OPTOSPLIT II BYPASS

Manual

This guide details initial set up and installation of your OptoSplit II Bypass (BP) image splitter. Each unit is serial numbered, calibrated and QC'd prior to delivery, therefore minimal setup is required providing you are familiar with the key controls.

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

A supplementary Troubleshooting Guide is also available to address frequently asked questions during OptoSplit operation.

Please do not hesitate to contact us if you have any questions or issues during installation or operation (tech@cairn-research.co.uk).

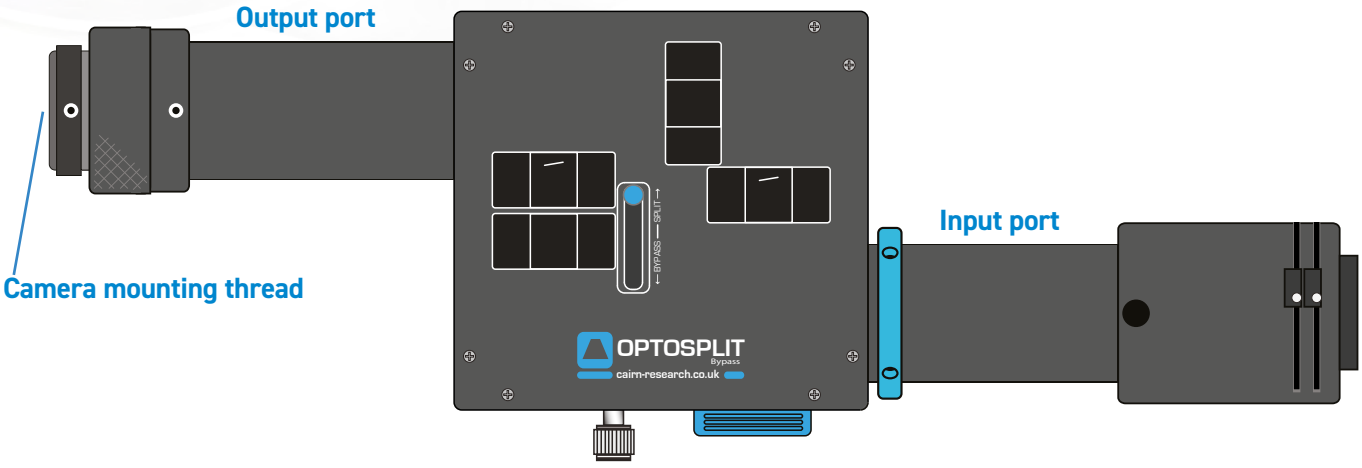
1) Mounting to your microscope

It is best practice to firstly ensure you have optimised your microscope light source in the absence of the OptoSplit, with the camera mounted directly on the c-mount. Many technical queries are raised relating to uneven illumination, which is often not introduced by the image splitter. Once both your transmitted and fluorescent light source have been correctly aligned and focussed, the OptoSplit can be mounted on a 1x c-mount; for deviations from this, please contact us for advice.

1.1) Correct camera orientation

The OptoSplit **output port** connects to your camera c-mount via a **camera mounting thread**. The silver knurled section on the output port can be rotated to secure the camera, whilst also ensuring any cables connected do not get tangled.

The OptoSplit **input port** then connects to your microscope c-mount.



For correct alignment of the two emission channels, the orientation of the camera once attached to the OptoSplit II BP unit is important. Always ensure the top of your camera lies parallel with the top of the OptoSplit. If fitting onto an upright microscope, this camera orientation remains the same.

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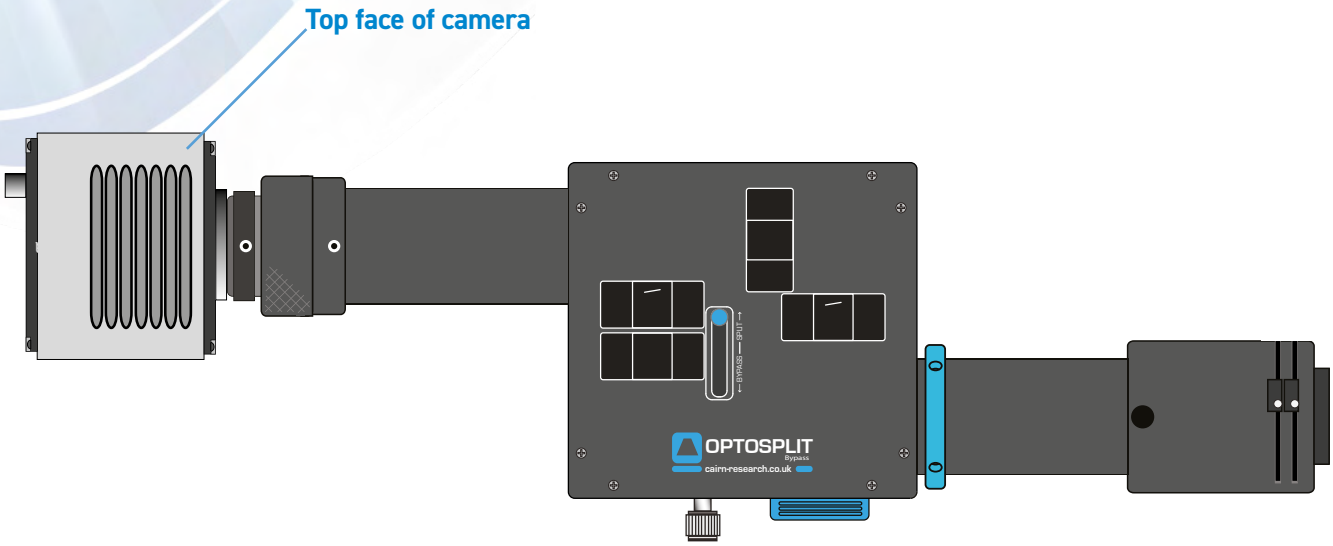
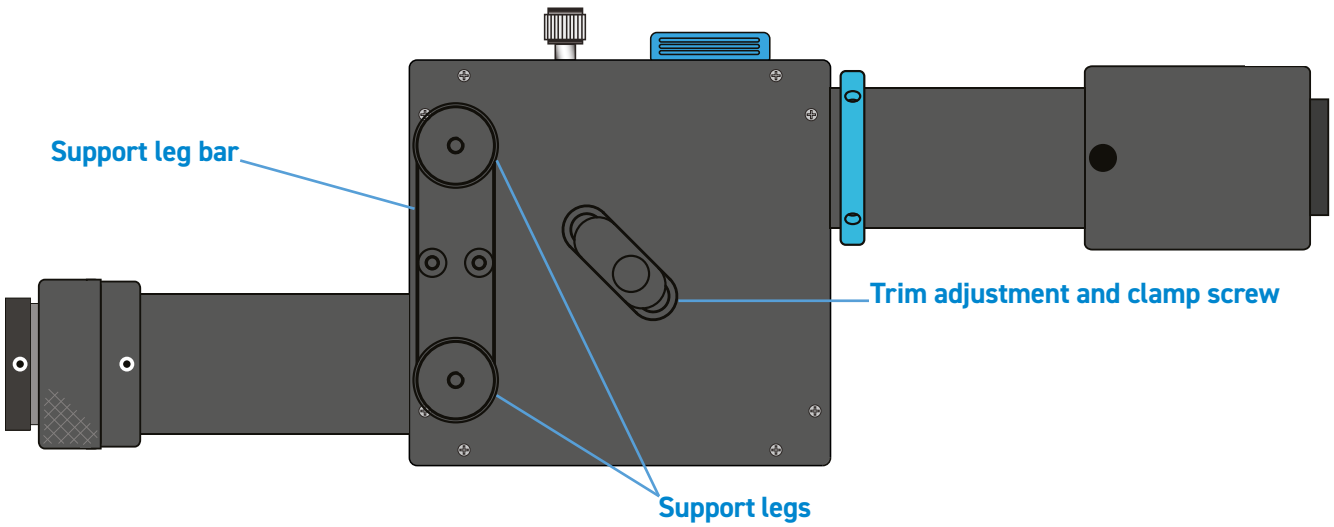


