CoolLED
Simply Better Control

User Manual

*p*E-300^{white}

White Light Fluorescence Illumination System

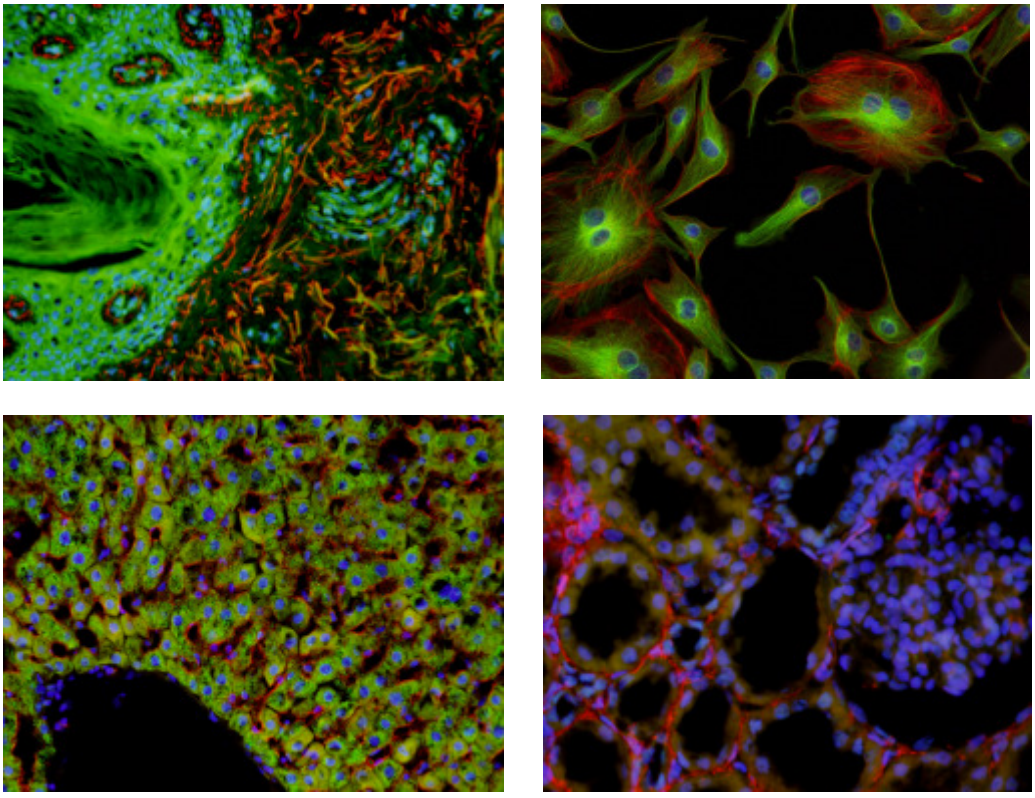


Table of Contents

1 Introduction

CoolLED's *p*E-300^{white} illumination system is designed to offer broad spectrum LED illumination for general use in fluorescence microscopy applications. It 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 *p*E-300^{white} 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 manual should give you all the information required to install and operate your new illumination system.

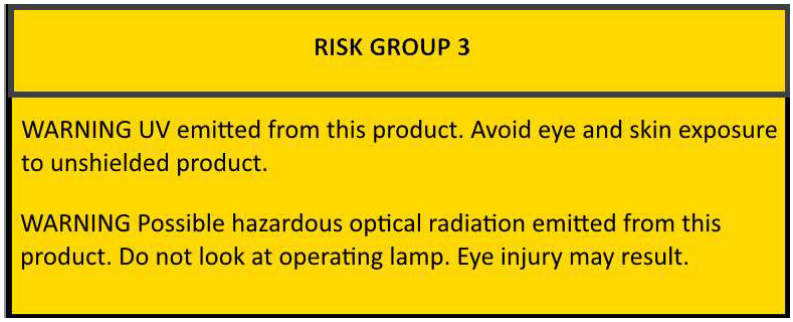
Additional information can be found on our website at www.coolled.com

2 Safety Precautions

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

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

IEC61010-1:2010, EN61010-1:2010	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.



This label is attached to the reverse panel of the light source.

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.

2.1 UV light is emitted from this product. Avoid eye and skin exposure. Never look directly into the light output beam from the LED light source. The emissions could damage the cornea and retina of the eye if the light is observed directly.

2.2 Always ensure that the LED light source is securely attached to the microscope 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. 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.

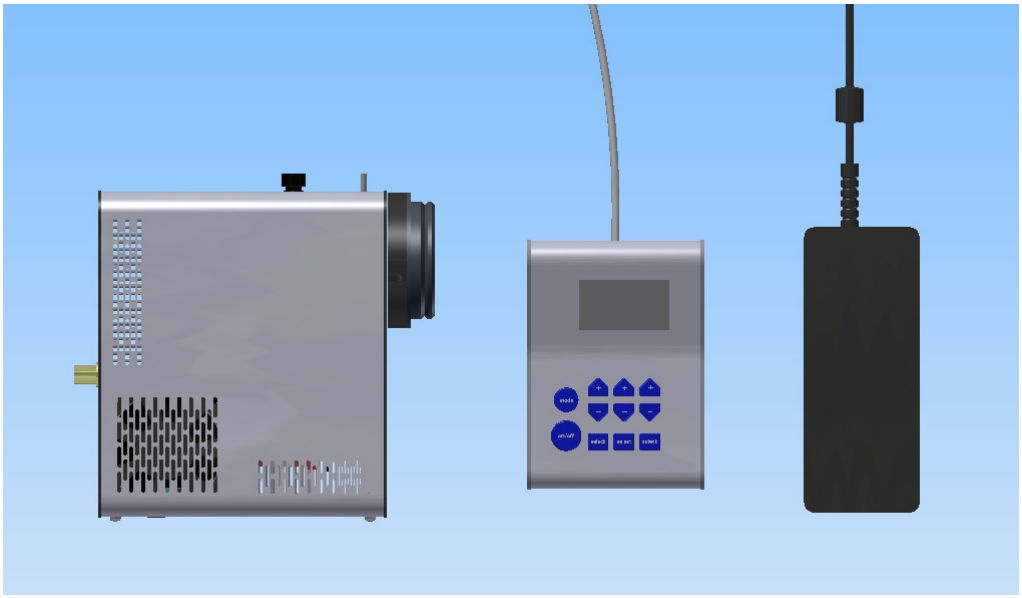
2.6 Any electronic equipment connected to this product must comply with the requirements of EN/IEC 60950.

