

## Two channel LED illuminator

The OptoLED power supply controls up to two OptoLED heads providing bright, stable illumination across the visible, UV and IR range via a closed-loop photodiode feedback design. With sub-millisecond, vibration free switching and intensity modulation, it's suitable for fast, demanding imaging requirements as well as routine fluorescence or transmitted light microscopy.

This manual provides guidance on setting up a new OptoLED system, operation via external control, and installation and alignment on your microscope. The FuraLED and TwinLED dual LED couplings are also included in this guide.

For the purpose of this manual, all words in **bold blue** refer to parts labelled in diagrams throughout this document.

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### 1. Important Safety Information

The OptoLED can produce very intense illumination.

The LED heads should **NEVER be viewed directly**. When testing the LEDs, always shine them at a white surface, such as a piece of paper and view the resultant reflection or fluorescence.

This is especially important with **ULTRAVIOLET LEDs** which will not appear bright to the human eye, but can cause permanent damage if not used with care.

## 2. Setting up your hardware

The OptoLED power supply is typically supplied with two interchangeable LED heads, an optional remote slider, coupling hardware for combining the LEDs and a suitable microscope mount.

The power supplies have two identical control sections on the rear and front panels. Typically, the shortest wavelength is connected to the left channel when viewing the front of the power supply, although all LEDs will function on any channel of the OptoLED controller.

When supplied with a remote slider, however, ensure the LEDs are connected according to the coloured LED stickers on the rear panel. This is due to the maximum current setting on the slider being set for this corresponding LED.

### 2.1 Hardware connections: OptoLED power supply rear panel



Figure 1 : OptoLED power supply rear panel

1. Connect the power supply to the mains
2. Connect the LED to the **LED head connector**, ensuring the connector is screwed in place for a secure connection.
3. If applicable, connect the remote slider to the **REMOTE connector**.
4. Turn the **FEEDBACK** switch 'ON'. See section 3.2 (Optical Feedback) for further details of this operating mode.
5. Ensure the switch next to **EXT./LEVEL** is 'OFF' by default. For further details of this operating mode, refer to section 3.3.2 (Analogue triggering).
6. The **MONITOR** connector is not required for general operation.

## 2.2 Hardware connections: OptoLED power supply front panel

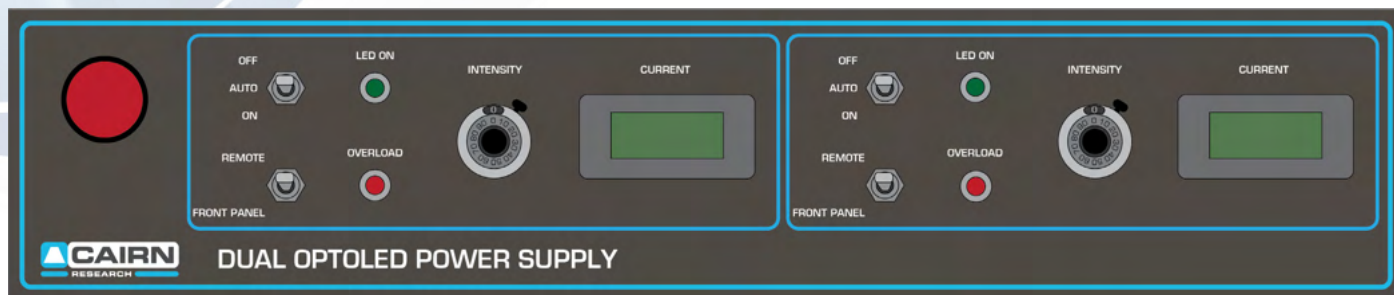


Figure 2 : OptoLED power supply front panel

1. Switch the red power button ON
2. Ensure the **REMOTE / FRONT PANEL switch** is set to **FRONT PANEL** unless you are using a remote slider, in which case select **REMOTE**. If **REMOTE** is selected and this is not connected on the rear panel, the LEDs will not come on under any operating conditions.
3. Turn the **INTENSITY** dial down to zero. This potentiometer has a 10-turn range, which sets the LED drive current, providing the **EXT/LEVEL** switch is OFF on the rear of the power supply (otherwise current is via an external voltage).
4. To test the unit, switch the **OFF / AUTO / ON** switch to 'ON' – the green **LED ON** indicator should now be illuminated.

**ENSURE YOU ARE NOT LOOKING DIRECTLY AT THE LED – ALWAYS VIEW THE FLUORESECENCE ON A WHITE PIECE OF PAPER, as described in section 1.**

5. Turn the **INTENSITY** dial to clockwise until you see light emitted from the corresponding LED head. The current being applied to the LED head will be given on the adjacent **CURRENT** display.

**ENSURE YOU ARE NOT LOOKING DIRECTLY AT THE LED – ALWAYS VIEW THE FLUORESECENCE ON A WHITE PIECE OF PAPER, as described in section 1.**

6. All LEDs have different maximal drive currents, and once this limit is reached on the dial, a protection feature triggers and the LED will transiently switch off and flash, accompanied by the red **OVERLOAD** coming on.

If this occurs, turn the **INTENSITY** dial anticlockwise until the **LED ON** (green) indicator stays green and the **OVERLOAD** (red) is no longer illuminated.

As general guidance on maximal current values – our highest rated LED heads are currently white (both 'cool' and 'warm') at 3A and will overload at settings higher than this value. By contrast, the 340nm UV LED will overload at >0.7A, so please take note of the value on the **current display** accordingly, rather than using the 10-turn potentiometer as an indicator of full range.

