CAIRN RESEARCH

Specialists in Fluorescence Imaging, Electrophysiology, and Microscopy

OptoSplit II

Instruction Manual

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Important Information

Please Read Before Installing and Operating Your Optosplit II

For maximum reliability we recommend using the equipment within certain guidelines. If in any doubt, then please feel free to contact our technical support department who are always willing to help. (e-mail tech@cairn-research.co.uk)

Note: Please do not adjust any control before reading this manual.

The Optosplit is supplied pre-aligned and centred, please refer to this manual before making any adjustments.

When changing the Cairn Filter Cube, care should be taken not to touch any optical surfaces. If any lenses, filters, or mirrors are marked then they should be wiped with a clean lens tissue.

Contents

1 Introduction

In fluorescence imaging applications it is often useful to acquire simultaneous images at two emission wavelengths. Traditionally such applications have been restricted by the speed of an electronic filter changer, or by the cost and complexity of adding a second camera to a system. In many instances the region of interest (ROI) does not require the full resolution of the camera, so the ideal solution would be to simultaneously image at two different wavelengths on the same camera chip. In conjunction with a research microscope and a suitable CCD camera the Cairn Optosplit II allows the researcher to do exactly this.

The Cairn Optosplit II is usually supplied with a rectangular aperture to define a ROI and includes controls to allow the two images to be positioned accurately and conveniently within the camera frame. Images can be acquired using any imaging software and processed either manually off-line or using an appropriate analysis tool such as the Splitview module in Universal Imaging's Meta series software or Field Split in Andor Bioimaging's iQ.

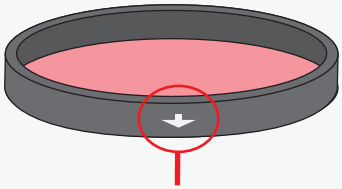
The instrument is usually configured to attach to the c-mount output port of a research microscope, with a c-mount CCD camera fitted to its output. The design allows for connection to a variety of alternative devices, so please consult with us if you intend using it in any other configuration.

2 Installation Guide

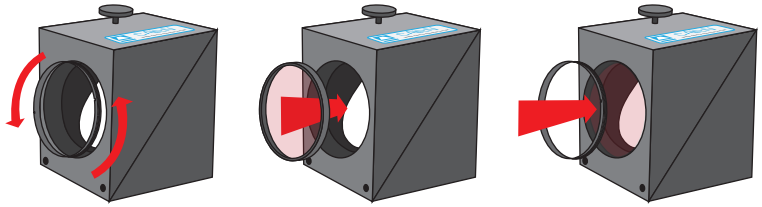
Before using the Optosplit II image splitter you will first need to install the appropriate set of filters and mirror for your application into the Cairn Filter Cube. Instructions for these procedures are in sections 2.1 and 2.2. Always take extreme care when changing or adjusting the filter and mirror sets to avoid damage or soiling. If you purchased your Optosplit II with a full filter set pre-installed then you may wish to go to section 2.4. as the following may not be pertenant.

2 .1 Installing Filters into the Cairn Filter Cube

The Cairn Filter Cube has spaces for two 25mm filters. These are held in place by locking rings which can be removed using the tool provided. To fit a filter, remove the locking ring and place the filter orientated so that the arrow points into the Cairn Filter Cube and towards the light path. Then simply replace and tighten the locking ring.



The arrow should be orientated into the filter cube and towards the light path.



Turn the locking ring anticlockwise to remove it.

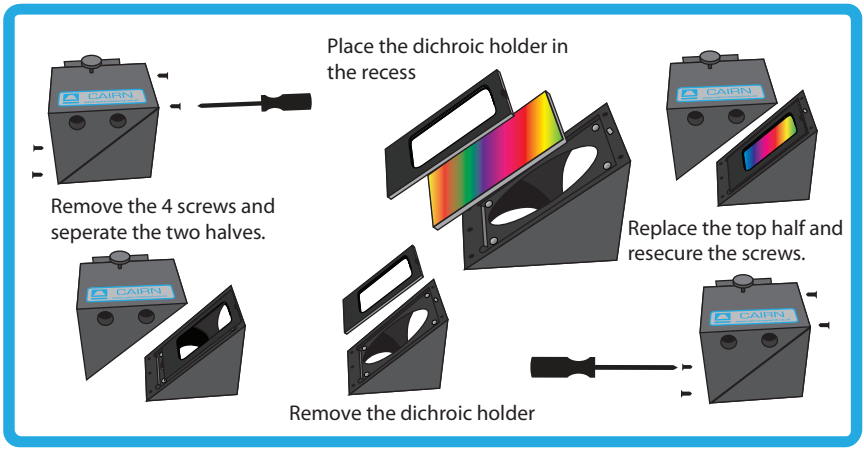
Carefully place the filter inside the recess.