1.2) Support legs

Two support legs are supplied for use with an inverted microscope to support the weight of the unit.

1. Secure the **support leg bar** to the underside of the OptoSplit using the two screws provided
2. Screw the **support legs** to the end of the bar
3. The height can be adjusted on each support leg – we recommend a spirit level is used for final adjustment.

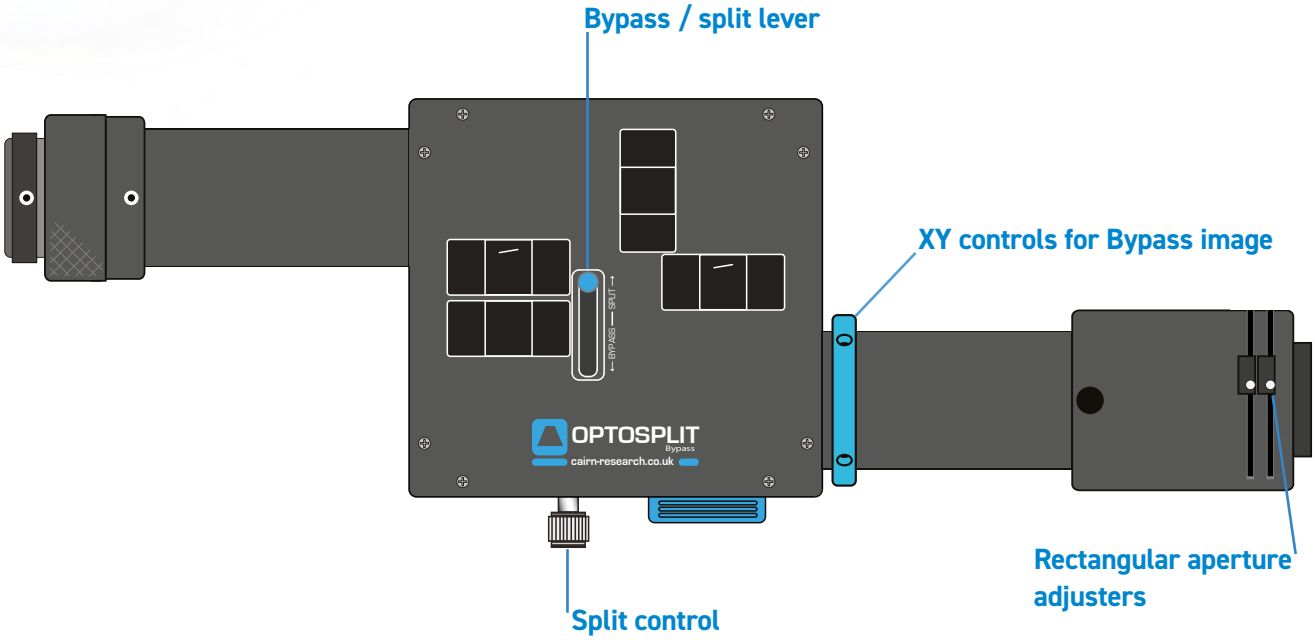


2) Key controls

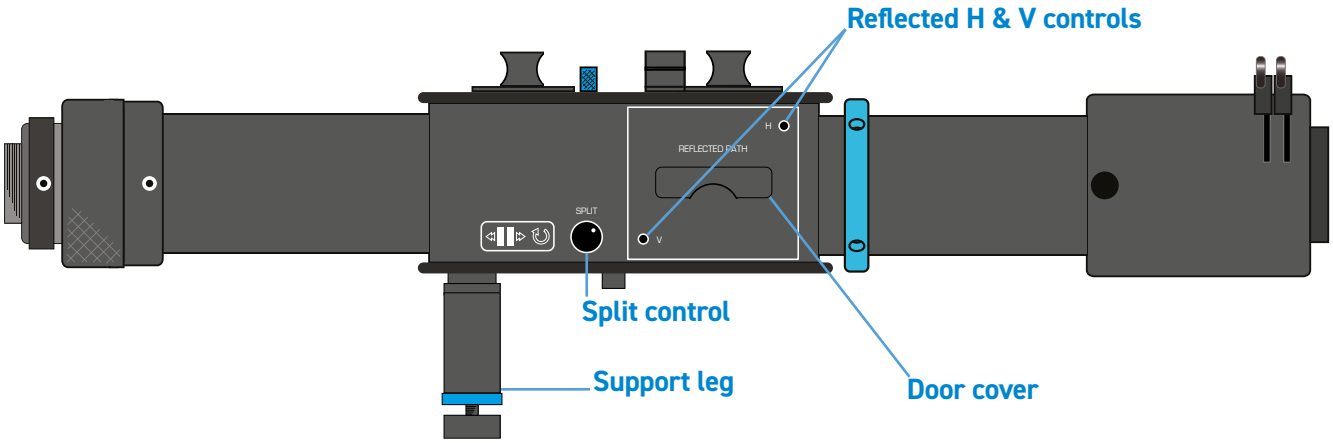
This section details the key controls for all operating modes – all labels are referred to in subsequent text in bold blue font.

