

User Manual

pE-2

LED Excitation for Fluorescence

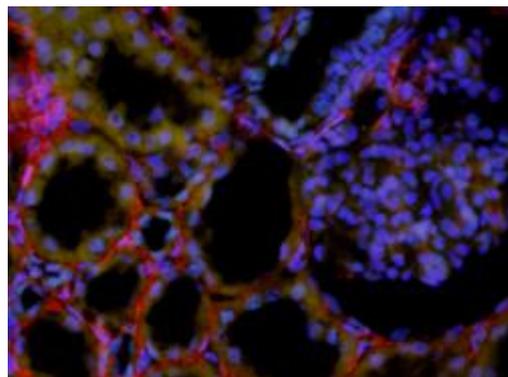
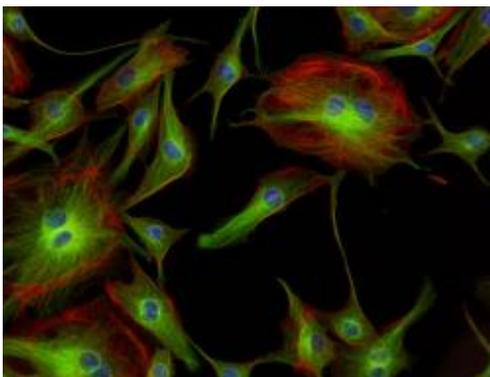
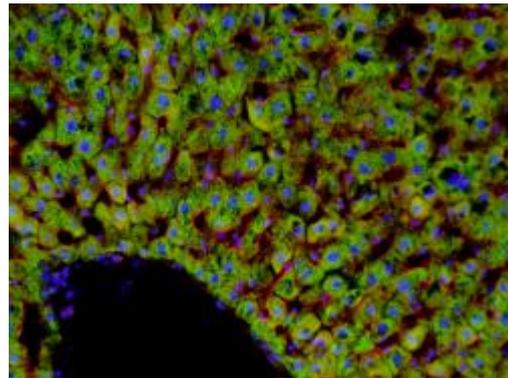
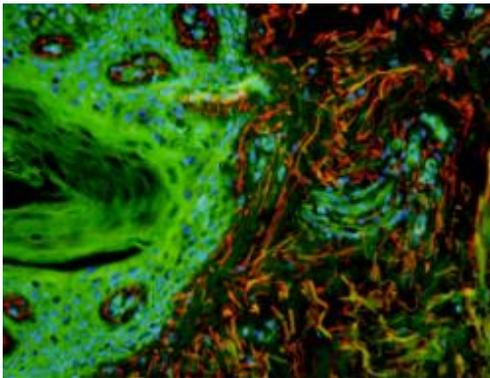
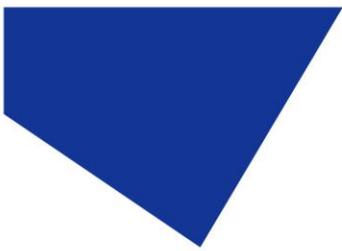




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1. Introduction

This manual covers some of the basic information on the CoolLED pE2 excitation system. However there is a wealth of more detailed information on our website www.cooled.com together with links to many videos which are useful guides to how to use and operate the light sources.

CoolLED pE excitation systems are practical LED light sources for fluorescence microscopy that deliver separate, controllable wavelength peaks to match common fluorophores. They benefit from patent-pending, high power collimated LED light source technology.

CoolLED pE excitation systems represent the very latest technology in fluorescence illumination. It is no longer necessary to expose broad spectrum light on your sensitive samples.

CoolLED pE excitation systems deliver intense light in three or four wavelengths. Comparable results to those produced with mercury and xenon bulb systems are achieved but without the restrictions, limitations and on-going running costs.

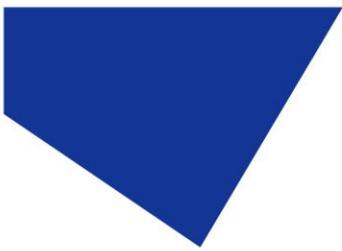
CoolLED pE excitation systems are modular and use interchangeable LAMs – LED Array Modules – to provide LED excitation at a range of wavelengths. A current list of available wavelengths can be found at <http://www.cooled.com/product-detail/led-wavelengths/>

As a solid-state LED light source, the output is stable over time with a very long working lifetime in excess of 10,000 hours on time. No warm up or cool down periods are required as the unit can be switched on and off at any time to produce a consistent, pre-defined output, time after time - ideal for a multi-user facility or tissue culture fluorescence system.

CoolLED pE excitation systems are safer than conventional sources as they do not use mercury. This avoids the risks associated with the use, over-heating, and potential explosion of mercury bulbs. In addition there are no disposal issues with LEDs. However they are bright light sources so care should be taken with their use – see [Section 3](#).

The output of each wavelength source is precisely controllable in 1% steps from 0-100%, removing the need for neutral density filters that only provide a coarse level of adjustment. This also benefits ratio-metric tests as each wavelength source can be balanced independently prior to a test and will remain consistent throughout the experiment.

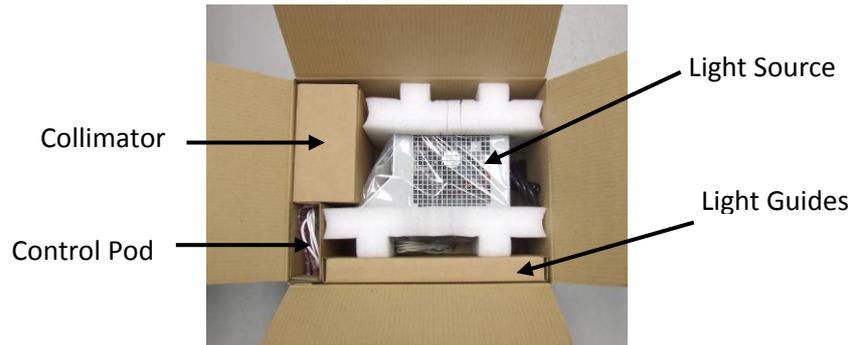
CoolLED pE excitation systems are supplied with an adaptor to your specified microscope. A simple once-only alignment is made to optimize for the optical path of your microscope.