Replace the locking ring and rotate it clockwise to secure.

In most applications the dichroic beamsplitter will have longpass characteristics, so the longer wavelength of the two filters should be located in the straight through filter position, and the shorter wavelength filter in the second position. (Please refer to the technical summary in section 4 for details of the light path.)

2 .2 Installing Dichroic Mirrors into the Cairn Filter Cube

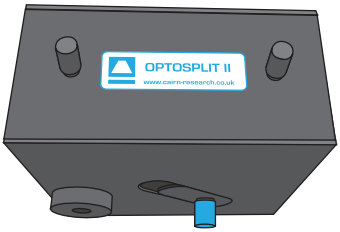
To fit your dichroic mirror into the Cairn Filter Cube remove each of the four screws that secure the two halves together. You will then be able to gently pull the two halves apart. There is one steel locating rod in each half of the cube to ensure the correct alignment when it is reassembled. Once the two halves of the cube are seperated place the dichroic mirror (active side down) into the rectangular recess. Then simply reassemble the cube and tighten the screws.

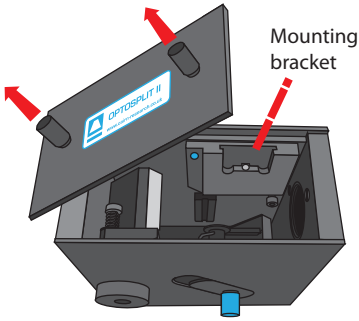


2.3 Inserting the Cairn Filter Cube into the Optosplit II

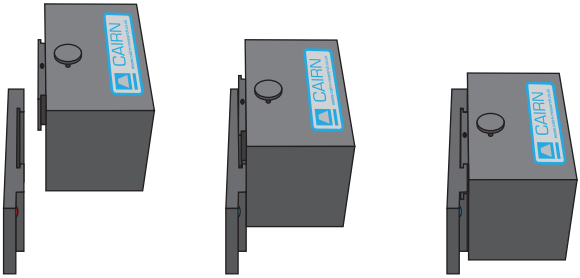
The Cairn filter cube is designed to be easily accessible in order to facilitate quick and easy changes of filter sets. Access to the filter cube mount is gained by removing the access panel using the two handles to pull gently and firmly. The filter cube mount will now be visible. The mount attached to the rear of the Cairn Filter Cube is designed to mate with the bracket on the internal wall of the Optosplit II so that the small handle on top of the cube will be facing out. Once the Cairn Filter Cube is mounted successfully replace the access panel using the two handles.

(See over for diagram.)

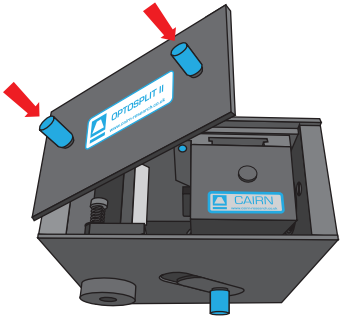


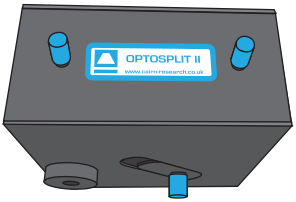


Remove the access panel using the two handles.



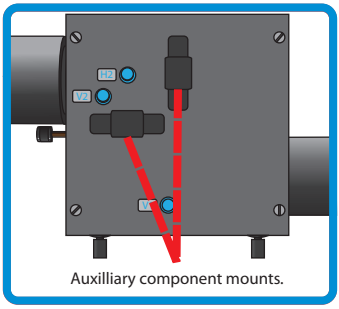
Mount the filter cube onto the brackect on the interior of the Optosplit II.





Replace the access panel using the two handles.