Feel free to contact us with specific questions regarding the maximal current for each LED supplied with your OptoLED [tech@cairn-research.co.uk](mailto:tech@cairn-research.co.uk)

### 3. General Operation

The OptoLED LED heads can be operated from either the controller front panel, the remote slider or externally via TTL and/or an analogue voltage. The front panel control is described in section 2.2 and is often used for continual illumination, such as checking cell transfections.

The remote slider offers a convenient microscope-side intensity control which overrides the front panel controls.

The most common form of operation is via software control; although currently no dedicated OptoLED USB operation is available, triggering on/off via 5V TTL provides fast LED switching and when used in Feedback mode, an ultra-stable output shows the OptoLED's true potential for quantitative imaging.

#### 3.1 Remote slider control (if applicable)

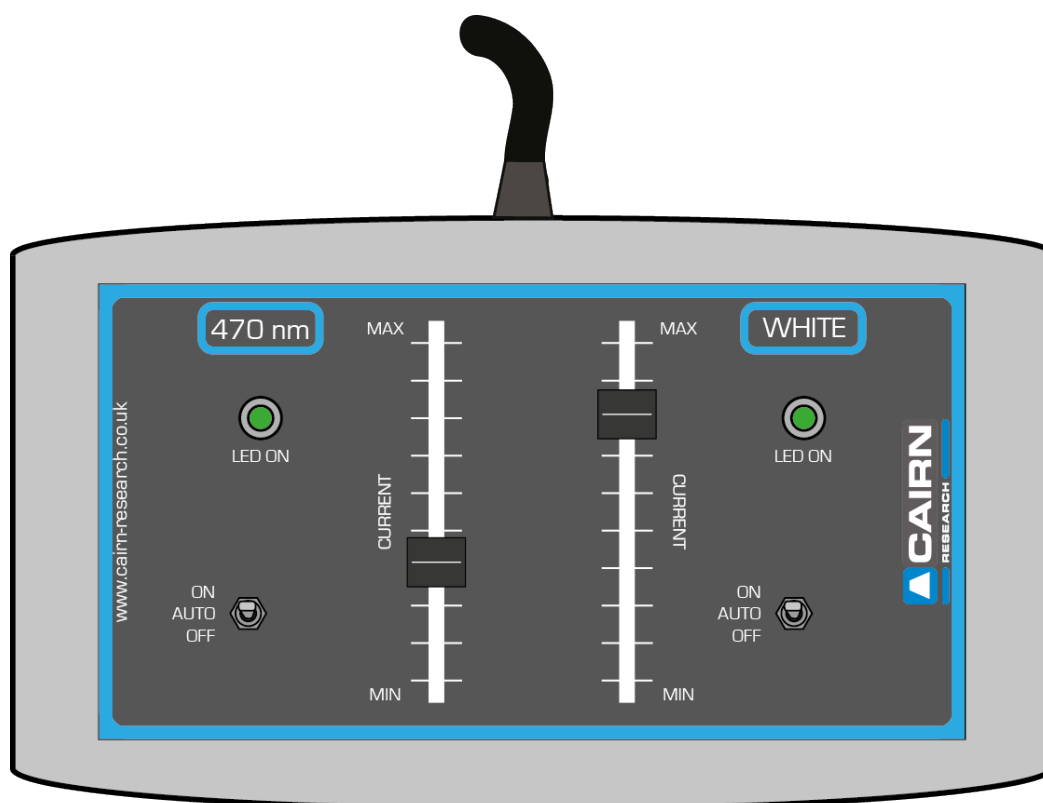


Figure 3: OptoLED (optional) remote slider

- 1 Ensure the **REMOTE / FRONT PANEL** switch is set to **REMOTE** on the OptoLED power supply front panel.
- 2 To test the unit, switch the **OFF / AUTO / ON** switch to 'ON' on the remote slider– the green **LED ON** indicator should now be illuminated.
- 3 Slide the **CURRENT** slider up to increase intensity. The '**MAX**' value is set prior to unit shipping, and the LED will not overload providing the corresponding LED colour is connected on this channel.
- 4 When using a remote slider with a variety of LEDs, the maximum current value can be adjusted easily with guidance, so please contact us. If this preset is not adjusted, the overload protection for the LED may be triggered below maximum on the slider, however, no damage will be caused.

### 3.2 Optical Feedback

When the **FEEDBACK** switch is set to 'ON' on the OptoLED rear panel, a feedback loop uses the output of a photodiode amplifier built into the head to set the control voltage, rather than just the intensity dial or slider. LED chip manufacturers and specifications vary, and when you factor in the influence of temperature, LEDs tend to be less efficient as they get hotter.

A comparison of LED output both with and without feedback can be seen in figures 4 and 5.