2. Unpacking

Have your Packing List available to verify that you have received all items ordered.

System Items:



pE Excitation Light Source



Control Pod



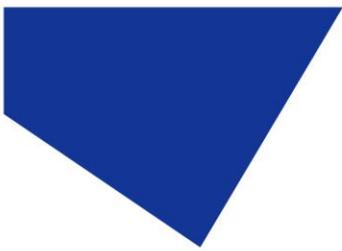
pE-2 Modular Collimator



Liquid light guide(s)

- Mains power connector
- User manual

If there are any parts missing from the package please contact CoolLED.



3. 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.

CoolLED Ltd. assumes no liability for damage caused by, or any risk arising from, using the System in a manner for which it is not intended or using it outside of the specifications of CoolLED Ltd.

3.1.

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.

3.2.

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.

3.3.

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.

3.4.

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

3.5.

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.

3.6.

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



3.7.

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 power cord should be removed from the Light Source prior to cleaning.

3.8.

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

3.9.



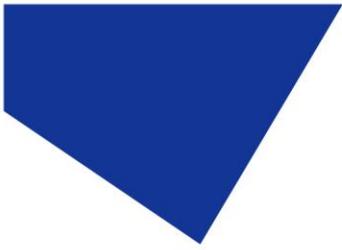
Caution!

Do not remove the liquid light guides from the Light Source during operation. High intensity light is emitted from the System at a variety of wavelengths, from UV to IR. To ensure safe operation, the user must follow the instructions within this User Manual.

3.10.

The Mains power connector may only be plugged into an electrical outlet that is equipped with a grounding contact.

Do not interfere with the grounding of the System by using any equipment (for example an extension cord) that does not have necessary grounding.



4. Installation

Only a qualified technical person should carry out the installation of the CoolLED pE excitation system.

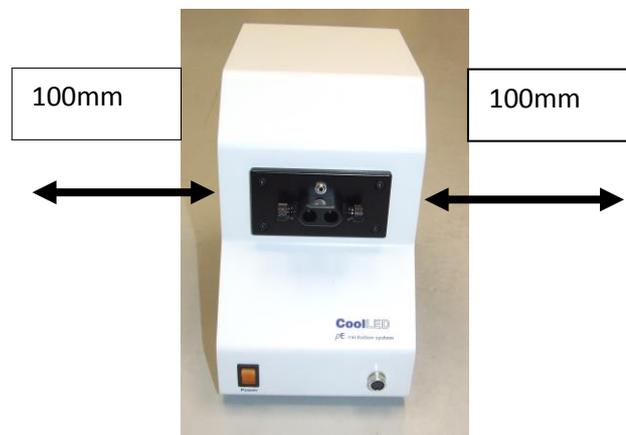
It is recommended that the following sequence is followed by the installer.

4.1. Positioning the Light Source

CoolLED Illumination Systems have been designed to take up as little room as possible around the microscope. The operator only needs to access the Control Pod to operate the System, so the Light Source can be positioned in a convenient area within the reach of the light guides and Control Pod cable. The Light Source should not be positioned so that it is difficult to operate the Power switch.

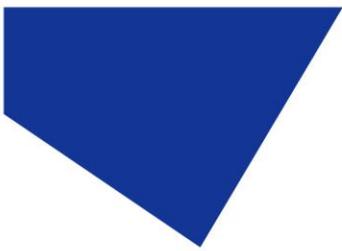
It is important to ensure a free flow of air on either side of the box to allow the internal cooling system to function correctly. A minimum gap of 100 mm should be allowed on both sides on unit. Do not position the Light Source close to any sources of heat.

- Place Light Source in a suitable position as described.



- Connect the power cable to the Light Source.
- Connect the power plug to an electrical outlet.
- Connect the communication cable of the Control Pod to the socket at the front of the Light Source.

Do NOT Switch on the Light Source at this stage



4.2. Connecting the pE-2 Collimator to a Microscope

- Ensure access to locate the Collimator in the microscope's epi-fluorescent port
- Remove any existing light source from the epi-fluorescent port
- Your Collimator will have been supplied with the adaptor you specified for your microscope see www.cooled.com/product-detail/adaptors-2 for adaptor lists
- Ensure that the red dust cover is removed from the Collimator.
- Fit the Collimator to the epi-fluorescent port and secure by the normal method for that microscope model.
- Excitation filters can be placed in the optical path of each channel within the pE-2 Collimator. For instruction on the use and fitting of filters, see [Appendix A](#).

4.3. Connecting the Light Guides between the Collimator and Light Source

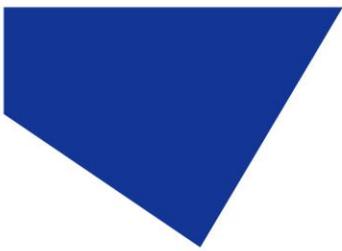
- Remove the red protection caps from each end of each light guide; ensure that there is no debris on the optical surface of each end.

Although the light guides are physically interchangeable, it is essential to fit them as shown below in order that the wavelengths match the combining Dichroic Mirror within the Collimator.

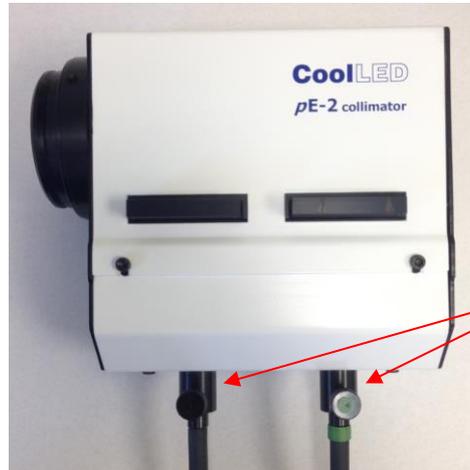
Left side port on Light Source - Front channel on the Collimator

Right side port on Light Source- Rear channel on the Collimator

Green bands are at each end of one light guide and green dots are located on the thumbscrews of the appropriate port on Light Source and Collimator to make this clear.