2.1) OptoSplit panels

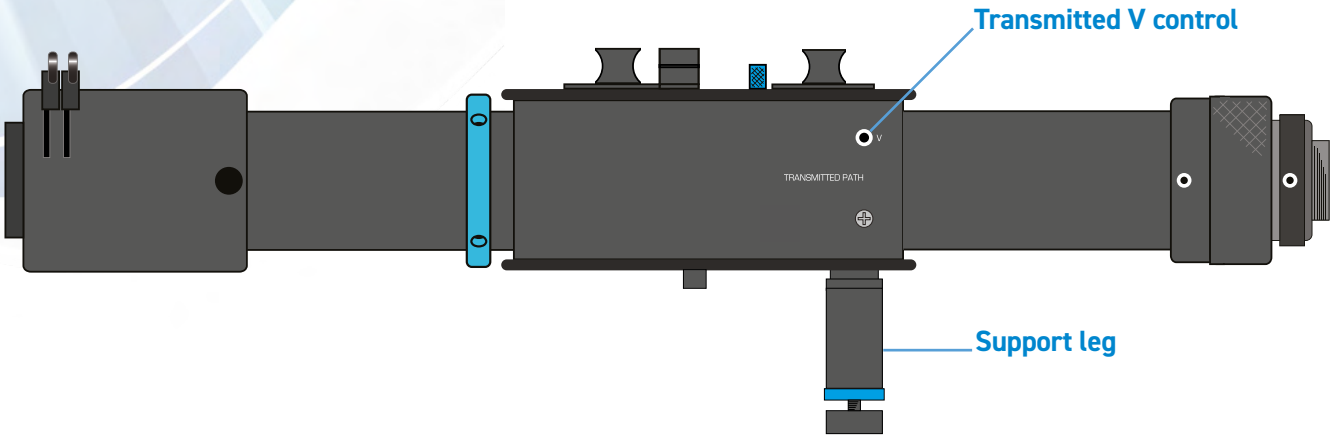
A: Top Panel



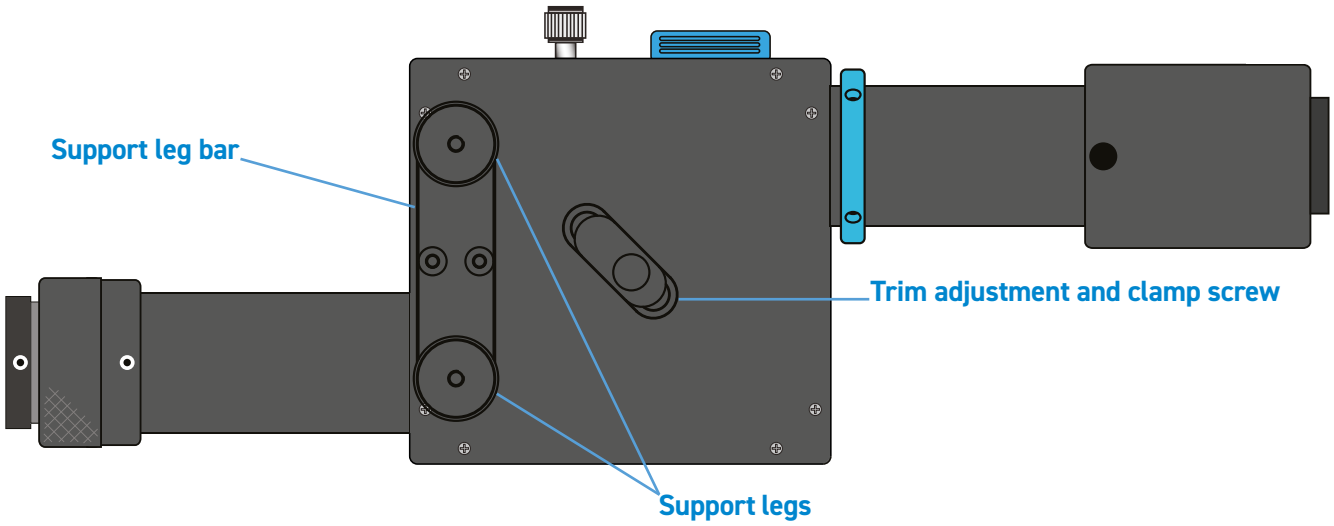
B: Left side panel



C: Right side panel



D: Underside



3) Centralising the Bypass image

The OptoSplit II Bypass unit requires a small adjustment once installed to ensure the Bypass image is projected onto the centre of the camera sensor. It's best practise to ensure this is carried out prior to performing dual channel imaging, but this adjustment can be checked periodically.