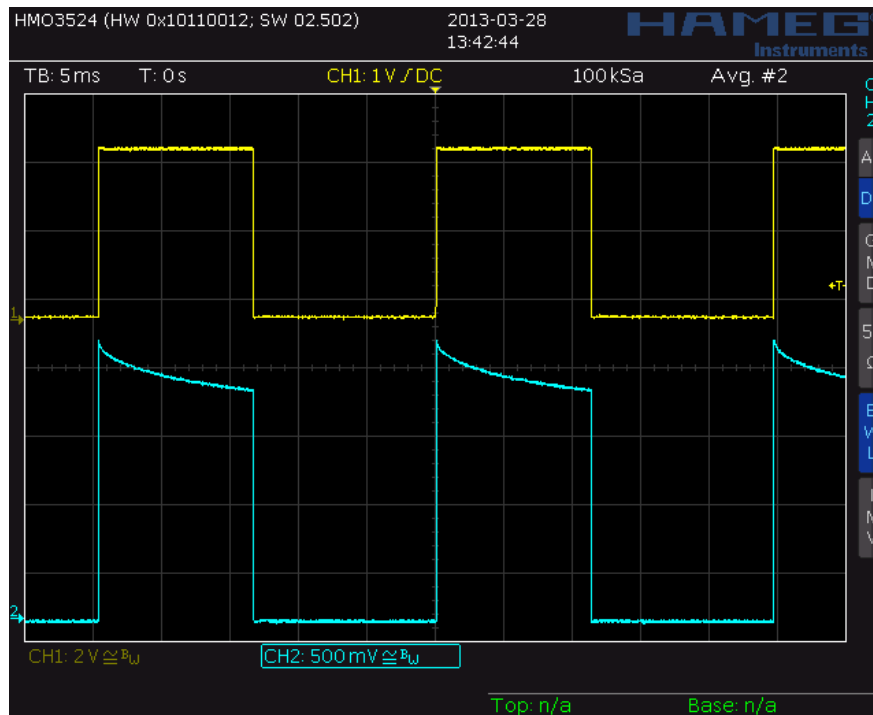


Figure 4: 590nm LED optical output without Feedback enabled

The yellow trace shows a pulsed current going through the LED. The timescale is 5ms/division, with the blue trace showing the optical output directly from the LED head, obtained via the **MONITOR** BNC on the OptoLED rear panel.

The optical output declines during the 'on' period, due to temperature effects of the LED. (The 590nm LED is a particularly pronounced example), again illustrating the differences between LED manufacturers.

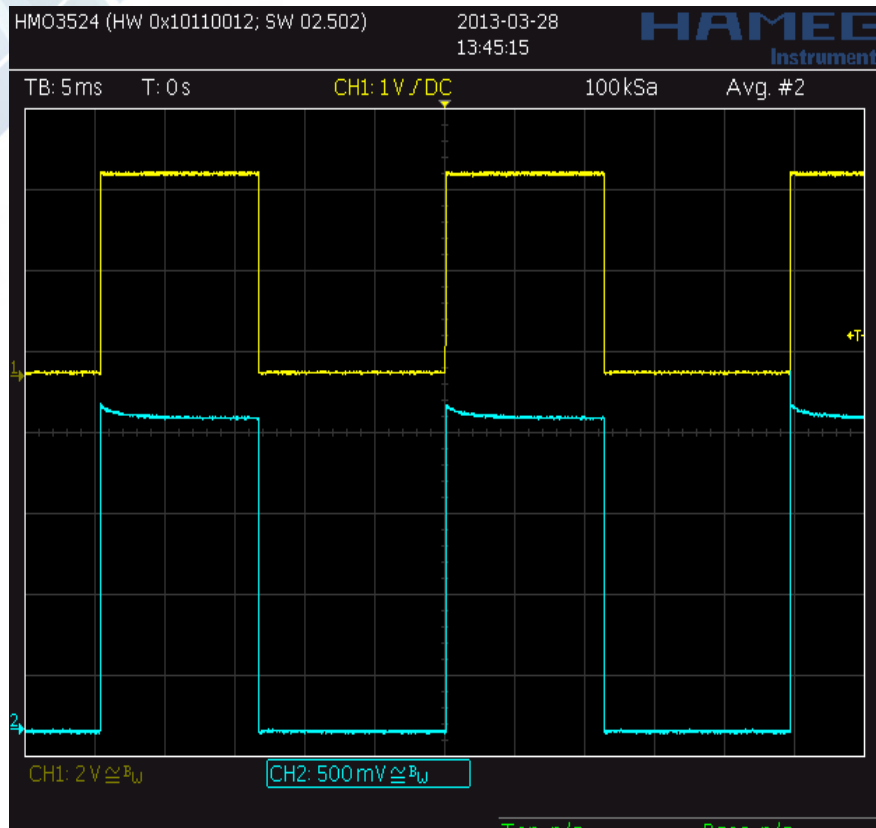


Figure 5: 590nm LED optical output with Feedback enabled

Again, the timescale is 5ms/division, with the blue trace showing the optical output directly from the 590nm LED head.

With feedback enabled, the traces are now square. The thermal effect has a time course on the order of milliseconds, whereas the optical feedback can be applied on a microsecond timescale, which is sufficiently faster to deal with it completely.

### **When is Feedback not useful?**

We would recommend switching Feedback on during general OptoLED operation, particularly considering the spectral output of an LED can also change with temperature. If you are pulsing an LED with a very short duty cycle however, having Feedback enabled will limit the switching speed to  $\sim 10\mu\text{s}$  (vs.  $< 100\text{ns}$  with Feedback off), which incidentally is still fast enough for most applications.

### 3.3 External Triggering

The OptoLED heads can be switched on or off, as well as the intensity being set by an external analogue voltage via BNC connections on the rear panel. We also supply a data interface as an optional extra with the system, plus the corresponding device drivers for a range of third-party acquisition software.

This combination is typically a Cairn boxed National Instruments USB device (details and connections of the exact model will be provided with the unit), for which we have written our own Micro-Manager drivers. Many other software packages also contain the National Instruments hardware drivers by default. Please contact us or refer to supplementary documentation for guidance installing and setting up these devices.

#### 3.3.1 TTL triggering

1. Connect **GATE 1** on the rear panel of the OptoLED controller to a DIG I/O channel of your data interface device via a BNC cable for each channel respectively.
2. Switch the **OFF / AUTO / ON** switch to 'AUTO' on the front panel for each channel.
3. If using a remote slider, ensure the **REMOTE / FRONT PANEL switch** is set to **REMOTE** on the OptoLED power supply front panel and switch the **OFF / AUTO / ON** switch on the remote to '**AUTO**'.
4. Ensure the **INTENSITY** dial (or slider on the remote) has been turned up sufficiently to ensure light is emitted from the LED head.
5. When a TTL logic high (+5V) signal is given, the LED head should start emitting light and the green **LED ON** indicator should now be illuminated.
6. Note: when solely using a TTL input, ensure the **EXT/LEVEL** switch is 'OFF' to ensure the intensity is determined by the front dial.