- Insert each light guide into a channel of the Collimator according to picture below. They will be free to move within the tube. The final position will be determined later.



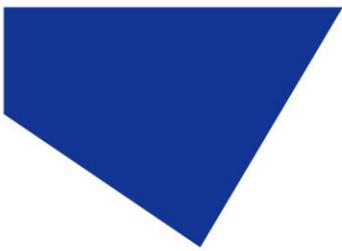
Channels for light guides

- Insert the other ends of the light guides into the Light Source, taking care to follow the orientation protocol highlighted above. The light guides should be inserted until they cannot travel further. The metal ferule should be entirely within the tube. Tighten the thumb screws until they engage on the light guide.



Channels for light guides

It is important to ensure that the light guides are not bent in any smaller radius than 50mm.



4.4. Powering the System

Before switching on the system, ensure the correct mains voltage and fuse current has been set. This can be identified on a panel at the rear of the Light Source.

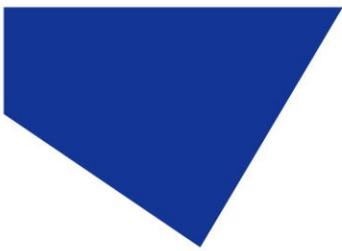
Ensure that the Mains supply cords provided by CoolLED are used with the System. If the cord is to be replaced, please either see the mains cord specification section of this User Manual ([Mains supply cord specifications](#)) or contact CoolLED to ensure that a replacement of the correct rating is used. Using a Mains supply cord outside of the advised ratings may impair the safety of the System.

- Check that the correct fuse has been fitted according to table below.

Power specifications			
Operation Voltage	Frequency	Maximum rated power	Installed fuse specification
120V	60Hz	6.3A	6.3A, 250V
240V	50Hz	3A	3A, 250V

- Switch on the mains switch at the front of the Light Source and allow 5 seconds for the processor to initialise.

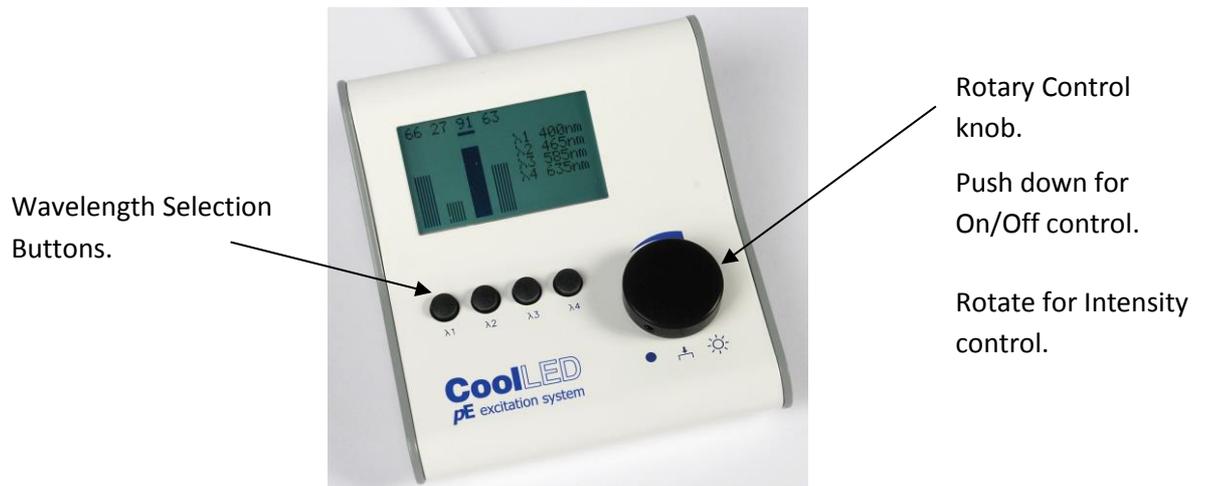
Familiarise yourself with the Manual Operation instructions and then complete the once-only Collimator Alignment. Instructions are provided in [Section 6](#). There is also a video which can be accessed via our web site www.coolled.com/product-detail/pe-2 which helps to explain this process.



5. Manual Operation

Operation of the CoolLED pE excitation system is by the Control Pod or under remote control. Remote control is discussed in [Section 7](#).

The Control Pod display indicates which LED wavelengths are installed. It has individual wavelength selection buttons and a Rotary Control On/Off & Intensity Control knob. Functions and display on the Control Pod are shown below.



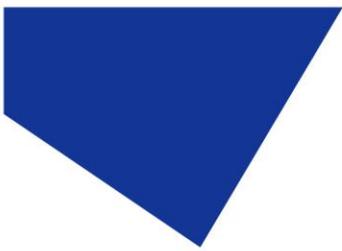
On/Off is achieved by pushing the Master Rotary Control knob down.

Rotation of the Master Control knob varies intensity from 0-100% in 1% increments. Intensity for each wavelength installed is individually set by selecting each wavelength and adjusting as desired. By default, the intensity setting is 50% for all wavelengths.

Select the wavelength by pressing the corresponding button below each colour bar on the display – selection indicated by the colour title being underlined.

Switching between the wavelengths is also possible while the Light Source is enabled by pressing the specific wavelength selection buttons.