2.4 Auxilliary Component Mounts



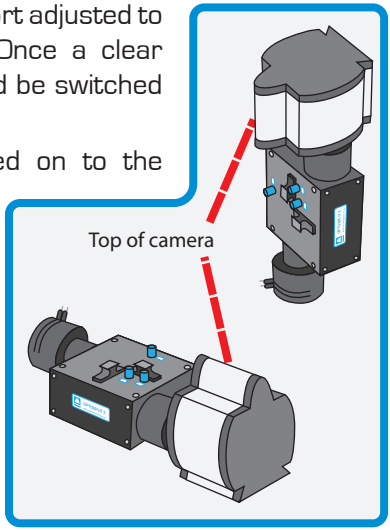
To control the relative intensity of the two pathways, we recommend using neutral density filtering in the brighter pathway using the auxilliary component mounts. This is required only when one image is disproportionately brighter than the other.

This auxilliary position can also be used for corrector lenses to reduce chromatic abberation and ensure both pathways are focussed on the camera sensor.

2.5 Installing the Optosplit II in the Light Path

Before installing the Optosplit II unit it is important to first set up the microscope, camera, and software to give a clear image of an object of less than half the size the camera frame. Ideally this should be a real sample with the appropriate optical properties for the installed filter set. Failing this, a standard bright field image can be used, but this may lead to arbitrary intensity differences between the spectrally resolved images.

Firstly the CCD camera should be mounted on the microscope C-mount output and the port adjusted to give the sharpest possible image. Once a clear image can be seen the camera should be switched off and removed from the microscope.

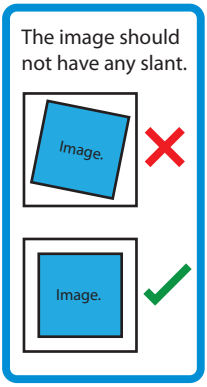


The Optosplit II should then be fitted on to the microscope with the diaphragm orientated toward the output of the microscope. The camera can then be fixed onto the output port of the Optosplit II, with the top orientated so that the top of the camera lies parallel with the top of the Optosplit II. If you are fitting onto a vertical mount then the tops should still be orientated in the same direction.*

* A 1mm nylon spacer is also included with each unit. This may be required on the camera output mount, particularly when mounting a shutter in addition to the OptoSplit.

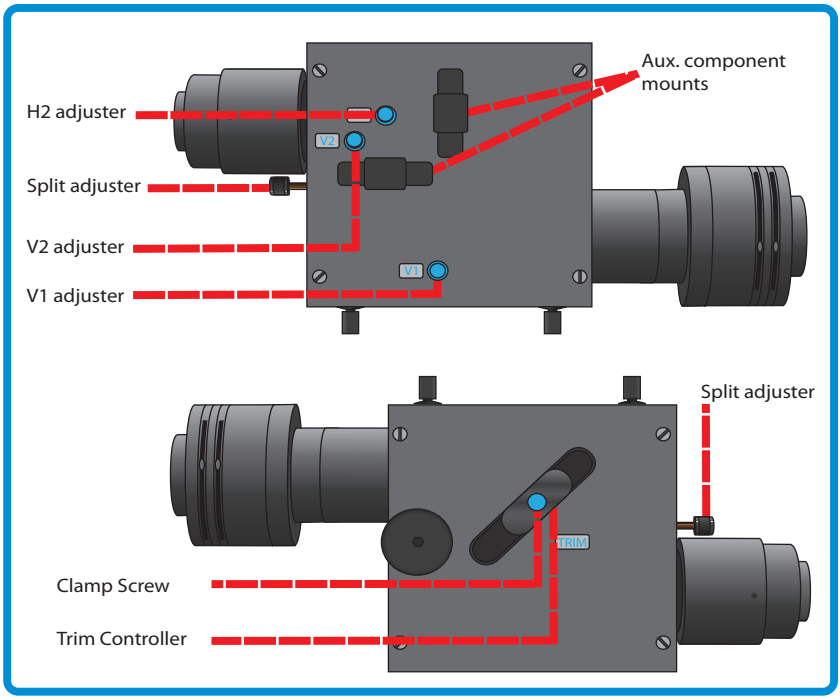
Having fitted the camera to the output port of the Optosplit II it should be possible to adjust the image to see a sharp picture of the aperture edges with the sample in focus. The image should line up with the edges of the aperture and should not have any rotation or slant in either direction.

During manufacture the Optosplit II is calibrated with the two images approximately super imposed, so when it is first installed a single central image is seen on screen.