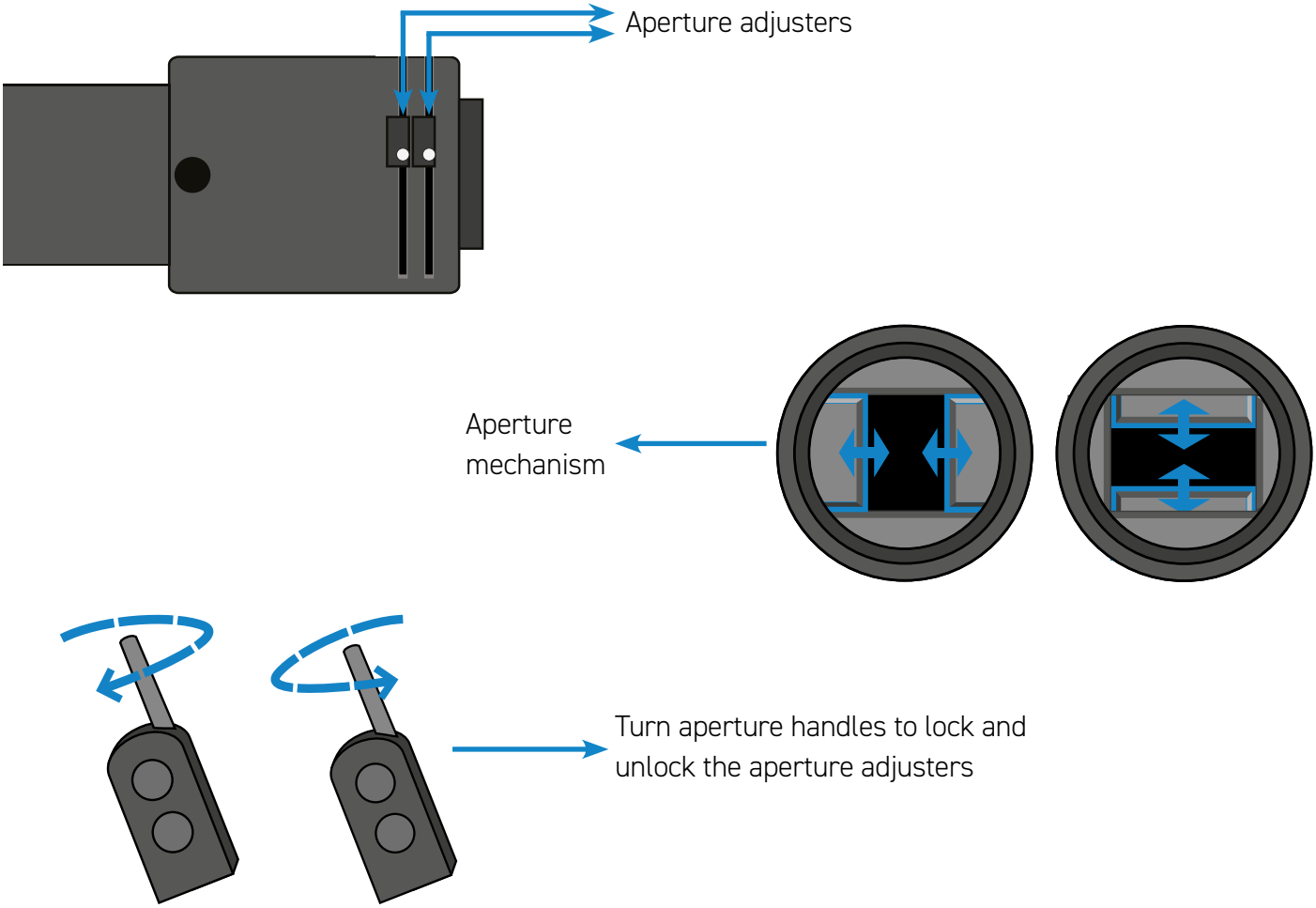
Firstly, it's useful to explore how to define a field of view and become familiar with the adjustable rectangular aperture.

3.1) Defining field of view

An adjustable **rectangular aperture** (or diaphragm) precisely defines the region of interest on half of the camera sensor. On occasions, the OptoSplit is supplied with a Cairn OptoMask diaphragm, which requires a specific setup procedure – please refer to supplementary documentation when appropriate.

1. Slide the **Bypass / Split Lever** to 'Bypass'
2. Ensure there is no Cairn filter cube installed in the unit by removing the magnetic **door cover** labelled REFLECTED PATH. Replace the door cover.
3. Adjust the two **rectangular aperture adjusters** (one horizontal, the other vertical) to create a single rectangular region of interest visible on your camera.
4. Ensure the image is square, with no rotation or slant in either direction. Slightly rotate the camera if required – a rectangular Region of Interest within your imaging software is useful as a reference for aligning the **rectangular aperture**.
5. The aperture edges should be in sharp focus, as the rectangular diaphragm is precisely located in the microscope focal plane.*

* If the rectangular diaphragm is not in sharp focus on your camera, please refer to the OptoSplit II BP Troubleshooting Guide in supplementary material to resolve.



3.2) Centring the Bypass Image

Once a rectangular region of interest has been defined, this image can then be centred in Bypass mode.

1. **The XY controls for the Bypass image** are located on the **input port** of the OptoSplit.
2. Using the 1.5mm allen key provided, adjust each of the two controls in turn whilst observing your single image on the camera. One control will result in a horizontal movement, the other vertical.
3. Centre the Bypass image using an iterative approach with the two controls. A rectangular Region of Interest within your imaging software is useful as a reference tool to ensure the image is centred and square.

4) Obtaining a split image

The predominant operating mode of two identical images projected onto each half of your camera sensor is achieved by installation of a Cairn filter cube.