Each OptoLED channel also has a second GATE input on the rear panel. When connected to your imaging camera, this allows the LEDs to also be turned on only when the camera is exposing and the relevant channel is selected in your software, as the logic level will be low when the camera is reading out the signal. This is a huge benefit for fluorescence microscopy, as damaging phototoxicity is reduced, in addition to any motion artefacts.

7. To utilise this mode, connect **GATE 1** to your data interface as described above, plus connect **GATE 2** to the 'Expose Out' BNC on your camera trigger cable.
8. Ensure **OFF / AUTO / ON** remains on '**AUTO**'
9. When using two channels, it's advisable to connect both **GATE 2** outputs to the camera via a BNC T-piece, straight from the EXPOSE OUT output.
10. Now, when your camera goes live, or you are acquiring a stack of images, the LED will appear to flash, signifying the low 'down time' during camera readout at the end of the exposure.

### 3.3.2 Analogue triggering

For intensity control in your software, the current provided to the OptoLED heads can be determined by an analogue voltage. Often, this is used in combination with the TTL on/off triggering, therefore the description below assumes the GATE inputs have already been determined.

1. Connect **EXT/LEVEL** on the rear panel of the OptoLED controller to an ANALOGUE OUT channel of your data interface device via a BNC cable for each channel respectively.
2. Switch the adjacent **EXT/LEVEL** switch to 'ON'.
3. Switch the **OFF / AUTO / ON** switch to 'AUTO' on the front panel for each channel. (Or on the remote slider if available). \*
4. Provide a TTL logic high in order to turn the LED on, plus an analogue voltage to determine the intensity of light emitted. This voltage will vary depending upon the maximal drive current of the head attached and will always match the current control on the front panel. Therefore, there may be some trial and error with analogue voltages applied when setting up this operating mode - too high and the overload protection will be enabled, so reduce the voltage accordingly.

\* If the only external operating mode is for analogue intensity (i.e. no additional TTL inputs), switch the **OFF / AUTO / ON** switch to 'ON' to enable this mode.

Feel free to contact us with specific questions regarding the maximal current for each LED supplied with your OptoLED [tech@cairn-research.co.uk](mailto:tech@cairn-research.co.uk)

## 4. LED alignment on your microscope

Typically, the OptoLED is supplied with a microscope adapter and the means to mount either one, two, three or four LEDs simultaneously. We also offer dedicated twin heads (namely the FuraLED and TwinLED heads), which are all covered in this manual.

As we have adapters for a large range of microscopes, both past and present, they are not covered in this document. Attaching them to your frame should be self-explanatory. If you are having issues, however, please feel free to [contact us!](#)

## 4.1 Single port coupling

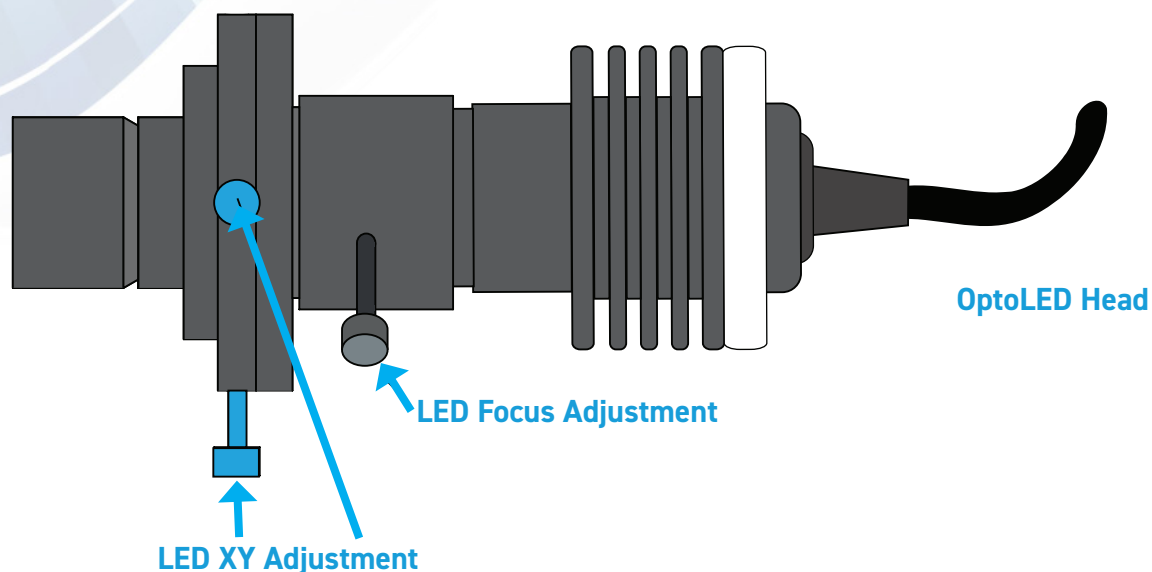


Figure 6: Single port coupling

1. Insert the **OptoLED Head** into the single port coupling, taking note of the notch section on the outer casing.
2. Secure the **LED Focus Adjustment** screw within this notch on the LED head – this acts as a safety measure, ensuring the LED cannot be easily removed during operation.
3. Move to a blank objective position on the microscope nosepiece and remove any cover caps.
4. Ensure the relevant fluorescent filter cube is in position in the epi-fluorescent cube turret.
5. Cover the objective aperture with small piece of white paper, or a centring tool provided by your microscope manufacturer.
6. Loosen the **LED Focus Adjustment** screw and move the LED head in Z within the coupling until the image of the LED comes into sharp focus on the piece of paper (Figure 7B).
7. Lock off this position by securing the thumb screw.
8. Centre the focussed image of the LED using the **LED XY Adjustment** screws (Figure 7C).