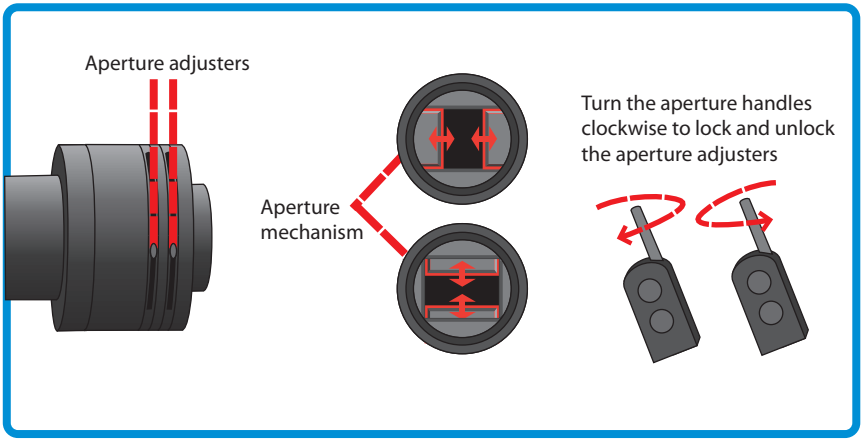
3 Operation of the Optosplit II

The Optosplit II uses a single control for adjusting image seperation and allows for different sizes of samples to be used. There are additional controls for refining the ROI, and centering the image.



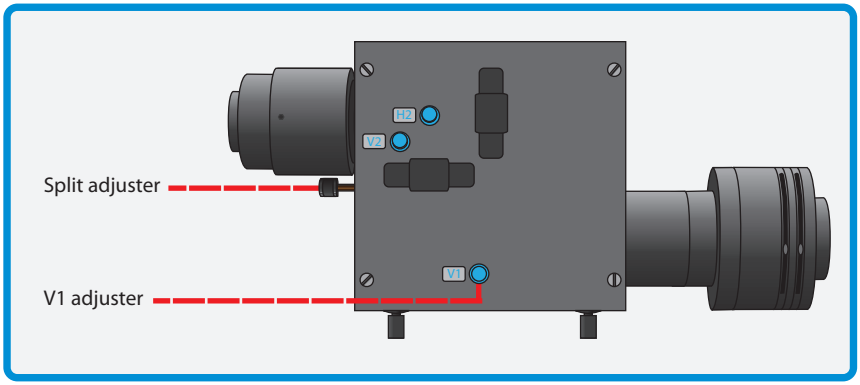
3.1 Adjusting and Locking the Aperture

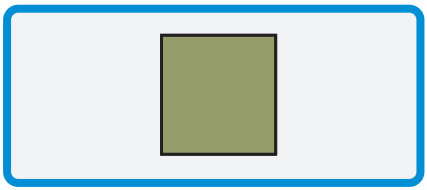
The Optosplit II is supplied with an adjustable rectangular aperture that allows the user to determine the ROI both vertically and horizontally. Once the ROI has been defined, the aperture can be locked in place using the aperture adjusters. (See diagram below.)
In order to adjust the ROI adjust each of the two levers on the input port of the Optosplit II until the desired area is defined on the camera. If you wish to lock the aperture in place then tighten the levers by rotating them clockwise, and to loosen them rotate anti-clockwise.



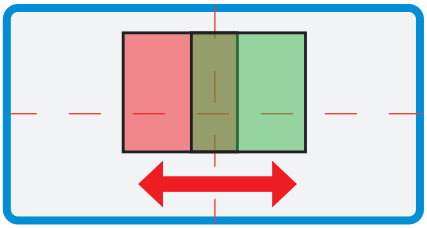
3.2 Adjusting the Position of the Images

Once the camera is set up it is generally a good idea to familiarise yourself with the operation of the alignment adjustments. Please note that the diagrams overleaf indicate the images for a system using an appropriately aligned and centred rectangular diaphragm. An image similar to that shown opposite will be observed when the Optosplit II is first used, with the two images being displayed superimposed.
When the camera is mounted correctly, the split adjuster and the aperture control are the only controls that will be frequently adjusted. The remaining adjusters on the body of the Optosplit II should remain untouched unless the filter set has become unaligned.

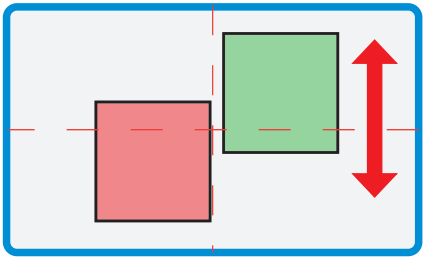




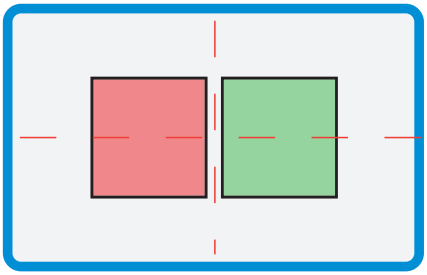
Here we see the two superimposed images. Although your image may be monochromatic, colours have been used here to define the two images.