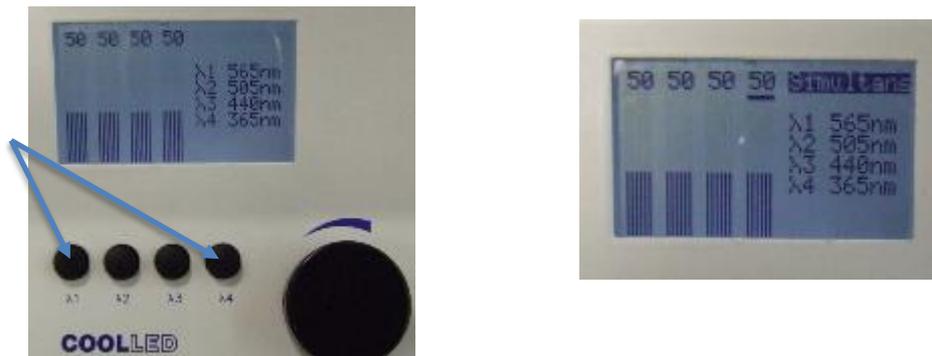
NOTE: Switching the Light Source On/Off does not adversely affect the lifetime of the LAMs



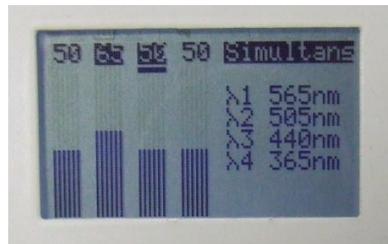
5.1. Simultaneous Mode

Under normal operation, only one LED wavelength is on at any time. However it is possible to operate the System in Simultaneous Mode where more than one wavelength is illuminated.

To enter or exit simultaneous operation, simultaneously press and hold the first and fourth buttons on the Control Pod.



Now you may select the wavelengths you wish to use by pressing each of the four buttons.



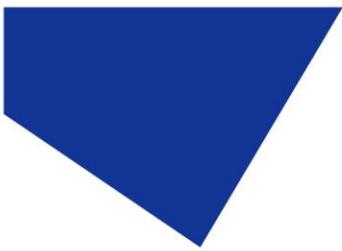
These buttons now have a dual purpose:

- Press a button to select its colour for intensity adjustment using the rotary control. Selection for adjustment is indicated by a bold underline under the intensity numbers on the Control Pod.
- Press a button to put the corresponding wavelength into a selected state. Selected for simultaneous use is indicated by the intensity numbers in white text on a black background.

If a channel is On but not selected for adjustment and you wish to adjust its intensity, press the button twice to get the condition you want. The light will go Off for a moment after the first press, then On again at the second press.

On the Control Pod in simultaneous mode, press the rotary control to turn all selected channels On or Off simultaneously.

Note, there are some restrictions to the use of Simultaneous Mode. Wavelengths greater than and including 595nm cannot be operated in this mode.



5.2. Display Control

The contrast and backlight of the Control Pod LCD display can be adjusted by the user.

By holding down buttons 1 and 2 together and adjusting the rotary control, the backlight intensity can be changed.

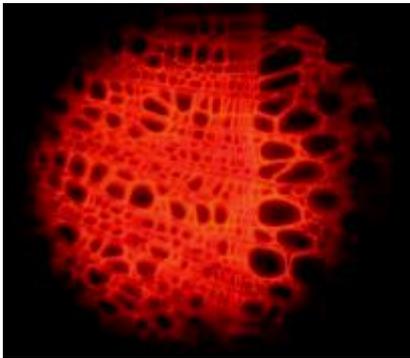
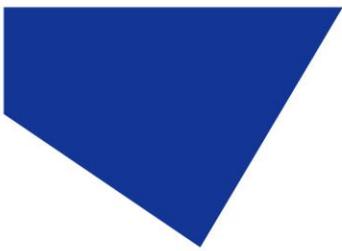
Likewise, by holding buttons 1 and 3 together and adjusting the rotary control, the contrast can be changed. Please note that the contrast can be reduced such that the display disappears. If this happens, just press buttons 1 and 3 and increase the contrast.

6. Optimizing the Collimator

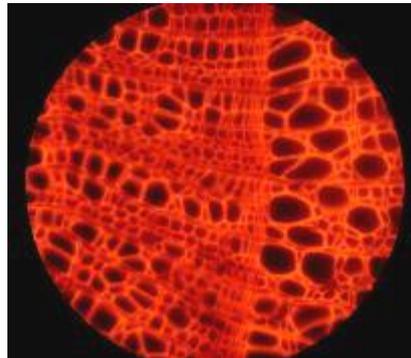
CoolLED pE excitation systems do not need regular alignment. All pE Collimators simply require a once only optimization to match the optics within the microscope to ensure optimum performance.

1. Place a fluorescent sample on microscope stage
2. Switch on the Light Source, select an LED wavelength and set intensity to 100%.
3. View sample
4. Draw out and adjust light guide until an even and bright image is achieved
5. Repeat with second light guide
6. Tighten thumb screws
7. Further adjustment is not required unless optical path is changed in microscope or Collimator is fitted to another microscope. In these circumstances, repeat Steps 1-6.

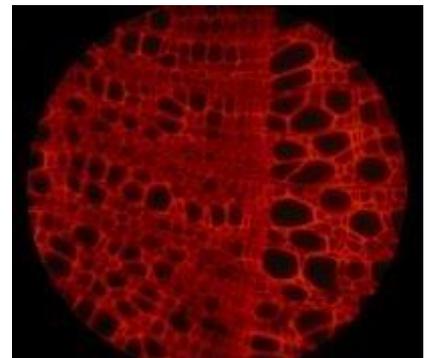




Light guide too far in.



Light guide optimum.



Light guide too far out.

Your CoolLED pE excitation system is now ready to be used.