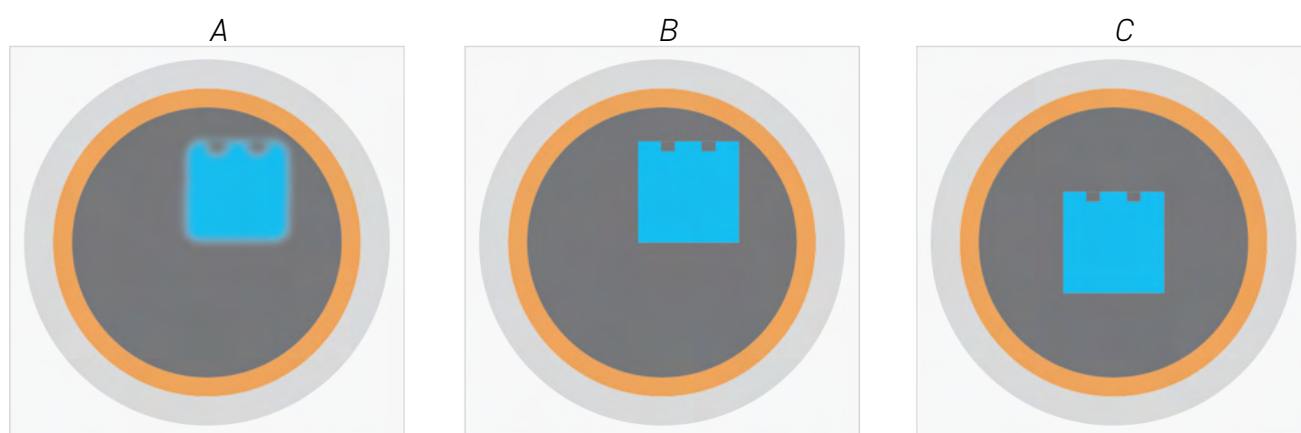


Figure 7: Focussing and aligning the LED image at the back aperture of the objective

## 4.2 Dual port coupling

When mounting two LEDs, our dual port coupling includes a Cairn filter cube, which houses a dichroic mirror (with suitable long pass characteristics) to reflect the LED of a shorter wavelength. Each LED has individual X, Y and Z adjustments to ensure optimal illumination.

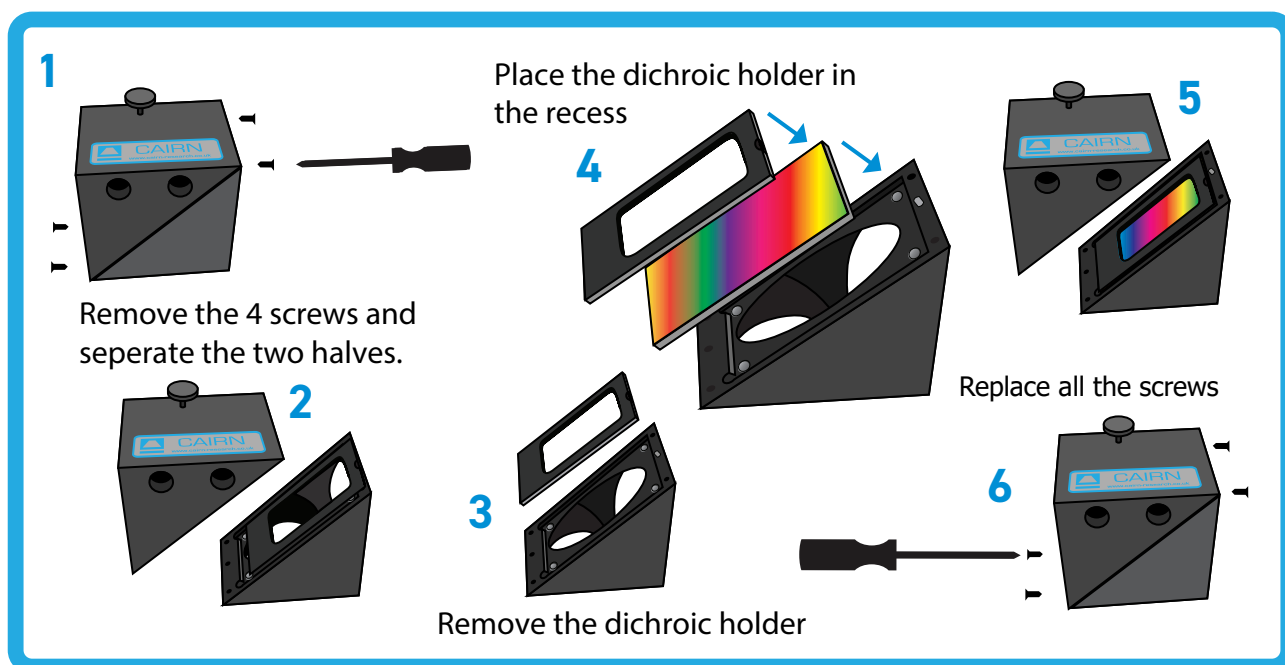


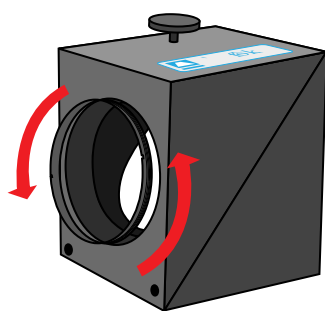
Figure 8: Cairn filter cube and filter removal tool

### 4.2.1 Installing filters and dichroic mirrors into Cairn filter cubes

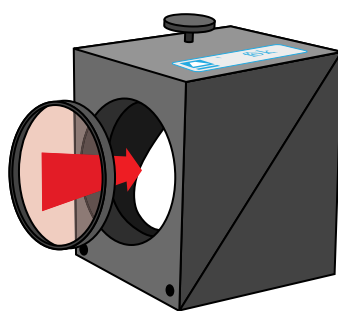
Typically, the all Cairn multi-port couplings come supplied with appropriate excitation filters and dichroic mirrors as per your specification. However, the Cairn filter cubes can be supplied empty and be easily fitted in the lab, as per the instructions below.