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.

3 Getting Started – System Components

The CoolLED *p*E-300^{white} Illumination System is supplied with the following components:

1. LED Light Source;
2. Manual Control Pod;
3. Microscope adaptor for specific microscope model (supplied already attached to LED Light Source);
4. DC Power Supply Type GS120 A12;
5. IEC Power Cable (not shown);
6. User Guide (not shown).



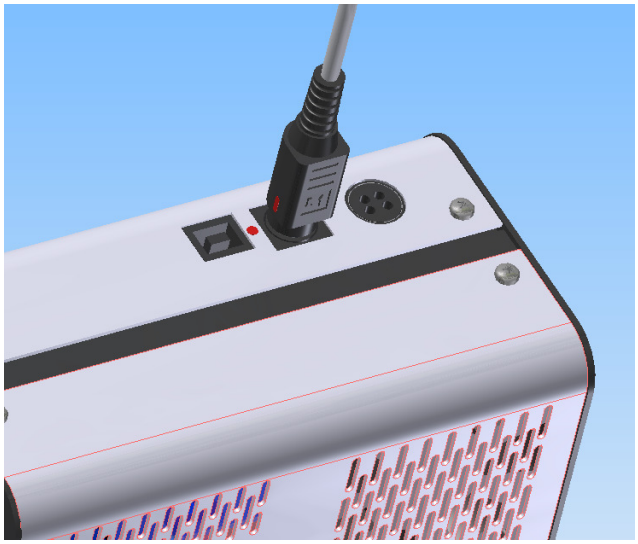
If any components are missing or appear damaged, please contact CoolLED immediately.

4 Installation and setup

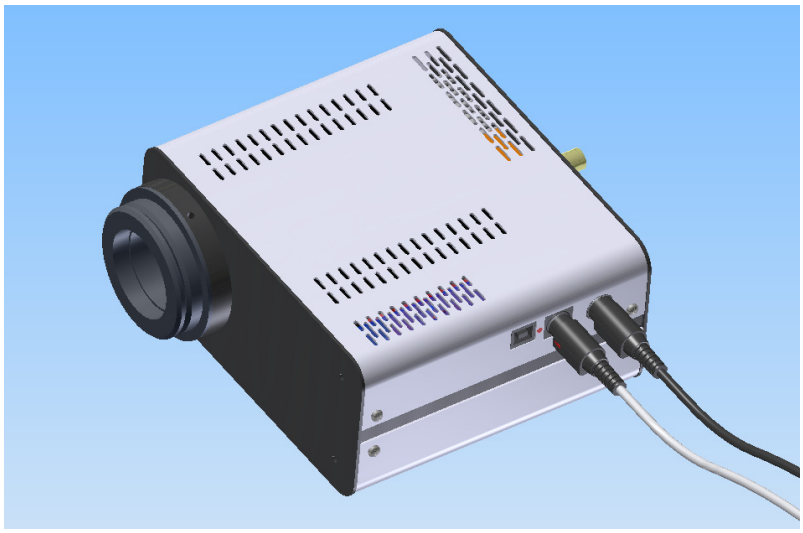
4.1 Carefully unpack the components from the shipping cartons.

4.2 Remove the protective cap from the end of the pod cable connector.

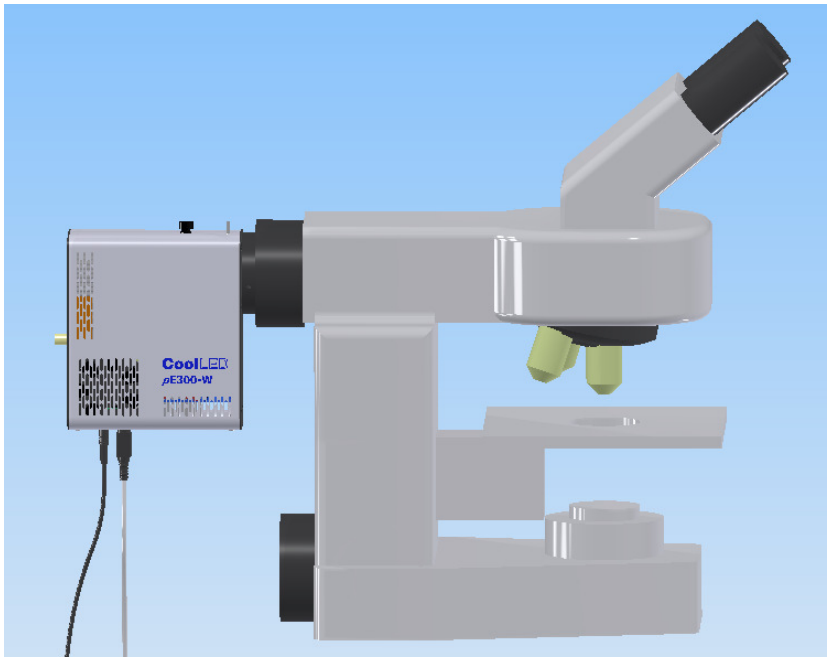
4.3 Insert the pod cable into the LED head using the red dots as a guide for orientation of the plug.