****See video on our website www.cooled.com/product-detail/pe-2/****

7. Remote Operation

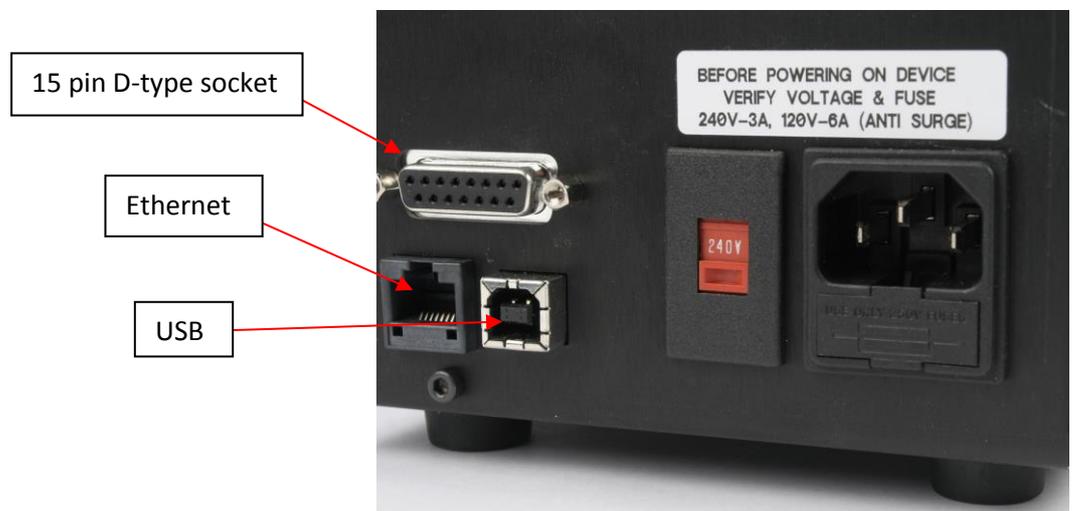
CoolLED pE excitation systems can be operated remotely using custom software or under many of the leading Imaging Software packages e.g. MetaMorph.

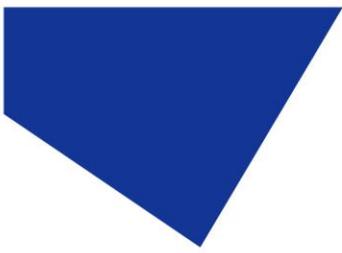
For a current list of imaging software packages which support CoolLED products, visit:

<http://www.cooled.com/product-detail/imaging-software/>

Control software which treats peripherals as a Virtual Coms port can control CoolLED products. Please contact CoolLED for further information and advice.

Hardware connections for control are shown below;

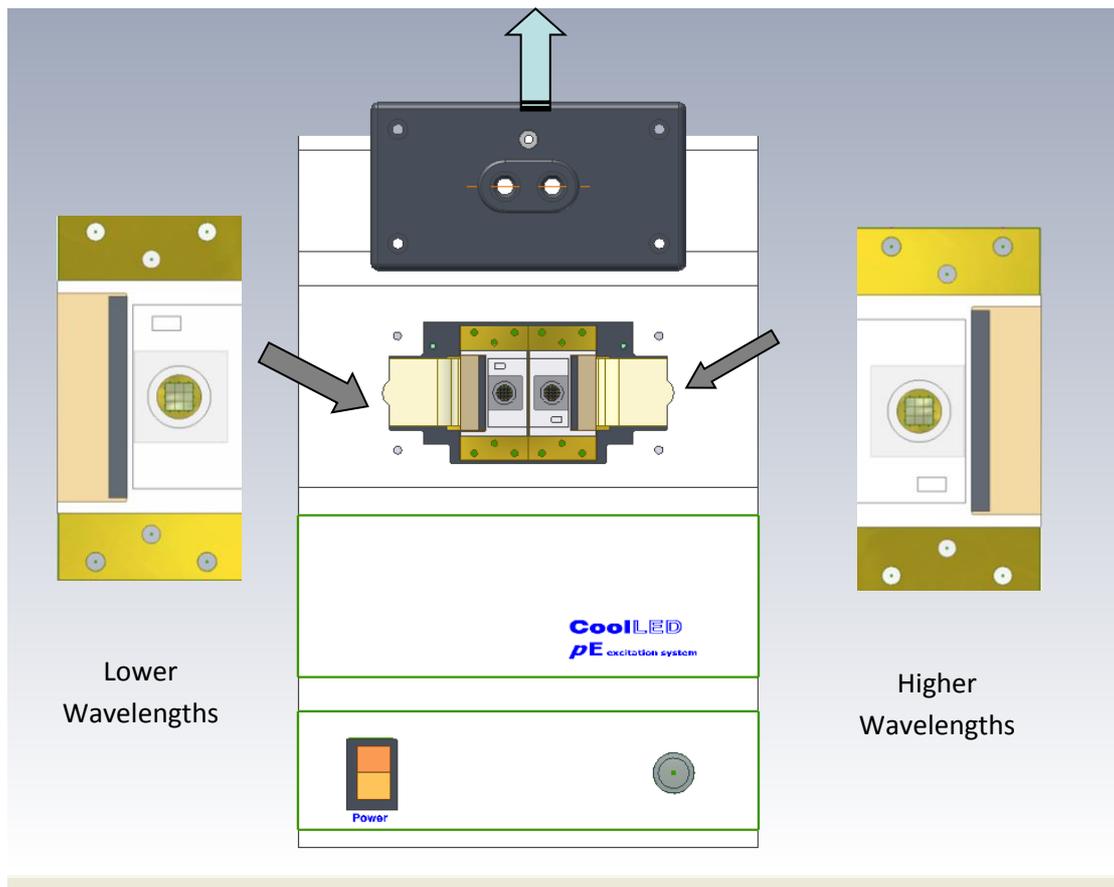




8. LAM location within Light Source

8.1. LAM position

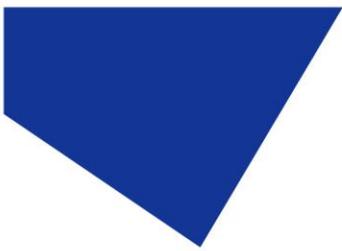
CoolLED pE excitation systems can accommodate up to two LAMs. Depending on what you purchased, one or two LAMs will have been installed in the Light Source at time of supply.



8.2. Collimator Dichroic Mirror

The excitation wavelengths generated on the LAMs are transmitted to the Collimator fitted to the microscope by light guides. Within the Collimator, the light outputs from each light guide are collimated and then combined using a Dichroic Mirror to create a single beam of light entering the epi-fluorescent port of the microscope.

When considering which position to install a new LAM within the Light Source, it is necessary to position:-



LAM with LED wavelengths below the Dichroic Mirror in the Left Side (LHS)

LAM with LED wavelengths above the Dichroic Mirror in the Right Side (RHS)

For example

LHS	Dichroic Mirror	RHS
365/490 nm LAM	515 nm	565/635 nm LAM

In a CoolLED pE-2 Collimator, a User-exchangeable combining Dichroic Mirror is supplied. Additional mounted dichroics can be purchased and easily exchanged.