Please [contact us](#) if you need any filter or mirror advice – we hold stock of all common Chroma filter sets, often available for next day UK delivery.

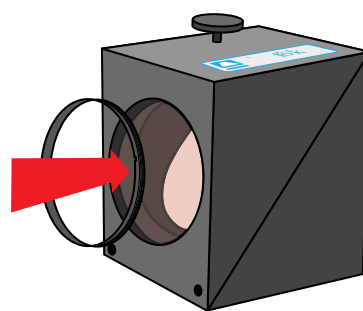




Turn the locking ring anticlockwise to remove it.



Carefully place the filter inside the recess.



Replace the locking ring and rotate it clockwise to secure.

*Figure 9: Installing dichroic mirrors and filters in Cairn filter cubes*

Please refer to specific guidance from your filter manufacturer for details on correct dichroic mirror and filter orientation.

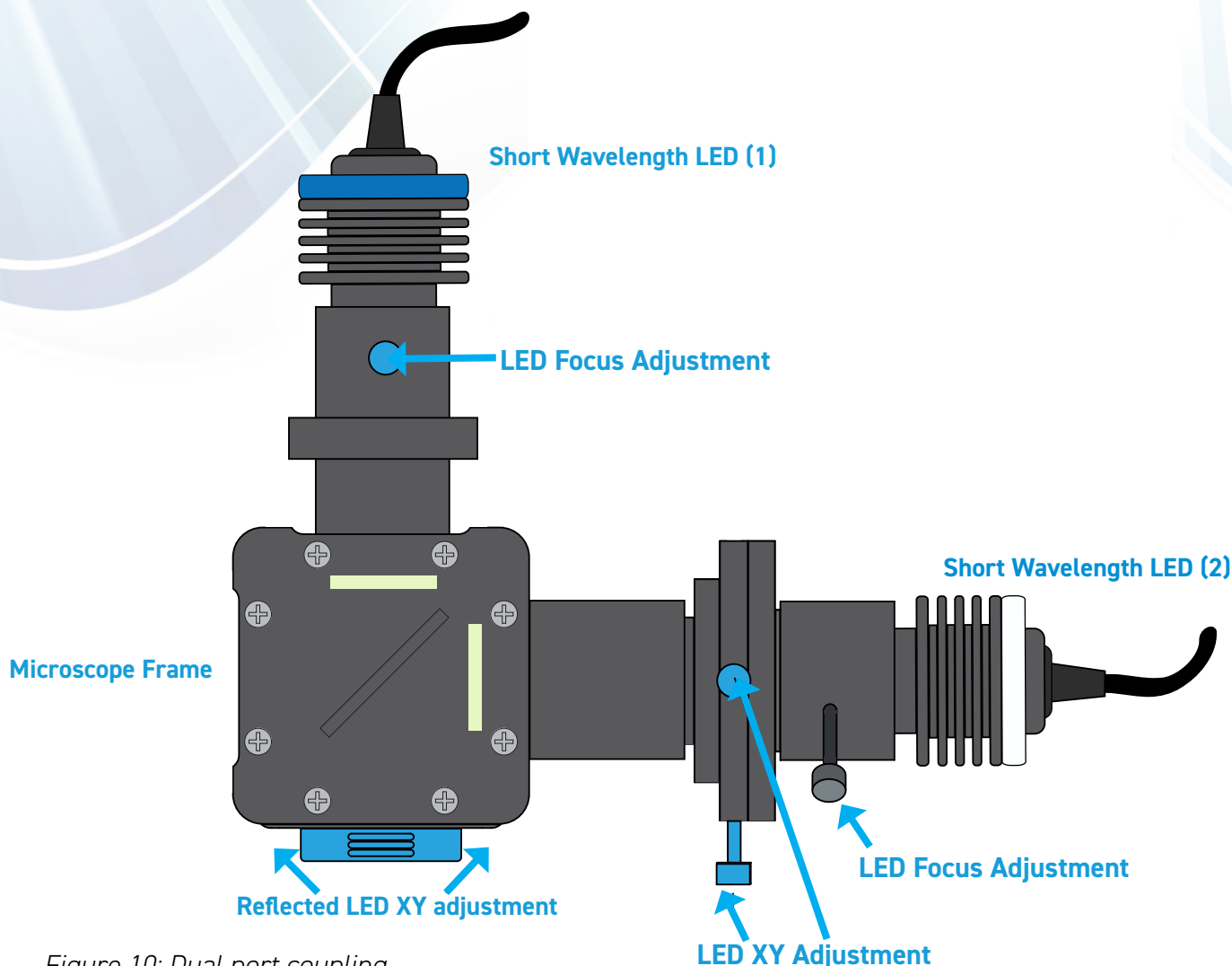


Figure 10: Dual port coupling

1. Mount the LEDs in wavelength order, with the shortest wavelength closest to the rear of the microscope frame.
2. Move to a blank objective position on the microscope nosepiece and remove any cover caps.
3. Ensure the relevant fluorescent filter cube is in position in the epi-fluorescent cube turret.
4. Cover the objective aperture with small piece of white paper, or a centring tool provided by your microscope manufacturer.
5. Follow the steps described in section 4.1 for alignment and focus adjustment of the **Long wavelength LED (2)**.
6. Remove the **cube cover magnetic door** to gain access to the Cairn filter cube, ensuring this contains a suitable dichroic mirror and is in place within the dual port coupling.
7. Adjust the focus of the **Short wavelength LED (1)** by moving the LED head in Z within the coupling and observing the image at the back aperture of the objective, as previously described
5. Lock off the **LED Focus adjustment** thumb screw.