4.4 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.



4.5 Attach the LED Light Source to the epi-fluorescence port on your microscope. Your *p*E-300^{white} will have been supplied with a compatible fitting for the microscope you specified at order. Attach the Light Source ensuring that it is secure and true/flush with the microscope.

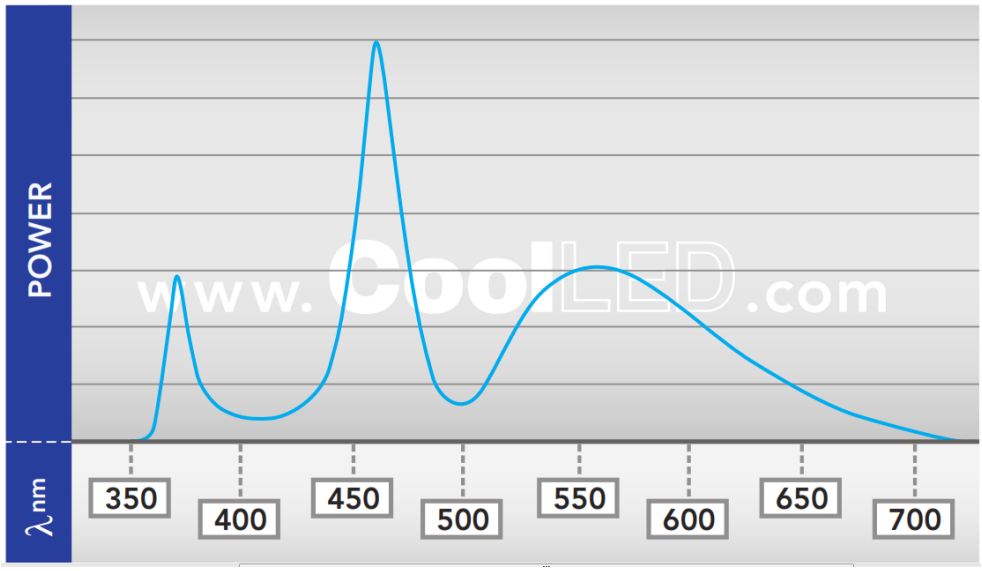


4.6 Ensure that there is free airflow around the LED Light Source so that the cooling system is not impaired. A gap of 200mm on either side is sufficient. The diagram shows the Light Source in the preferred orientation. However it may be set with the cables at the top or at either side.

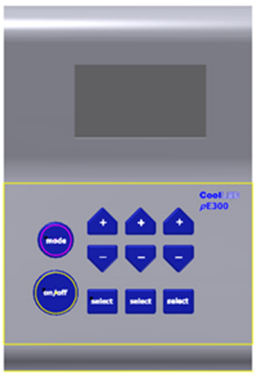
4.7 With the LED Light Source now attached to the microscope it is safe to connect the mains power. Connect the mains lead supplied to a convenient socket, plug in the IEC connector into the DC power supply and switch the power on at the socket.

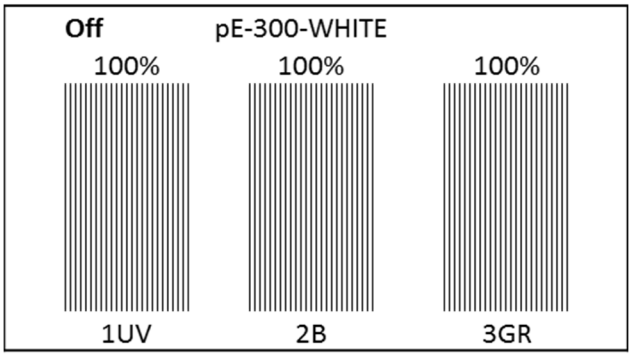
5 Configuration of LEDs as a White Light Source

5.1 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 *p*E-300^{white}, 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.

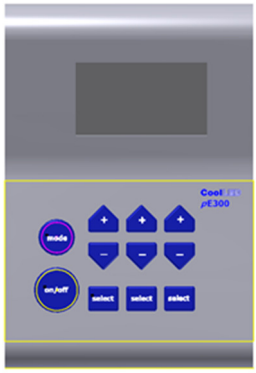


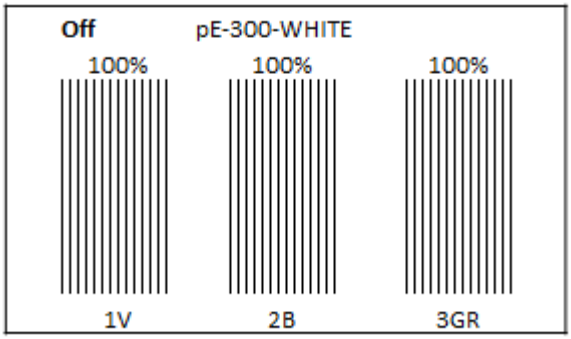
5.2 The *p*E-300^{white} has independent circuits giving the user control of the three main peaks of emissions. On the standard configuration, these are referred to as 1UV, 2B (blue) and 3GR (green, yellow, red).