For initial setup, we recommend using the supplied Calibration Cube (with a 50% / 50% mirror) and a focussed image of a graticule with transmitted light.

4.1) Installing the Calibration Cube

1. Slide the **Bypass / Split lever** to 'Split'.
2. Install the calibration cube, by removing the magnetic **door cover** labelled REFLECTED PATH.
3. Insert the calibration cube, which has a magnetic fixing and will easily locate into place, with the small handle facing outwards.
4. Replace the door cover.
5. Ensure the **rectangular aperture** is closed enough in both dimensions to see two distinct images.

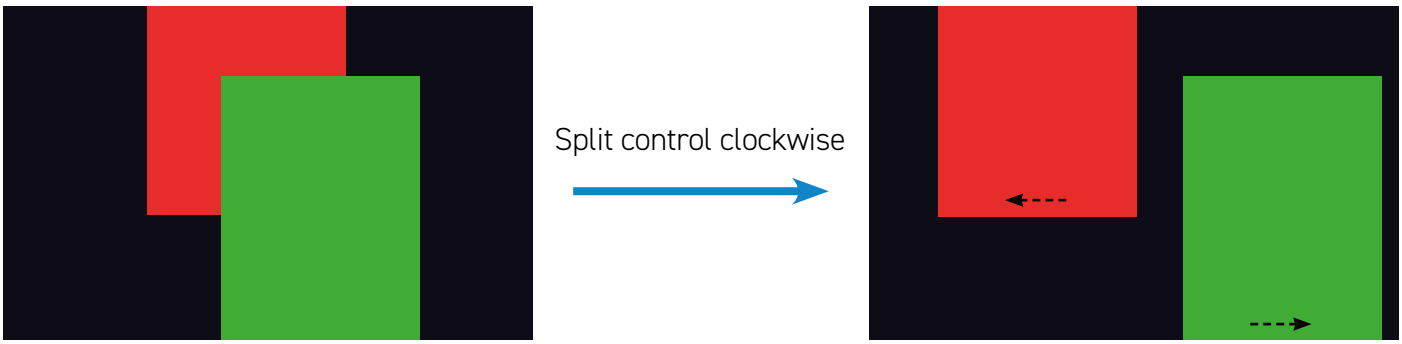
4.2) Split mode: image alignment

Once the calibration cube is in place, two identical images will be visible on a live camera image. It's best practice to setup the unit with the reflected (shorter) wavelength channel on the right and the transmitted (longer) wavelength channel on the left:

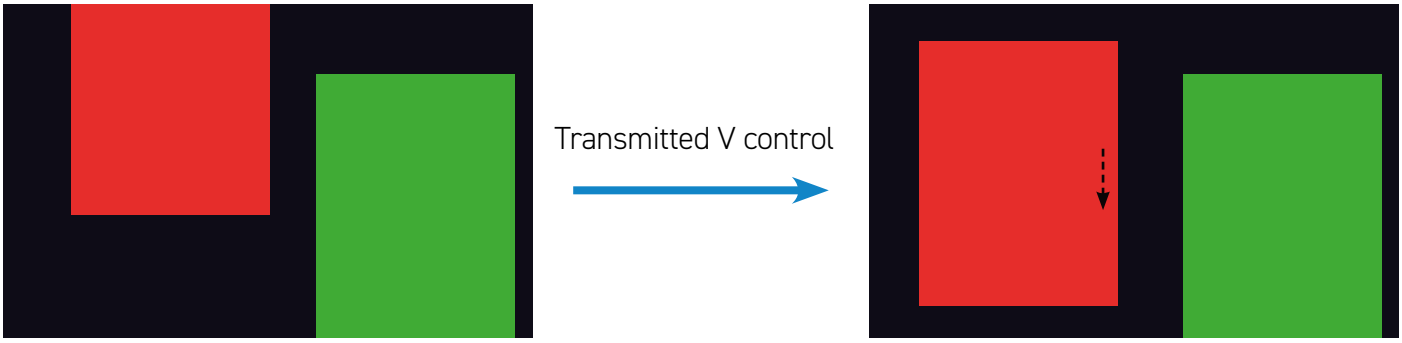
**Please note that the colours on the adjacent image are for illustration purposes only. On a monochrome camera, both will be monochrome.*

Firstly, identify each channel - the reflected image can be moved vertically and horizontally using the provided 1.5mm allen key on the **H&V controls** on the magnetic **door cover**.
Once you are aware which channel is which, turn your attention to the transmitted image and position this image on the left-hand side first.

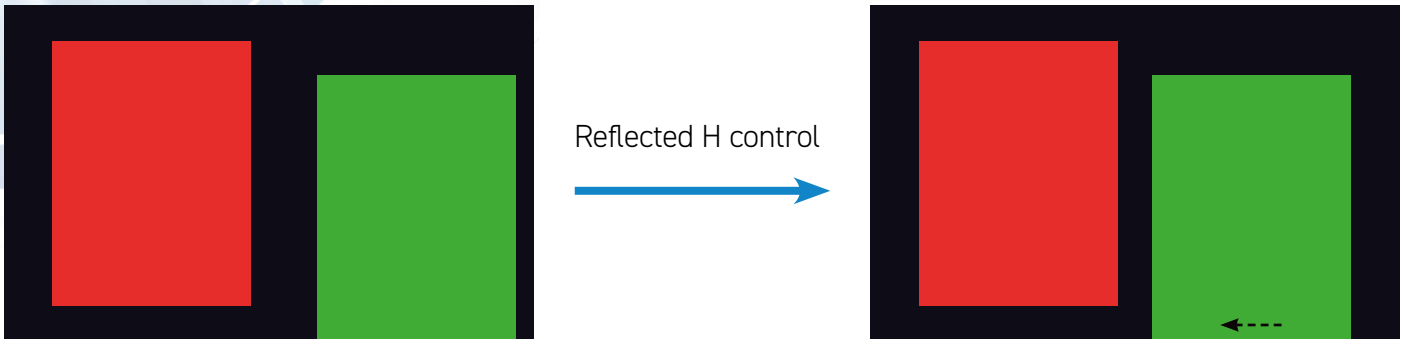
1. Turn the **split control** clockwise to position the transmitted image horizontally close to the edge of the camera sensor.