6. Using the 1.5mm Hex key provided, adjust the position of the **Short wavelength LED (1)** via the **Reflected LED XY adjustment** (note the magnetic door can remain in place). This mechanism tips and tilts the Cairn cube housing the dichroic mirror, acting as a precise alignment tool for the reflected pathway.

### 4.3 Triple port / Quad port coupling

When installing a triple port or quad port LED coupling to house three or four LEDs respectively, the coupling will be an extension of the dual port configuration.

Always ensure the LEDs are in wavelength order, with the shortest closest to the microscope frame and that suitable dichroic mirrors are present in the Cairn filter cubes with the shortest wavelength in the fully reflected position and the longest in the fully transmitted position. Please also ensure that suitable dichroic mirrors are fitted to the Cairn filter cubes.

The longest wavelength LED will have individual X, Y and Z controls and all reflected LEDs of shorter wavelengths will have an individual Z control, with XY adjustment on the appropriate cube cover door as described previously.

For quad port couplings, one dichroic mirror will reflect two LEDs, therefore both will move in XY simultaneously. All LEDs have individual controls, however, so can be aligned and focussed appropriately.

We recommend aligning the LEDs in wavelength order, from shortest to longest.

### 4.4 FuraLED / TwinLED coupling

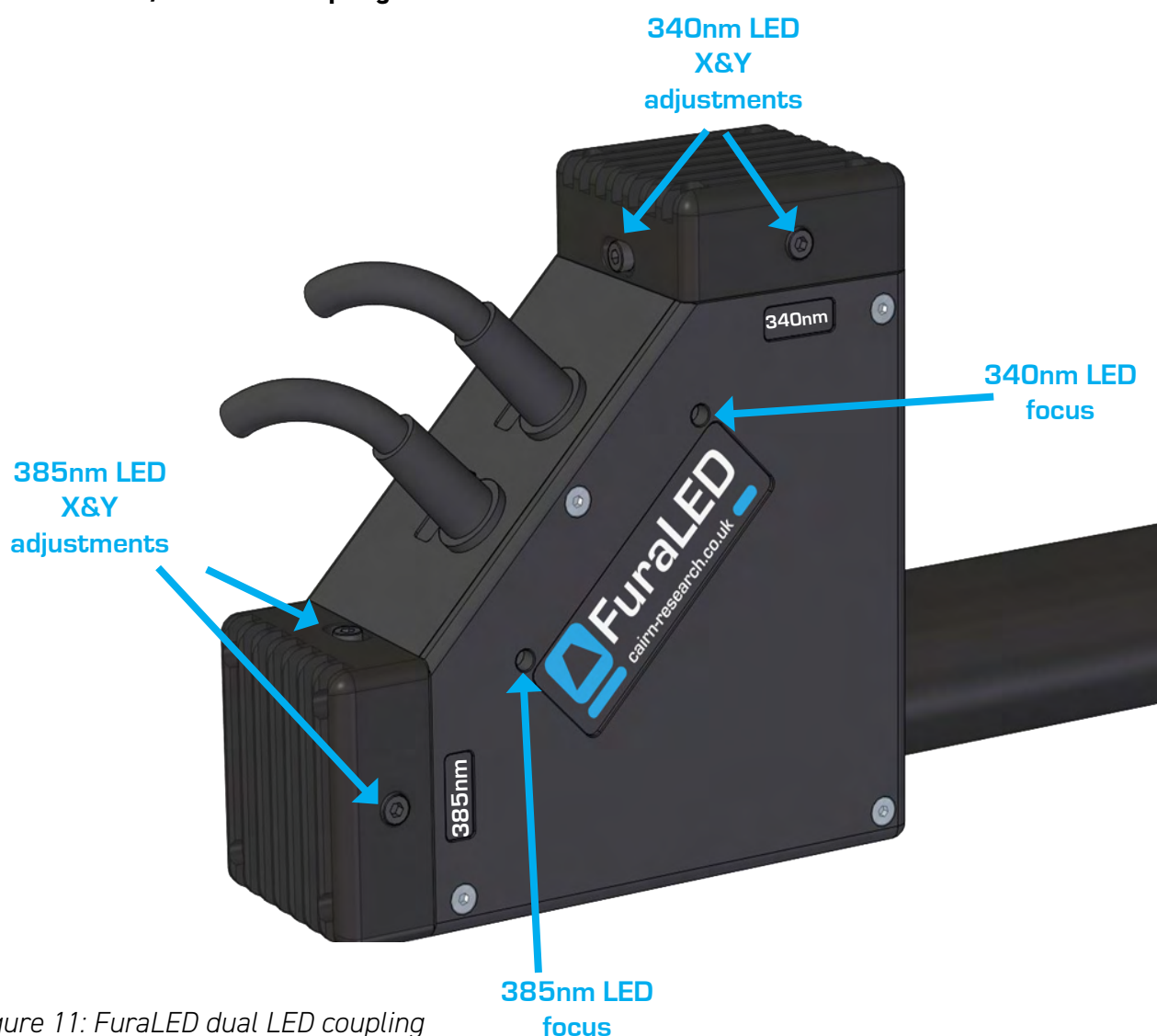


Figure 11: FuraLED dual LED coupling

The FuraLED dual port coupling houses two UV LEDs (typically 340nm and 385nm for Fura-2, although a Fura-8 variant is available), photodiodes providing optical feedback for each channel, excitation filters and a dichroic mirror to combine the two wavelengths. Similarly, the 'TwinLED' houses two non-UV LEDs for more general illumination, with the same adjustments available for setup.