5.3 There is a special variant of the *p*E-300^{white} 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.



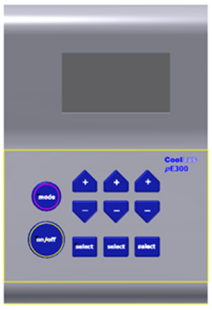


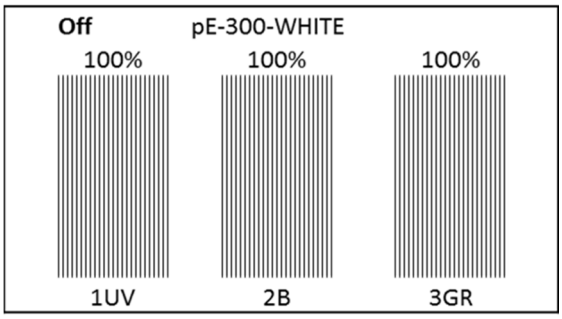
6 Operation – Manual Control

6.1 Manual Control Pod Operation on/off

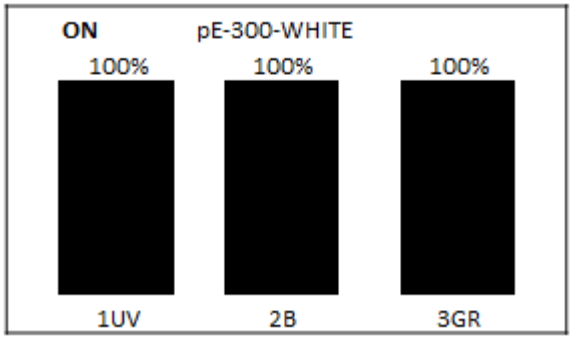
The *p*E-300^{white} is easily controlled from the manual control Pod. LEDs are switched on and off by pressing the ‘on/off’ button.

6.2 Start-up menu. 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.

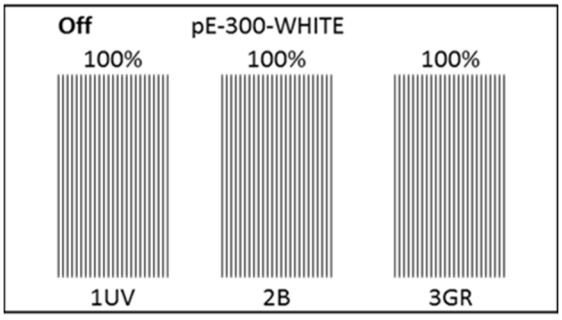




6.3 To switch on LEDs press ‘on/off’ once.



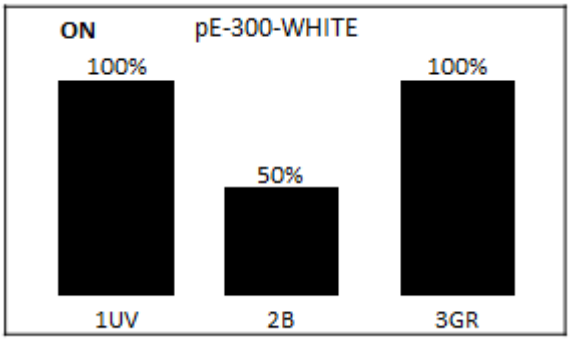
6.4 To switch off the LEDs, press the ‘on/off’ once again.



6.5 Intensity Control.

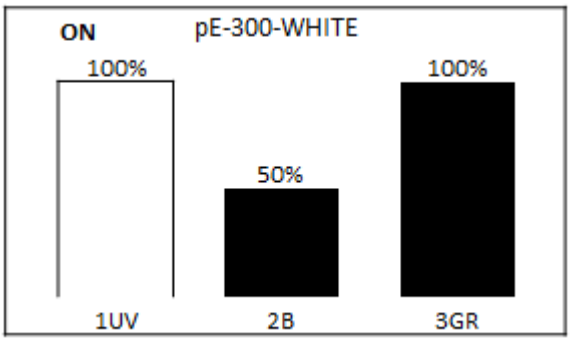
The control pod enables the user to control the intensity of the LEDs that are exciting different stains. This helps to balance the emissions so that one stain does not dominate another. This feature is very useful in multi-band work (see application note in Appendix 1).

6.6 Reduce intensity of one stain by pressing the down intensity button

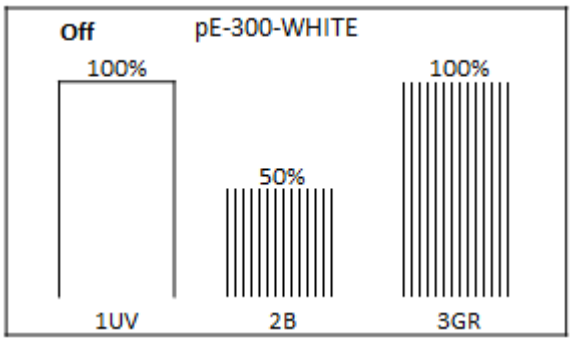


6.7 Individual bands can be switched off (de-selected) by pressing the 'select' button. Light is then only generated where it is required to excite the stains in use. This has many attractive benefits with improvements in contrast, cell viability and savings in energy.

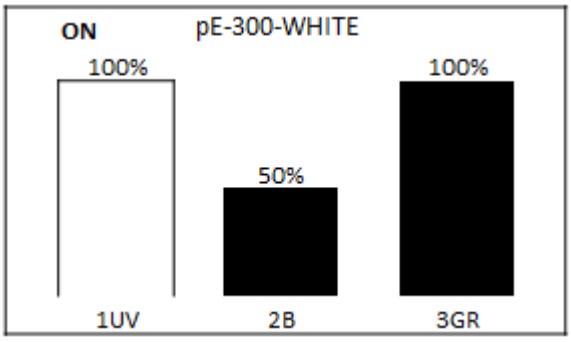
Switching off UV will help to reduce damage to cells through photo-bleaching.