Most configurations of wavelengths which are selected by the user can be satisfied by one of CoolLED's standard combining Dichroic Mirrors. These are listed below.

Dichroic Mirror	Wavelength
244-4312-490	490 nm
244-4312-515	515 nm

Most other wavelengths can be provided. Please contact CoolLED to verify availability.

Instructions on how to exchange LAMs are provided in [Section 9](#).

The wavelength of an Exchangeable Dichroic Mirror is identified on its mount within the CoolLED pE-2 Collimator.

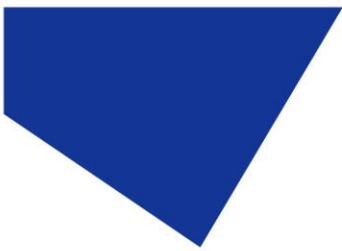
9. LAM change over procedure

Your CoolLED pE excitation system will have been supplied with LAMs already installed. If you have purchased additional LAMs – either with the system or subsequently – it is quite straight-forward to exchange these.

Simply follow the instructions below.

Alternatively go to www.cooled.com and take links to videos to see how it is done.

When you are finished, the Light Source and Control Pod will recognize that the wavelengths have been changed. Operating parameters and Control Pod display will also have been changed.



9.1. Removing a LAM

9.1.1.

Disconnect mains and Control Pod cable from Light Source and place it on its back.

9.1.2.

Remove the 4 Hex screws holding the LAM panel in place.



9.1.3.

Lift off the LAM panel to reveal the LAMs and flex connectors

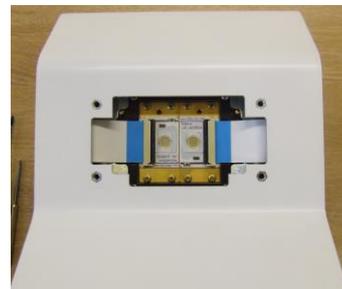


9.1.4.

The Light Source can accommodate 2 LAMs. Each LAM is secured to the main cooling system by four screws.

Position of the LAM is dependent on the wavelength of the combining Dichroic Mirror in the Collimator. For further explanation, see section below on Dichroic Mirror and LAM position.

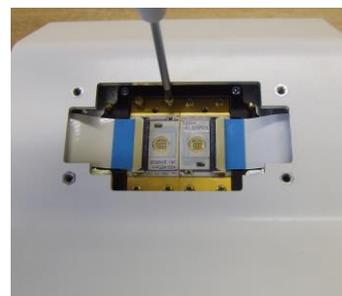
Electrical connection to the LAM is made by the flex circuits (blue bands on their ends)

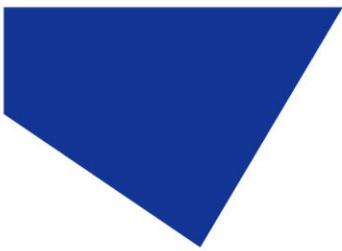


9.1.5.

With a Pozidrive point size 0, unscrew the 4 screws securing the LAM to be changed.

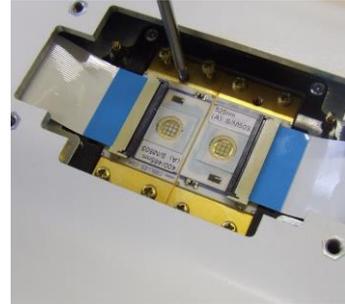
It is not necessary to remove the screws, just unscrew until they are no longer held by the threads.





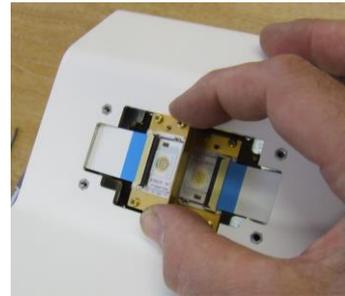
9.1.6.

Insert the LAM removal tool into the threaded hole as shown and screw in until the LAM is released from its mounting.



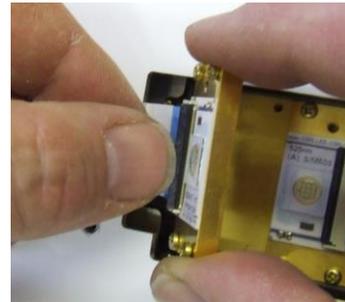
9.1.7.

Unscrew the LAM removal tool from the LAM while holding on to the LAM.

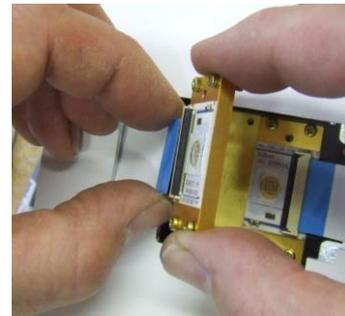


9.1.8.

The LAM now needs to be disconnected from the flex circuit. This is achieved by lifting up the back of the black release bar on the connector. The easiest way is to use a finger nail as shown.



The flex circuit can now be removed from the connector to release the LAM.

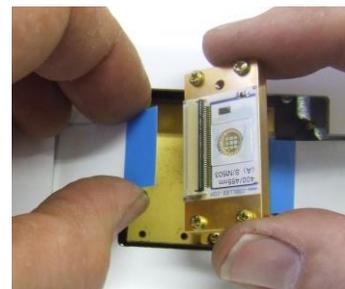


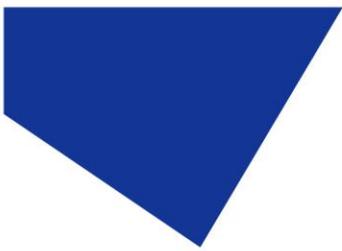
9.2. Fitting a new LAM

9.2.1.

Fit the 4 screws into the LAM.

Insert the flex circuit into the connector. The flex end slides approximately 1mm into the jaws of the connector. Push the black locking bar down ensuring that it has clicked back into the locked position along its full length.