1. Move to a blank objective position on the microscope nosepiece and remove any cover caps.
2. Ensure the relevant fluorescent filter cube is in position in the epi-fluorescent cube turret.
3. Cover the objective aperture with small piece of white paper, or a centring tool provided by your microscope manufacturer.
4. Turn on the 340nm OptoLED channel at a low intensity.
5. Using the 2.5mm hex key provided, adjust the **340nm LED focus** until the image of the LED comes into sharp focus on the piece of paper.
6. Centre the focussed image of the LED using the **340nm LED X & Y adjustment** screws, using the same hex key.
7. Repeat the steps above for X, Y and Z adjustment for the 385nm LED

#### 4.5 Coupling variation – FuraLED in reflected position of dual port coupling

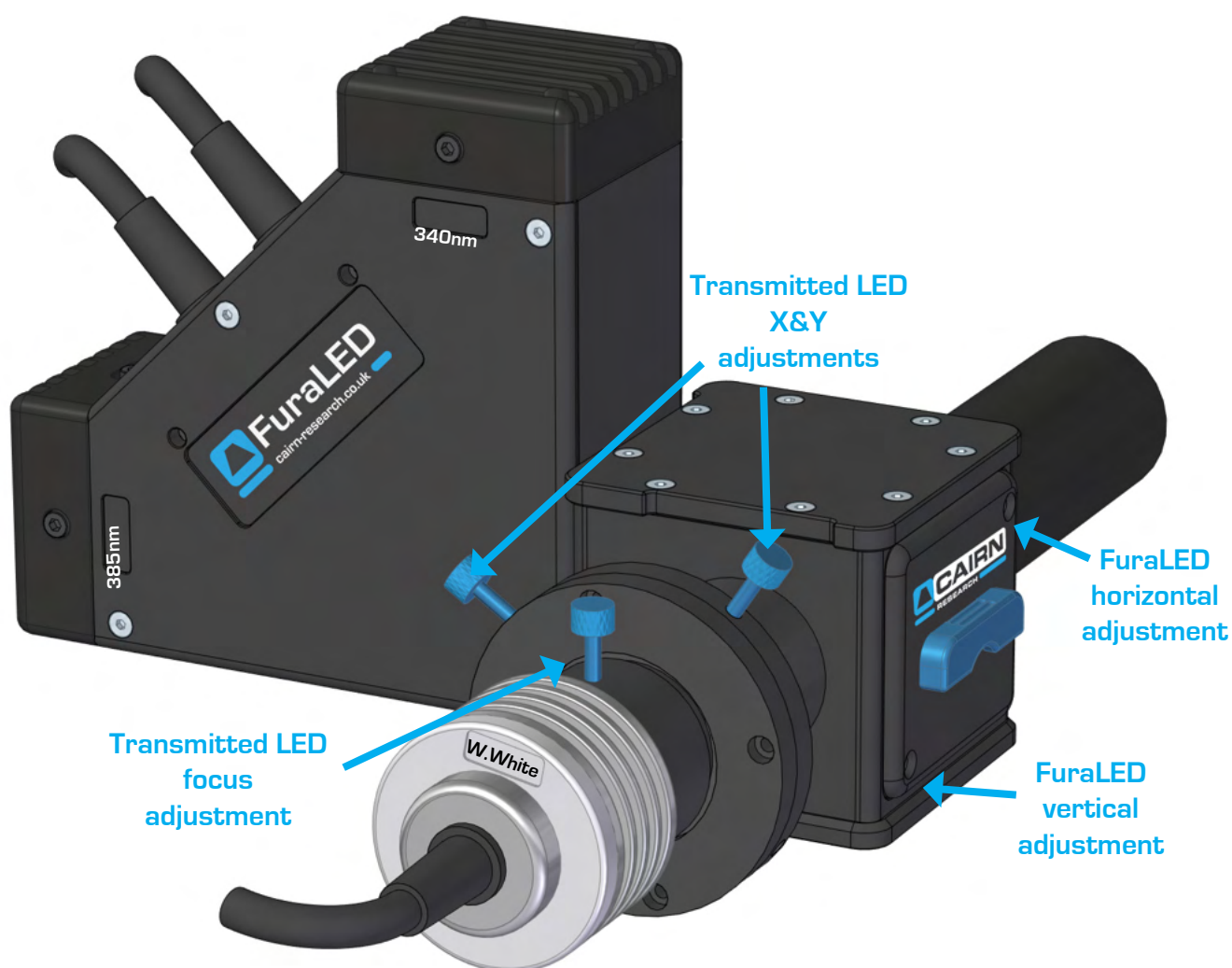


Figure 12: FuraLED and dual port coupling configuration

1. Align and focus the FuraLED 340nm and 385nm LEDs as per the previous instructions in section 4.4.
2. Align and focus the transmitted LED by referring to the previous instructions in section 4.1.
3. Perform any final positional adjustment (moving both UV LEDs simultaneously) via the dichroic mirror. Using the 1.5mm allen key provided, ensure both LEDs are central using the **FuraLED horizontal and vertical adjustments**.

**Please contact us if you have any questions or issues during OptoLED setup or operation and we will be happy to help! [tech@cairn-research.co.uk](mailto:tech@cairn-research.co.uk)**