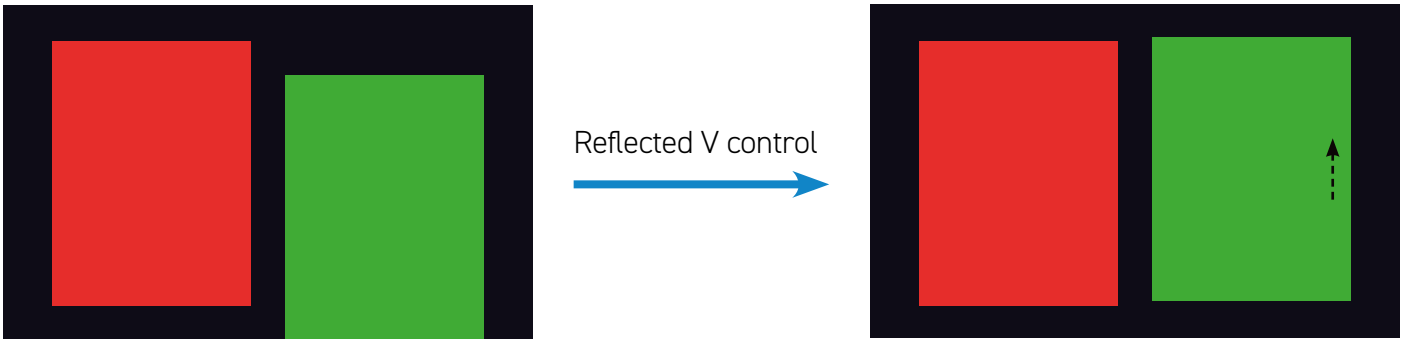
2. Adjust the **Transmitted V control** (using the 1.5mm allen key provided) to optimise the vertical position of this (red) image.



3. Adjust the **Reflected H control** to optimise the horizontal position of the reflected (green) image.



4. Adjust the **Reflected V control** to optimise the vertical position of the reflected (green) image.



Final tweaks of the **rectangular aperture** and **Split control** will ensure maximal field of view is projected onto each half of your camera sensor.

4.3) Split mode: Trim adjustment

'Vignetting' may be present once split mode has been optimised. This is characterised by one image appearing darker on one edge.

1. To remove vignetting, identify the **TRIM adjustment** on the underside of the unit.
2. A **clamp screw** holds the adjustment in place, therefore this may need to be turned anticlockwise to release the slider.
3. Slide the **TRIM adjustment** in one direction (diagonally) until the vignetting is removed. When moved too far, vignetting will begin to appear on the opposite image.

4. Slide the control until vignetting is removed from both images.
5. Re-tighten the **clamp screw**.

5) Filter cube assembly

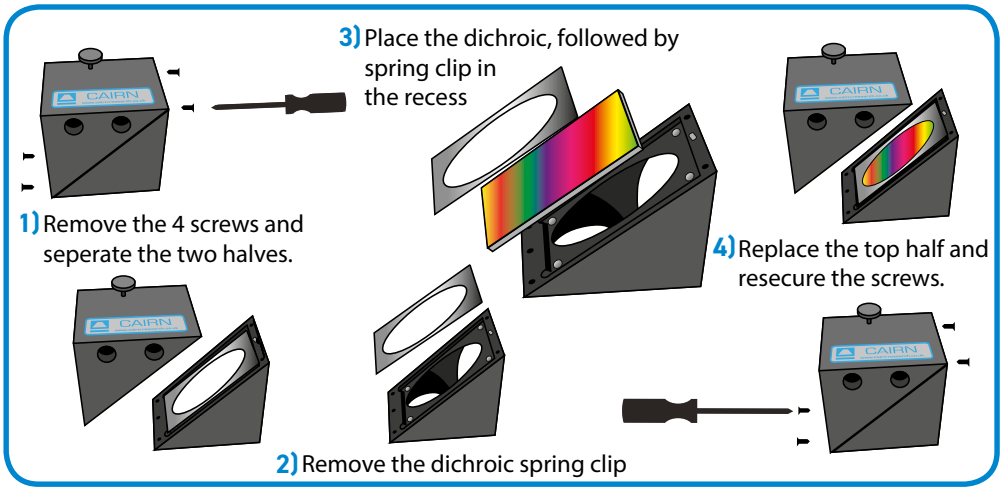
Often OptoSplit II BP units are supplied with populated filter cubes, however (if required), these can be installed using the guidance notes below.

As UK distributors, we have worked very closely with Chroma to develop a dichroic mirror range for use in our image splitters with minimal image distortion. As a result, we only recommend the Chroma Ultra-Flat (UF-2) 2mm thick dichroic mirror range.

The Cairn Filter Cube accepts dichroic mirrors of the following size: 26mm x 38mm x 2mm. Other dichroic mirrors will fit, but may not be optimal.