9.2.2.

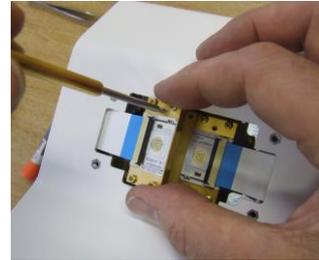
Ensure that there is no debris on either the back of the LAM or on the cooling surface in the Light Source.

It is important that the LAM is secured flat to the cooling surface for maximum thermal transfer.

Use the LAM removal tool to help with positioning the LAM back on to the cooling unit. Remove tool

To ensure full contact across the LAM surfaces, press down at both ends of the LAM to verify that there is no rocking movement.

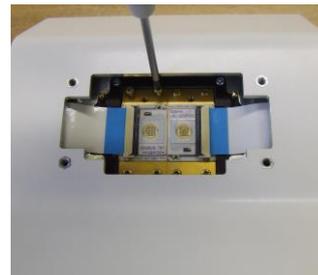
If this does occur take the LAM out again and find and remove the debris causing the LAM not to sit flat.



9.2.3.

Tighten up the 4 screws.

Rather than tightening one up fully at one time, gently tighten each one up and then go round the 4 screws again tightening them fully. Do not over tighten them as these are small screws.



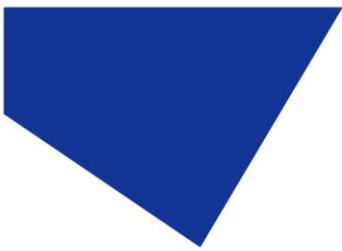
9.2.4.

Refit the LAM panel and reconnect the Control Pod and mains lead.

9.2.5.

Switch on the power and the Light Source will recognize the new LAM and display the wavelength on the Control Pod.

If the LAM has not previously been used in this Light Source, it may be necessary to update the software.



10. Filters

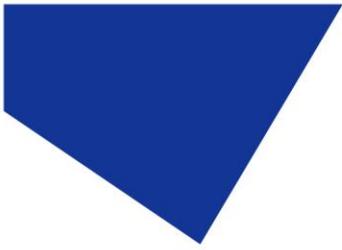
The selection of optical filters is essential to fluorescence microscopy. Their purpose is to separate the excitation wavelength(s) from the light source from the resulting emission wavelength(s) from the fluorophore. Filters are typically fitted within a cube in the microscope or on a filter wheel. A dichroic (colour separation) filter is also required and is situated within the microscope cube.

Conventional light sources provide a broad spectrum of energy from UV through to IR. In this case, the excitation filter's function is to exclude a wide band of undesired wavelengths above and below the fluorophore's excitation wavelength. LEDs have a discrete band of excitation around the nominal peak. This means that there is far less unwanted light (particularly over the UV spectrum) to be filtered. However, there is still a small amount of excitation light which can affect performance. For this reason, CoolLED recommends that excitation filters are still used.

It is desirable to excite the fluorophore with an LED wavelength as close to its optimal excitation wavelength as possible to achieve the maximum emission. CoolLED has a continually growing range of excitation wavelengths designed to match the majority of fluorophores available. A current list can be seen at <http://www.cooled.com/product-detail/led-wavelengths/>

CoolLED can provide advice and assistance on the selection of appropriate filters. In the first instance, please refer to the information on our website which identifies the optimal filter for each LED wavelength / fluorophore. Links are then provided to a number of leading filter manufacturer's websites where filter performance and ordering details can be viewed.

Filter information is at <http://www.cooled.com/product-detail/optical-filters/>



11. Product Specifications

11.1. Dimensions

pE-2 Light Source:	140mm(w) x 250mm(d) x 215mm(h)
Weight:	5.6kg
pE-2 Collimator:	75mm(w) x 143mm(d) x 134mm(h)
Weight:	1.16kg
pE-2 Control Pod	95mm(w) x 110mm(d) x 40mm(h)
Weight:	0.55kg

11.2. Environmental Operating Conditions

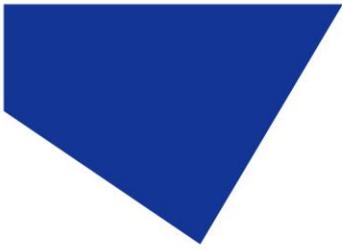
Operating: 5-35°C

11.3. Mains supply cord specifications

Region	Rated Voltage	Rated Current	Plug fuse rating
UK	250V	10A	5A, 250V
USA	125V	10A	-
Australia	250V	10A	-
Europe	250V	16A	-

12. Product options and order codes

See website ([Microscope Illuminators](#) | [LED Illumination Systems](#) | [CoolLED](#)) for full details of product options and order codes.



13. Warranty and Repairs

Please refer to CoolLED's current Warranty Policy available on our website [CoolLED Warranty - CoolLED](#). 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.

14. Compliance

14.1. 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

14.2. 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.



14.3. 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?

15. Contact Information

Further information and support is available from CoolLED. Please contact us by phone or email. Further information is also provided on our website.

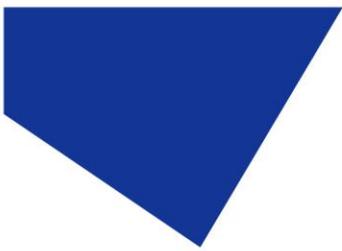
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Appendix A

pE-2 Collimator.

There are additional features and benefits to the **pE-2** Collimator which were not available with the **pE-1** Collimator. See videos via links on our web site <http://www.cooled.com/>

Excitation filters can be removed from the microscope cube or filter wheel and placed directly in the optical path within the Collimator. This offers the benefits of not having an excitation filter wheel, no vibration or delay, and selecting individual excitation filters optimised for each channel.

The combining Dichroic Mirror within the collimator can be exchanged to allow full modularity and compatibility with whichever LED wavelengths are selected.

A.1) **Fitting Excitation Filters**

Follow instructions below to fit a 25mm diameter excitation filter

Verify Filter Orientation.