6.8 Switch off selected bands by pressing the ‘on/off’ button.



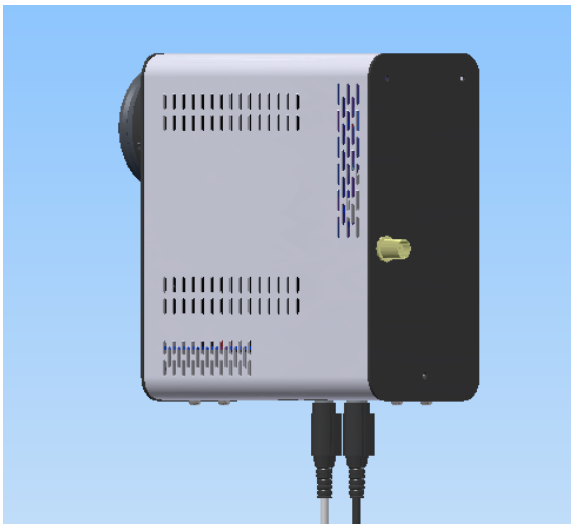
6.9 Switch selected bands back on by pressing ‘on/off’ button again.



7 Remote Operation – TTL and USB

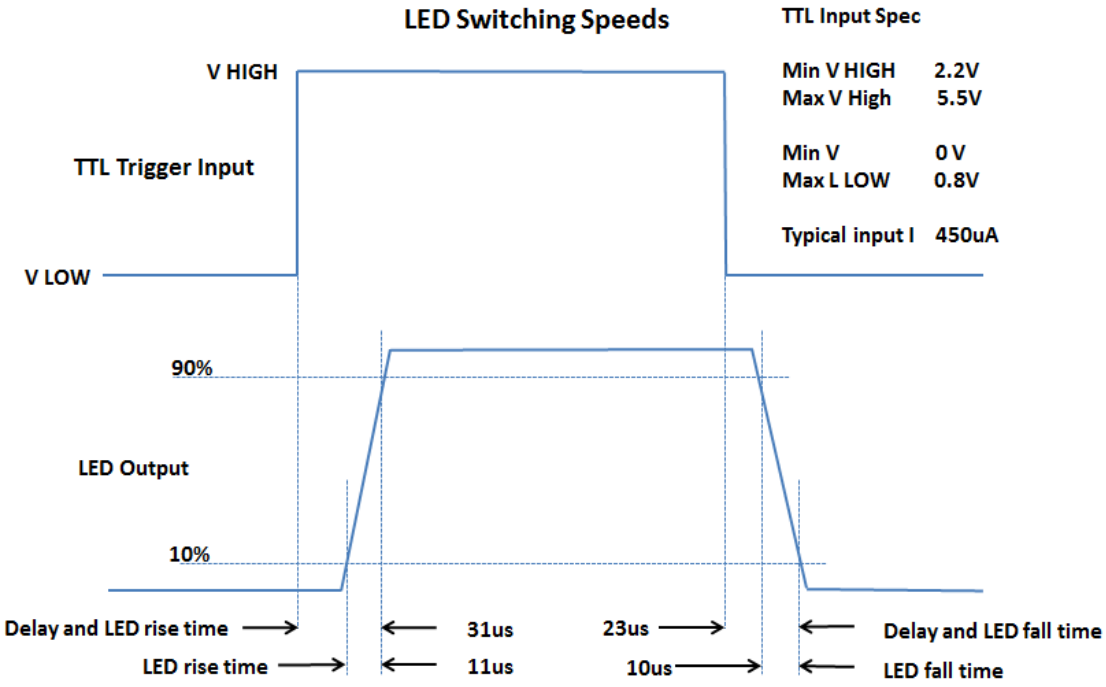
7.1 The *p*E-300^{white} can be controlled remotely via a TTL signal or through software via the USB interface.

7.2 TTL control uses the single BNC socket on the reverse of the Light Source.



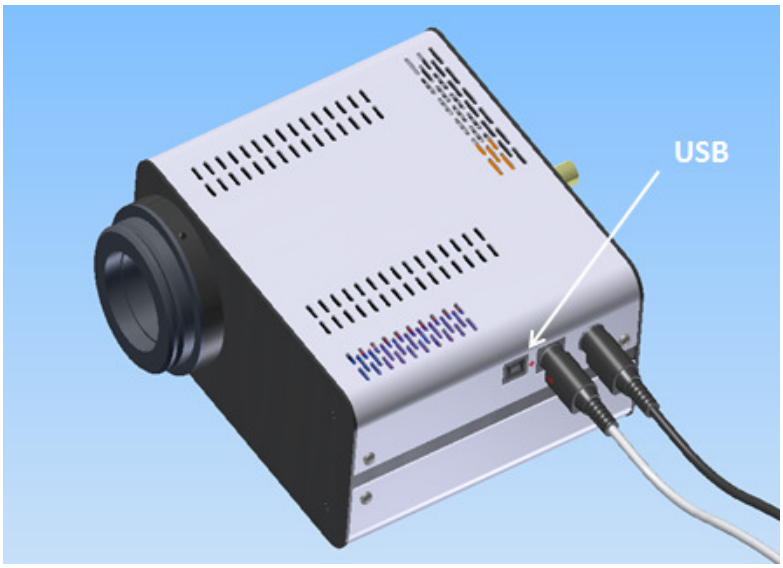
7.3 The TTL signal controls the on/off function of the Light Source. A TTL 'high' will cause the LEDs to be on, independent of the state of the on/off button. Only those bands which have been manually selected on the control Pod will be switched by the TTL signal. The intensities of the selected bands are manually set on the control pod.

7.4 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.



7.5 With fast repetitive switching, the Control pod display will not be able to respond at the same speed and so can get out of synch. If after a train of pulsing, the display on the Control pod indicates that the LEDs are on while they are actually off, simply press the 'on/off' button to reset the display correctly.

7.6 For remote control using software connection between the host computer and the *p*E-300^{white} uses the USB interface with the Light Source having a Type'B' connector socket located adjacent to the Control Pod socket.



