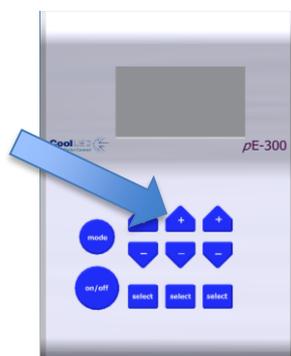
The Control Pod display settings can be adjusted to suit the lighting environment that the instrument is being operated in. To make adjustments, press and hold the 'mode' button for 3 seconds.

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

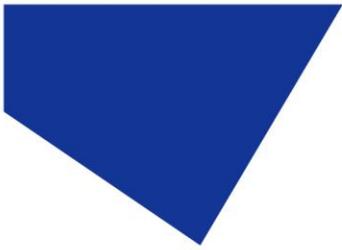
Use the first column up/down buttons to adjust the backlight to the desired level.



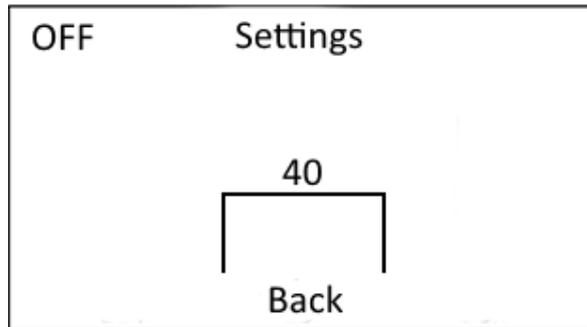
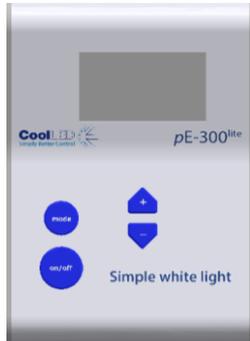
Use the second column up/down buttons to adjust the display contrast as required.



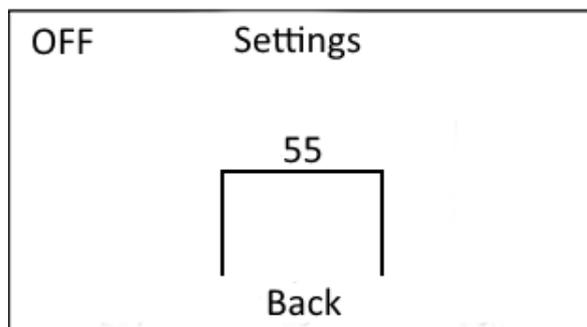
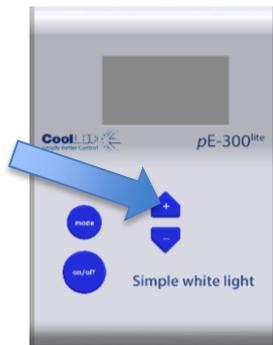
To return to the main screen, either press and hold the mode button for 3 seconds again or wait 10 seconds for the screen to automatically return.



12.1.2. pE-300^{lite}



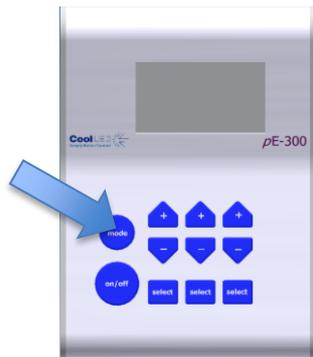
Use the “+” and “-” buttons to increase and decrease the backlight intensity. To return to the main screen, either press and hold the mode button for 3 seconds again or wait 10 seconds for the screen to automatically return.





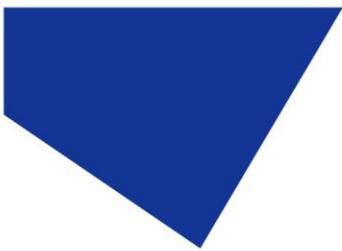
12.2. System Information

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 12.1, release the 'mode' button and then give it a second short duration press. The following display will appear.