Verify filter orientation



Place filter in Holder.

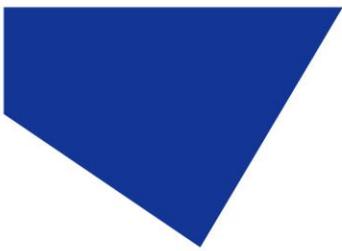
Filter is held by a sprung-loaded bearing.

Tighten only until contact is made.

Do not tighten to the point of rotation stopping.



Do NOT over-tighten.



A.2) **Inserting Filter Holders**

Filter holders slide directly into ports on the pE-2 collimator.

Push firmly in the centre of the holder.

You do not need to fit (empty) holders if excitation filters are in microscope cube.



A.3) **Changing over the Dichroic Mirror**

This operation should only be carried out by a trained technician.

Ensure LEDs are switched off prior to removing cover.

Removing the Combining Dichroic

Loosen retaining screws on collimator (4 off).

Lift cover up and away from the collimator.

Grip combining dichroic firmly between finger and thumb.

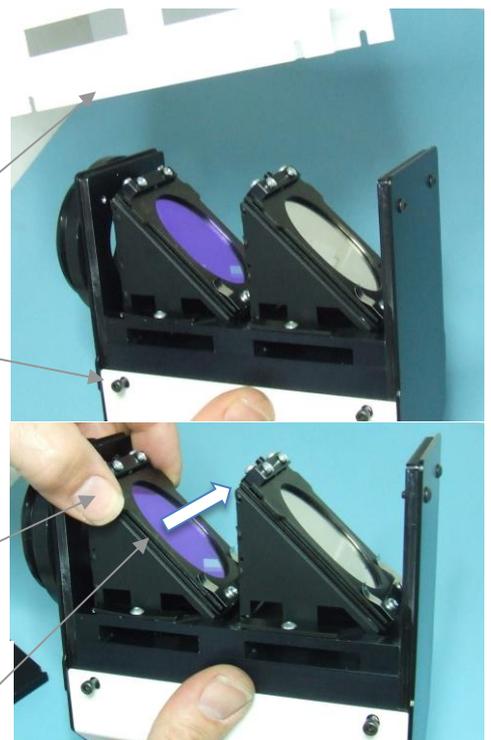
Push back until combining dichroic unclips from position.

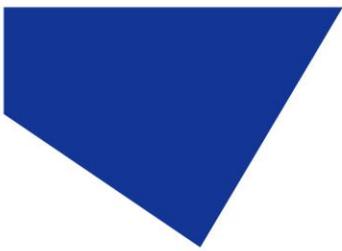
Remove cover

Loosen retaining screws

Hold top of Combining Dichroic firmly

Arrow indicates direction to push





Lift upwards to remove from Collimator.



Replacing the combining dichroic

Grip the combining dichroic as before.

Reference Surface

Orientate such that the optical surface of the combining dichroic will make direct contact with the reference surface.

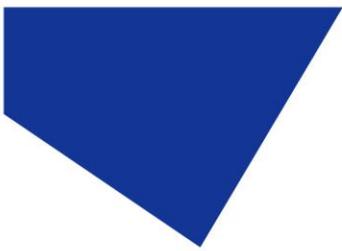
Place the bottom of the combining dichroic into the retaining clips.

Retaining clips

Gently push the dichroic against the reference surface until it clicks into place.



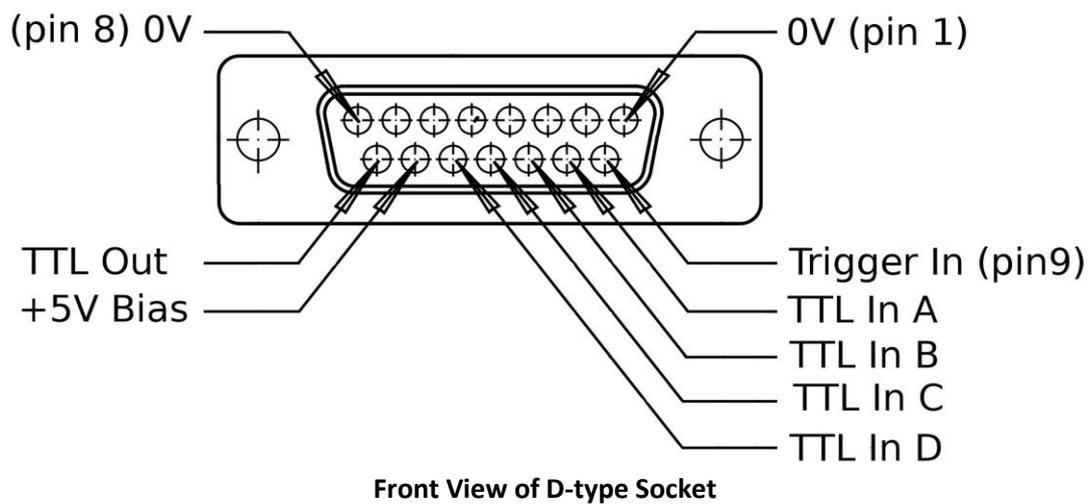
Replace the cover and tighten the screws.



Appendix B

pE excitation systems

Parallel TTL Port



TTL Drive	
Channel A TTL Input	Pin 10 +ve
Channel B TTL Input	Pin 11 +ve
Channel C TTL Input	Pin 12 +ve
Channel D TTL Input	Pin 13 +ve
Trigger TTL Input Pin 9 +ve	
TTL Output Pin 15 +ve	
Pin 1, 8 Gnd	

Switch Contact Drive	
(Internal 5V)	
Channel A Switch Input	Pin 10 and Pin 14
Channel B Switch Input	Pin 11 and Pin 14
Channel C Switch Input	Pin 12 and Pin 14
Channel D Switch Input	Pin 13 and Pin 14
Pin 1, 8 Gnd	
Connecting a switch contacts between the switch input pin and the internal 5V pin14 will turn on that channel when the switch is closed	

LED response time in approx. 150 microseconds

Full off-on-off period approx. 300 microseconds