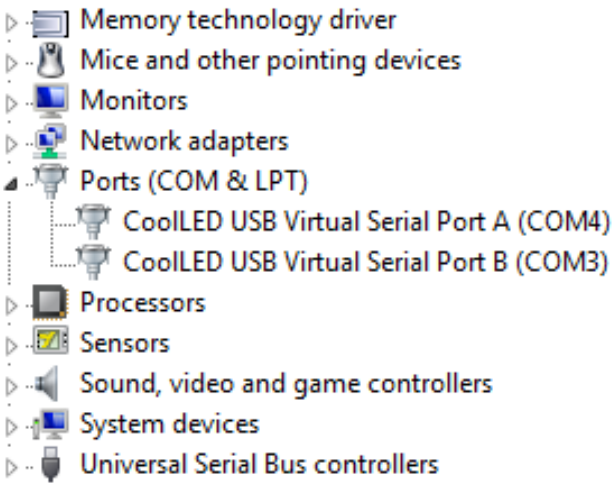
7.7 Connect the Light Source to your computer using a USB cable. As with all USB remotely controlled devices, it will be necessary to set up the driver files on your system to allow the *p*E-300^{white} to be recognised.

7.8 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.

7.9 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/

7.10 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.

7.11 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).

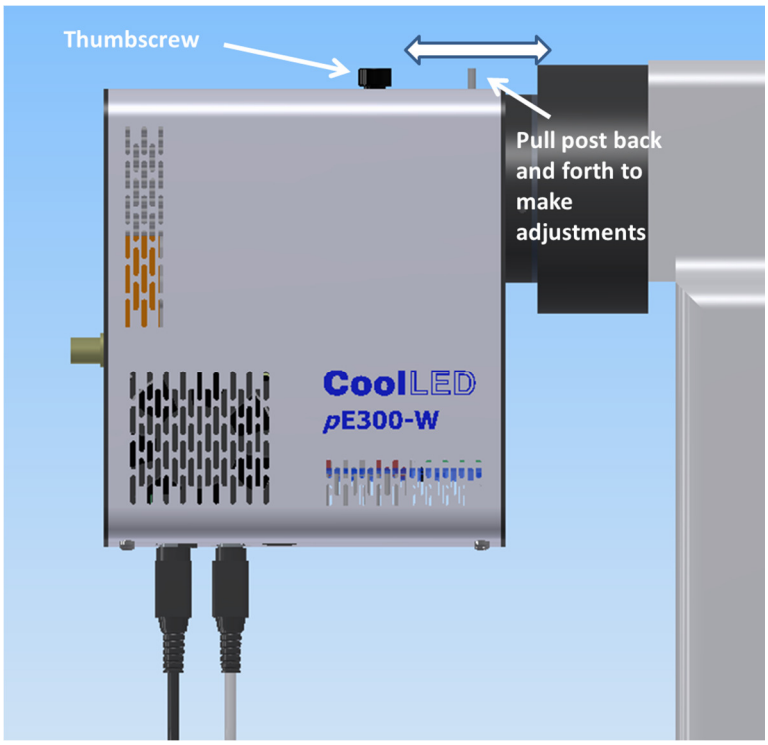


In this example the *p*E-300^{white} has been assigned two COM ports, COM3 and COM4. You may need this information to connect to the light source from your software control package. Either COM port may be used for control. Two COM ports have been assigned to allow for diagnostics to take place in parallel with communication and also allow for dual communication should that ever be desired.

7.12 The majority of microscopy imaging software systems have integrated the *p*E-300^{white} 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@coolled.com and request access to this information.

8 Optical setup

8.1 The *p*E-300^{white} 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 *p*E-300^{white} 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.



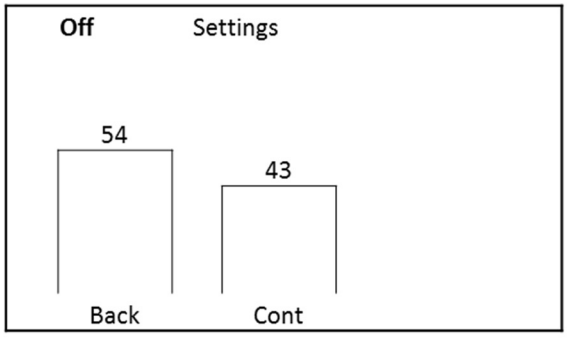
8.2 To make the adjustment, set up a typical sample on the microscope that gives an image over the whole field of view. Loosen the thumbscrew and slide the post back and forth until you achieve the maximum brightness with

an even field of view. Tighten the thumbscrew to prevent the setting from changing.

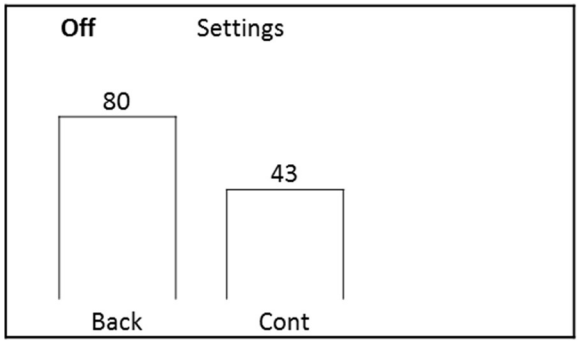
9 Settings / Additional Information

9.1 Display Backlight and Contrast settings.

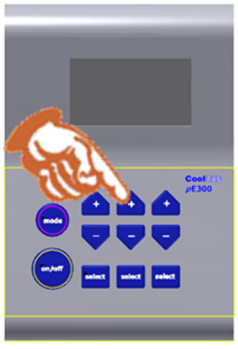
The Control pod display settings can be adjusted to suit the lighting environment that the instrument is being operated in. To make adjustments, press and hold the 'mode' button for 3 seconds. The following display will appear on the Control pod screen.