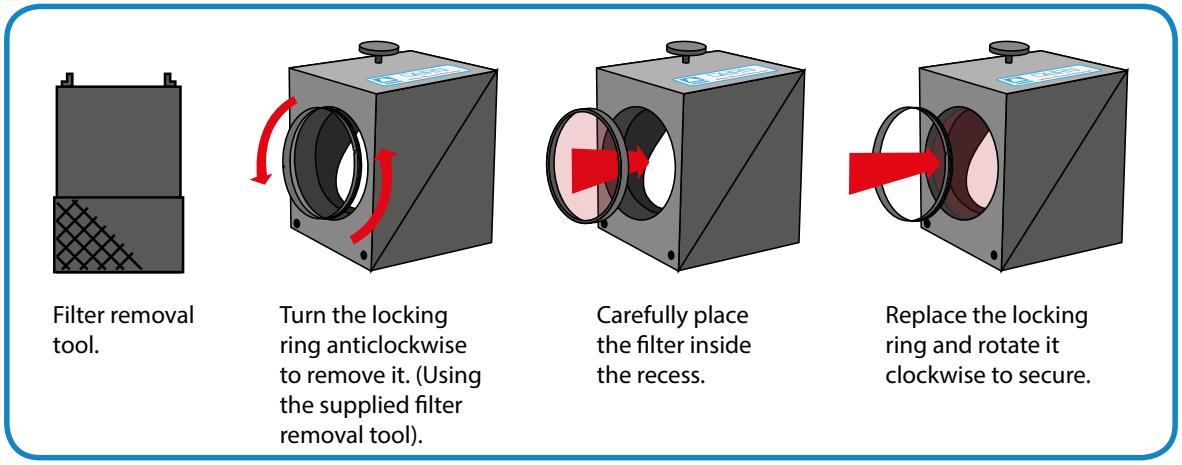
We are of course happy to provide filter and dichroic mirror advice for your specific application, so please get in touch.

5.1) Installing dichroic mirrors and emission filters



Installing Dichroic Mirrors into the Cairn Filter Cube

Please consult filter manufacturers instructions regarding orientation of the filter.

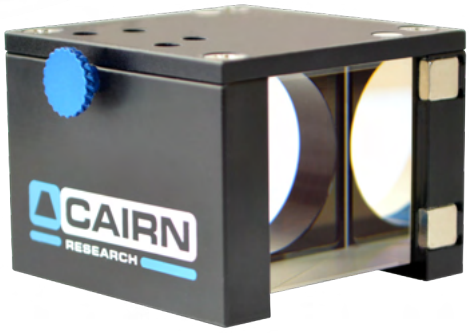


Installing Filters into the Cairn Filter Cube

5.2) Polarisation (anisotropy) studies

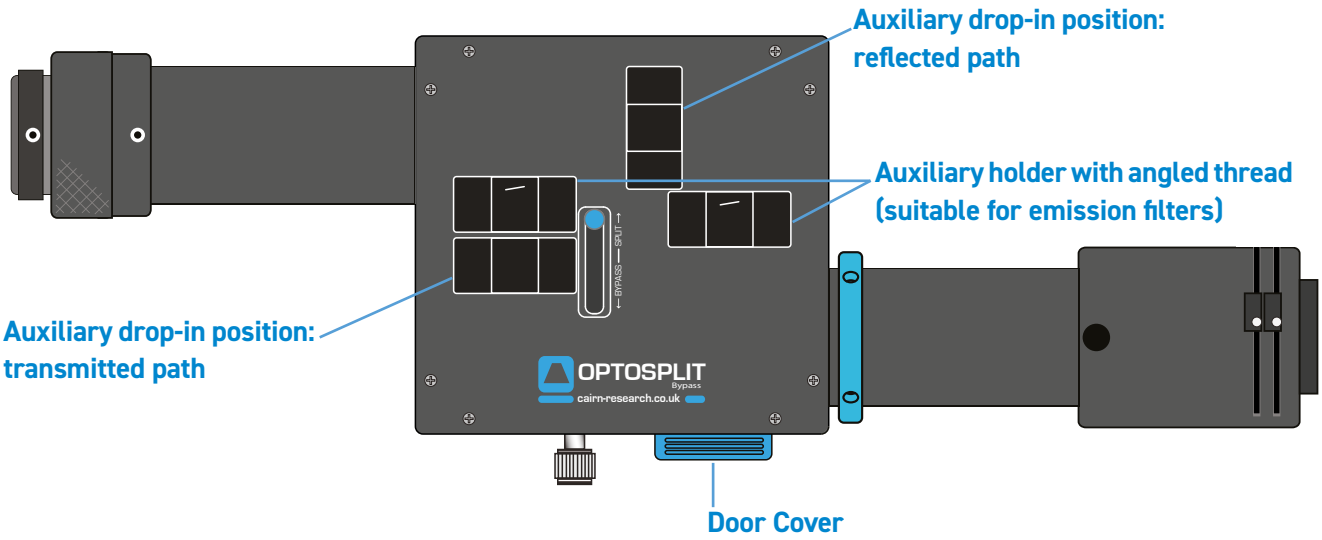
For polarisation experiments, a Cairn filter cube with an integrated polarising beamsplitter cube* is also available as an additional accessory. In addition, a rotating 25mm polariser is available to act as a 'clean-up' for the reflected S wave.

* Please note the polarising beamsplitter cube is very fragile!