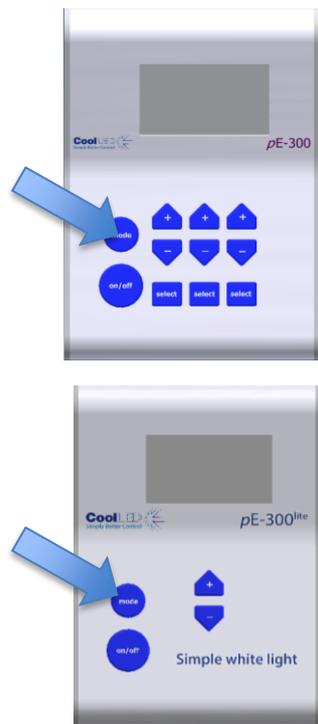
Off	Info
Model:	pE-300-Wh
Serial:	
Firmware:	1.0.10
Hardware:	1
Pod Ver:	1.0.6
Pod H/W:	3

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 12.2 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
	GYR:	0.8h

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

OFF	Info 2	
LED Usage:		12.2h

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 may be 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.

Fingerprints 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.

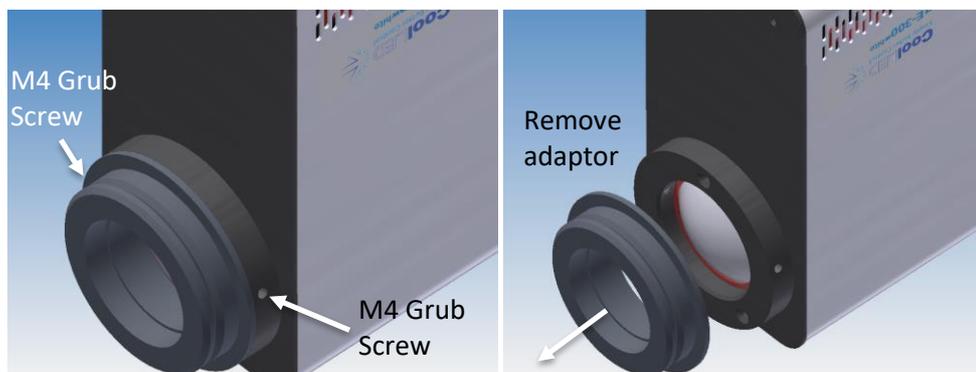
The pE-300 Series can be easily fitted to most fluorescence microscopes, both new and old. Every microscope manufacturer has one or a number of methods of attaching the fluorescence light source. CoolLED has designed a comprehensive range of adaptors to match these microscopes.

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@cooled.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.





14.4.

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: www.coolled.com/product-detail/adaptors-2/

14.6.

The simple optical setup procedure will need to be followed when fitting the pE-300 to a different microscope. See the [Optical setup](#) section.

15. Product specifications

15.1.

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

15.2.

Power consumption

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

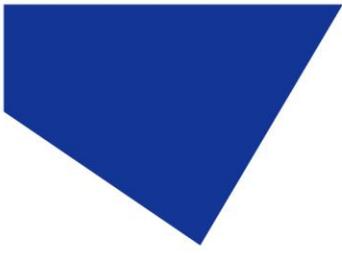
15.3.

Dimensions

Light Source	77 mm (w) x 186 mm (d) x 162 mm (h)
-weight	1.40 kg
Control Pod	88 mm (w) x 125 mm (d) x 37 mm (h)
-weight	0.32 kg
Power Supply	167 mm (w) x 67 mm (d) x 35 mm (h)
-weight	0.62 kg

15.4.

Environmental Operating Conditions
Operating 5 – 35 °C



16. Product options and order codes

See website ([Microscope Illuminators | LED Illumination Systems | CoolLED](#)) 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 <https://www.cooled.com/support/cooled-warranty/>. 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@cooled.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

For current compliance statements and environmental information please refer to our website <https://www.cooled.com/support/environment/>

18.1. 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?