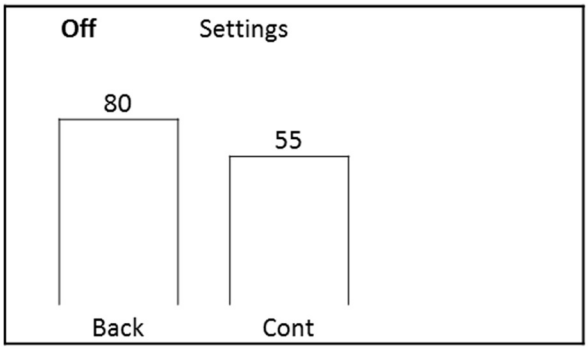


Use the first column up/down buttons to adjust the backlight to the desired level.



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.

9.2 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.

9.3 LED hours 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
GYR:	0.8h

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.

10 Troubleshooting

The *p*E-300^{white} is a relatively simple system and is easy to operate. The following is a checklist should the product not operate as expected.

10.1	Symptom:	Light Source does not power up, nothing appears on the control pod screen.
	Check:	Is the power connector fully inserted in the *p*E-300^{white} Light Source? Is the mains lead fully inserted in the DC power supply? Is the mains supply switched on?
10.2	Symptom:	Display does not respond to any button presses.
	Check:	Is the Control pod connector fully inserted in the *p*E-300^{white} Light Source? Was the Control pod connector inserted after power up? If so, power down and power up again once Control pod connector is fully inserted to reboot the system.
10.3	Symptom:	LEDs fail to switch on with message on screen 'Hot Lams'.
	Check:	LEDs are over heating so check that there is sufficient clearance for airflow around the *p*E-300^{white} Light Source (see section 4.6). Check that the ambient temperature is below the maximum operating temperature (see section 14.4). Check that there are no local heat sources close to the Light Source.
	Check:	Listen for the fan switching on. It should not run continuously - only when deemed necessary by the system's thermal management controls. If the fan does not operate with all the LEDs on and set at maximum intensity, then there is an internal fault and the Illumination System must be returned to CoolLED for repair. (see section 16)
10.4	Symptom:	Illumination appears to be weak
	Check:	Are the intensity settings on the Control pod turned up? Has the single optical setup procedure for the *p*E-300^{white}

been followed (see section 8)? Is the microscope set up correctly? Check for shutters and apertures being open and for appropriate filters and cubes. If microscope previously used a mercury or metal halide lamp, check for damage or frosting of optics in light path.

10.5	Symptom:	Illumination on sample is not flat and even.
	Check:	Has the single setup procedure been followed (see section 8)?
10.6	Symptom:	The Illumination is not centralised over the field of view
	Check:	Carefully slacken off the fixing retaining the *p*E-300^{white} on to the epi-port and rotate the Light Source while viewing the field of view. If the illumination offset follows the rotation, then the optics within the *p*E-300^{white} have lost their alignment, possibly through mis-handling and the product will need to be returned to CoolLED for re-aligning. If the illumination offset remains unchanged when rotating the light source then the problem is within the microscope and a person competent with microscope servicing will need to investigate the cause of the problem.

11 Routine Care and Maintenance

11.1 The *p*E-300^{white} requires little or no maintenance throughout its life. There are no field serviceable parts so there is no need to remove the covers.

11.2 Cleaning of the external surfaces can be carried out with a mild soap and water 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.

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

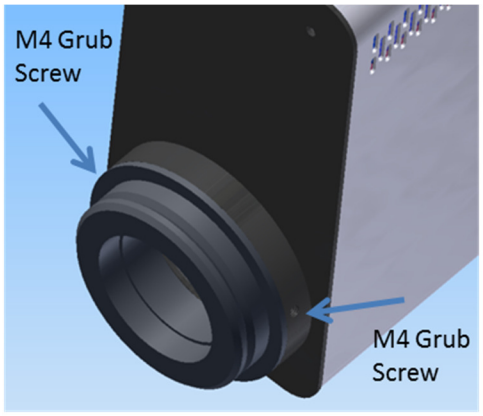
11.4 Finger prints or other liquid type contaminants should be removed using standard lens cleaning procedures. Do not flood the lens surfaces with fluid as liquid could enter the product and cause damage.

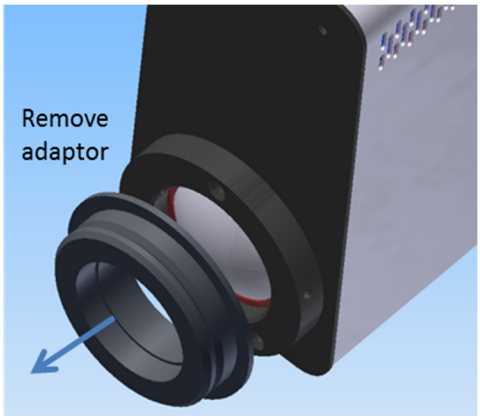
12 Fitting *p*E-300^{white} to different microscope

12.1 The *p*E-300^{white} 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.

12.2 There are a small number of microscopes which require additional optics or special settings internal to the *p*E-300^{white}. 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 an light source needs this modification and ensure that the complete Illumination System is returned.