Cairn polarising beamsplitter cube

Rotating 18mm polariser drop-in

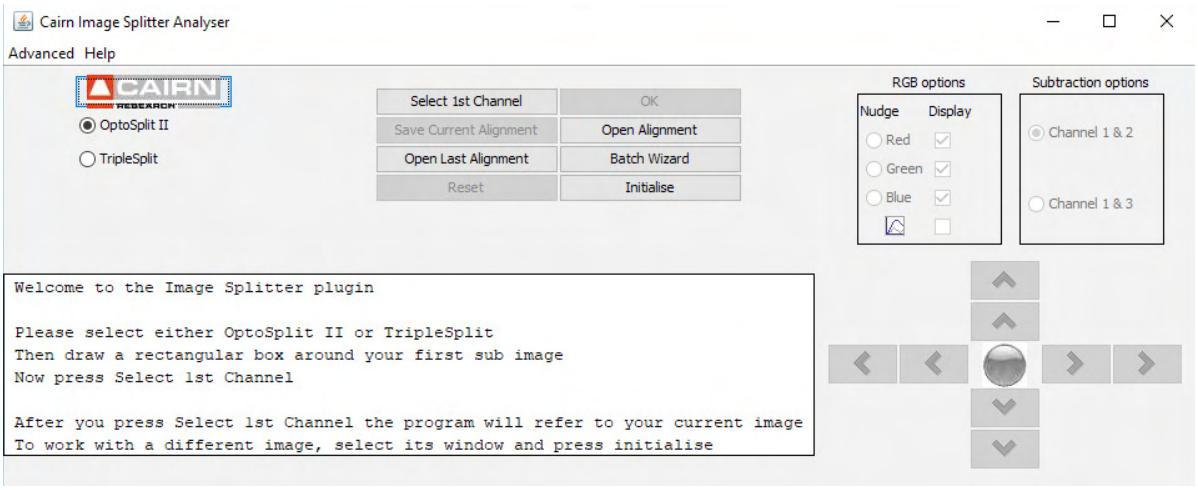


1. Insert the **polarising beamsplitter cube** by removing the **cube door** and allowing the magnetic cube to locate within the OptoSplit.
2. Replace the **door cover**.
3. Remove the blank cover for the **auxiliary drop-in position: reflected path**. (Note the blank holders will have no angled thread indictor and act as a protector for this pocket).
4. Insert the **rotating polariser drop-in** into the reflected auxiliary position.
5. The half-width blank auxiliary holder supplied will also be required to fill the space once the rotating polariser is in place.
6. Rotate the polariser until the reflected image (on the right-hand side) achieves maximal intensity, indicating the correct orientation.

6) Analysing dual channel data

Additional plugins and modules are available in a variety of software packages for analysis of dual channel data. For example, the 'Splitview' module in MetaMorph (Molecular Devices) or 'Field Split' in iQ (Andor) are useful tools.

We have also written an ImageJ plugin to generate a composite RGB image with the ability to pixel nudge each image in X and Y.



The .jar file can be downloaded from the 'Software' page on our website (https://www.cairn-research.co.uk/support/software/).
Paste the (unzipped) file into the 'plugins' folder in your root MicroManager folder on the C drive.

A MicroManager plugin for use with version 2.0 is also currently in development. Keep an eye on our website for more details.

Please contact us for any further assistance regarding data analysis (tech@cairn-research.co.uk)

7) Bypass Mode

The OptoSplit II Bypass is designed with a quick, simple mechanism for full field imaging without the need to remove the image splitter. A 100% mirror moves into position in 'Bypass' mode which blocks the reflected (shorter) wavelength, resulting in a single, central image projected onto the camera sensor.

In Bypass mode with the Cairn filter cube in place, the single image observed will be the transmitted (longer) wavelength channel. Depending upon your experimental requirement in Bypass mode, there are several scenarios for optimal operation.

7.1) Obtaining a full field image of your longest channel split conditions

e.g. A full field mCherry image when splitting GFP / mCherry

1. Leave the Cairn filter cube and existing microscope cube in place
2. Slide the **Bypass / Split lever** to 'Bypass'
3. Centre the Bypass image (if required) – refer to section 3.2
4. Open the **rectangular aperture**. Please note, it's best practise to only open the aperture enough to be just outside of your camera field of view to minimise scattered light.

To return to 'Split' conditions:

5. Slide the **Bypass / Split lever** to 'Split'
6. Close the **rectangular aperture** to ensure only half the camera chip is illuminated.
7. As the bypass mechanism does not interfere with any split controls, no further adjustment of the two split images should be necessary.

7.2) Obtaining a full field image of a longer emission wavelength than your split conditions

e.g. A full field Cy5 image when splitting GFP / mCherry

1. Leave the Cairn filter cube in place and change your microscope cube to a position suitable for Cy5 imaging.
2. The mCherry emission filter will need to be removed as it will impair the Cy5 channel. For ease of removal, we recommend mounting the mCherry emission filter in the **auxiliary drop-in position: transmitted path**, rather than in the Cairn filter cube.
   - Locate the **auxiliary holder with angled thread** indicated by the angled line on the handle.
   - Remove the retaining ring (using the tool provided).
   - Mount the emission filter with the active face (indicated with an arrow) pointing upwards.
   - Replace the retaining ring.
   - Replace the auxiliary holder in the transmitted path, maintaining the orientation of the active face towards the microscope.

3. Slide the **Bypass / Split lever** to 'Bypass'.
4. Centre the Bypass image (if required) – refer to section 3.2.
5. Open the **rectangular aperture**.

To return to 'Split' conditions:

6. Slide the **Bypass / Split lever** to 'Split'.
7. Replace the mCherry emission filter in the transmitted path.
8. Return your microscope cube to a position suitable for use with the OptoSplit.
9. Close the **rectangular aperture** to ensure only half the camera chip is illuminated.
10. As the bypass mechanism does not interfere with any split controls, no further adjustment of the two split images should be necessary.

7.3) Obtaining a full field image of a shorter emission wavelength than your split conditions OR imaging a variety of dyes utilising the microscope cubes.

e.g. A full field DAPI image when splitting GFP / mCherry

1. Remove the Cairn filter cube, gaining access via the magnetic **door cover**.
2. Change your microscope cube to a position suitable for DAPI imaging.
3. Slide the **Bypass / Split lever** to 'Bypass'
4. Centre the Bypass image (if required) – refer to section 3.2
5. Open the **rectangular aperture**.

To return to 'Split' conditions:

6. Slide the **Bypass / Split lever** to 'Split'
7. Replace the Cairn filter cube
8. Return your microscope cube to a position suitable for use with the OptoSplit
9. Close the **rectangular aperture** to ensure only half the camera chip is illuminated.
10. Although designed to generate minimal displacement when replacing the Cairn filter cube, final adjustment of split conditions may be necessary – please refer to section 4.2

Please refer to the supplementary Troubleshooting Guide for additional information not described in this manual.

Do not hesitate to contact us if you have any questions tech@cairn-research.co.uk