19. Contact Details

CoolLED Ltd
26 Focus Way
Andover
Hants
SP10 5NY
UK

Phone +44 (0)1264 323040 (Worldwide)
 1-800-877-0128 (USA + Canada)

Email info@cooled.com

Online www.cooled.com

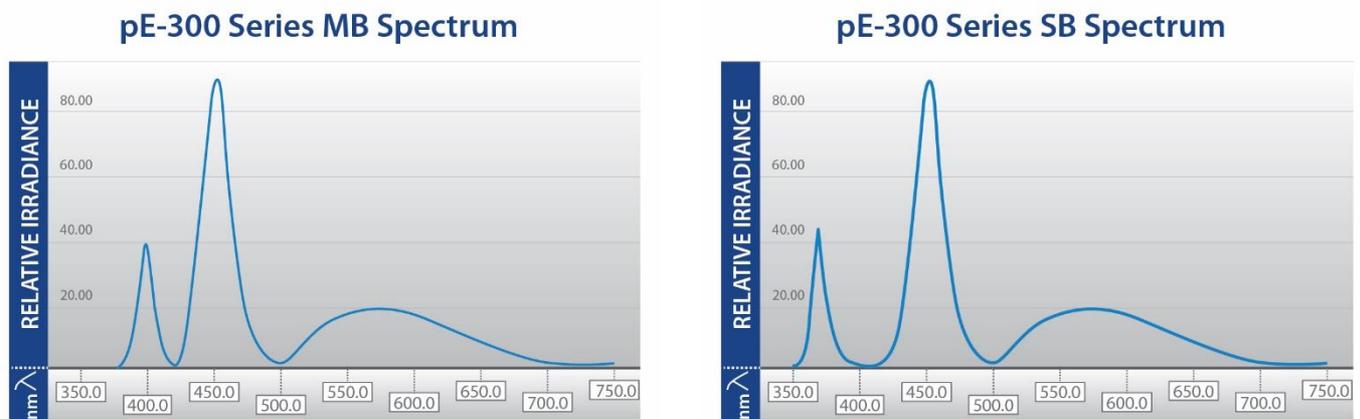


20. Appendix 1

Improved multiplexing for fluorescence microscopy 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.

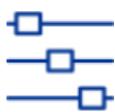
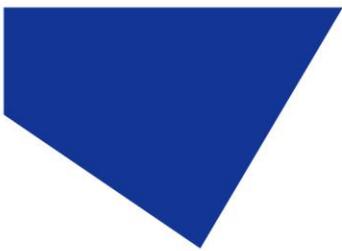


MB (400 nm LED)

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. More information on SB vs MB can be found [here](#).

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 μ s 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](#).

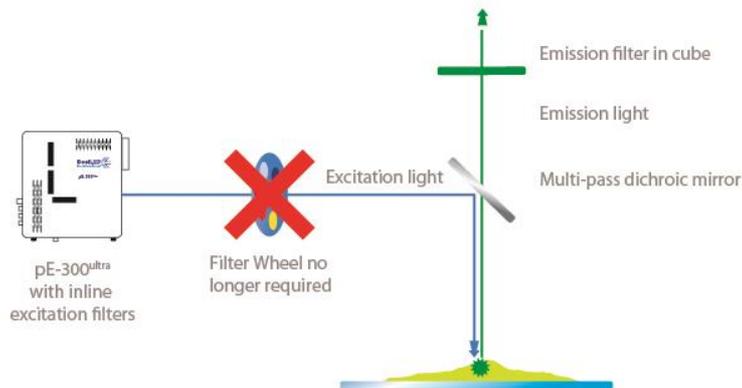
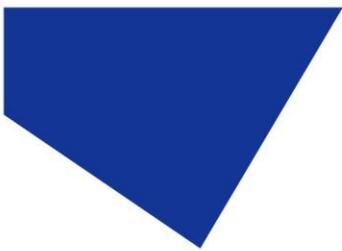


Figure 2: Capturing fast events with an LED Illumination System and Pinkel filter setup.

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.

Find out more information on the pE-300 Series [here](#) or contact us at info@cooled.com
More information on optical filters and a list of recommended filter sets can be found [here](#).

About COOLED 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