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





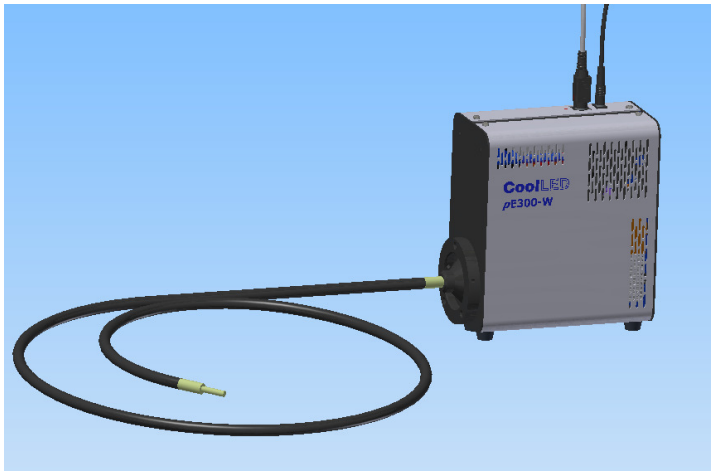
12.4 Fit the new adaptor and tighten grub screws.

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

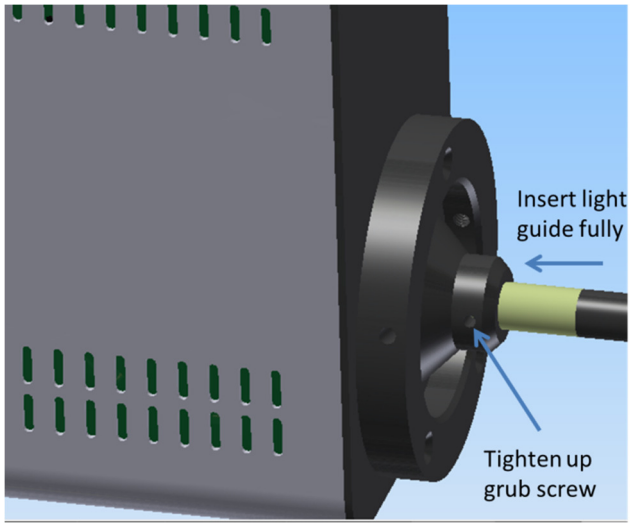
12.6 The simple optical setup procedure will need to be followed when fitting the *p*E-300^{white} to a different microscope. See section 8.

13 *p*E-300^{white} with liquid light guide option

13.1 The *p*E-300^{white} is available for use with a 3mm liquid light guide rather than using a direct attachment adaptor. This enables the Illumination System to be used on microscopes which just have a liquid light guide input, the collimating optics being an integral part of the microscope.

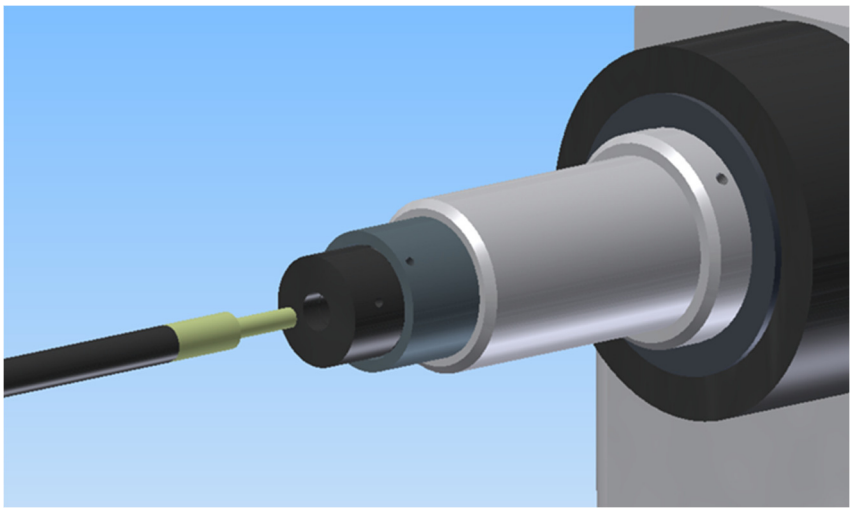


13.2 Fully insert light guide as shown and tighten up grub screw to ensure end of light guide is prevented from sliding out.



13.3 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.

13.4 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 electrical noise close up to the samples. The pE- Universal Collimator is available for these applications. See section 15 for details and order codes.



13.5 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.

14 Product specifications

14.1 Power requirements

110-240Va.c 50/60Hz 2A

14.2 Power consumption

Standby mode	max 2W
White (all 3 bands at 100% intensity)	max 80W
Two bands at 100%	max 63W
Single band at 100%	max 38W

14.3 Dimensions

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

14.4 Environmental Operating Conditions

Operating 5 – 35 deg C

15 Product options and order codes

See website www.coolled.com for full details of product options and order codes.

16 Warranty and Repairs

This product is supplied with a 12 month warranty which is calculated from the date of invoicing, covering defects in materials and workmanship. It does not cover or form the basis for any claims for damages or consequential losses. In addition the warranty covering the LEDs is extended from 12 months to 36 months or 25,000 hours whichever is first. Any repair that is carried out by CoolLED Ltd, or its approved agent is warranted for 100 days.

The warranty does not cover excessive wear and tear, poor handling or any fault caused by operating the product outside the advice of this User Guide.

In the event of the product developing a fault, make contact with support@coolled.com and provide a brief description of the problem. It is useful to have the product serial number available. If a repair is required, a support team member will provide a RMA number to log the incident. All returned units, including warranty claims should be packed up carefully and adequately (preferably in the original packaging), and sent postage and carriage paid to the service centre as specified by the support team member.

17 Contact Details

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	1-800-877-0128	(USA + Canada)
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18 Appendix 1

CoolLED *p*E-300^{white} with Multi-band Filter Sets

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

While the *p*E-300^{white} delivers a wide spectral output covering most filters 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 *p*E-300^{white} 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 *p*E-300^{white} allow the user to vary the brightness of the individual fluorescence stains on a multi-stained 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 *p*E-300^{white} 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 *p*E-300^{white} 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 *p*E-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 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 *p*E-300^{white}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 *p*E-300^{white} Violet, Blue and GYR.

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