Turning the Split control anti-clockwise will seperate the two images along the horizontal axis. Only small adjustments will be required when using the horizontal image seperation adjustment.



Should the left and right images be at different vertical heights then adjust the images using the V1 and V2 adjuster. Turning V1 will alter the position of the shorter wavelength image, and V2 will alter the position of the longer wavelength image.



When the spectrally resolved images are side by side on the camera chip you are ready to record. When carrying out experiments the aperture should be set to mask the region of interest tightly so that the two images are located as closely as possible on the camera chip.

3.3 Focussing the Camera on the Aperture

If the Optosplit II aperture is not in sharp focus, then adjust the fine focus on the camera as follows:

1. Set an aperture size of less than half the width of the camera frame.

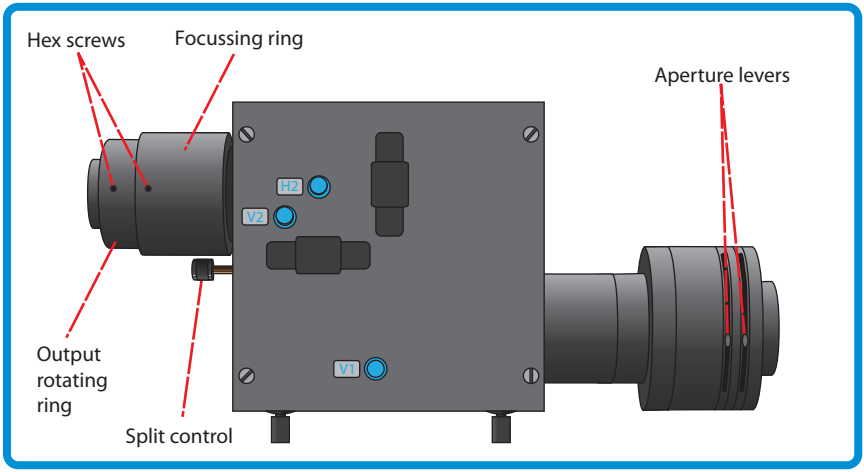
2. Slacken the two hex screws that retain the output rotating ring to allow the camera to rotate.

3. Separate the two images slightly with the Split control.

4. Slightly loosen the hex screws on the focussing ring. Adjust the focus whilst ensuring the optosplit is secure, and holding the camera, until the apertures edges are in sharp focus.

5. Then lock off the focussing ring by re-tightening the hex screws. Ensure the camera is again lined up with its top orientated the same way as the top of the Optosplit II and then lock off the output rotating ring.

6. Tighten the two hex screws that lock the camera rotating ring.



3.4 Centering Images on the Camera

When the Optosplit II is initially installed, or if the filter block goes out of alignment, the Optosplit II images can show visible aberrations, or in extreme cases only a single image may be observable for any setting of the separation control. In these cases the following procedure will enable the filter set to be reconfigured.

1. Define a small area using the Optosplit II aperture and centre the shorter wavelength image horizontally using the Split control.

2. Centre the same image vertically using the vertical offset adjustment marked V1 on the Optosplit II body.

3. The short wavelength is now correctly positioned.

4. To centre the long wavelength pathway, we superimpose the longer wavelength image on the shorter wavelength image we have just centred. Do this using the V2 and H2 adjusters.

5. Centre the image horizontally by smaller adjustments of H2. Once the image is exactly superimposed on the long wavelength image in the centre of the field of view we can adjust the vertical position of the image.

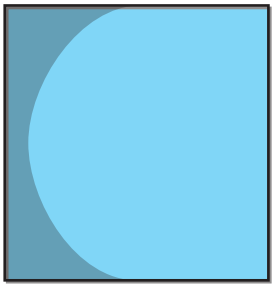
6. Adjust the vertical position with V2.

7. Recheck image horizontal alignment and repeat steps 5 & 6 if required.

Note: If the images are superimposed off-centre then image aberrations can result, so ensure the short wavelength image is correctly centered before positioning the long wavelength image

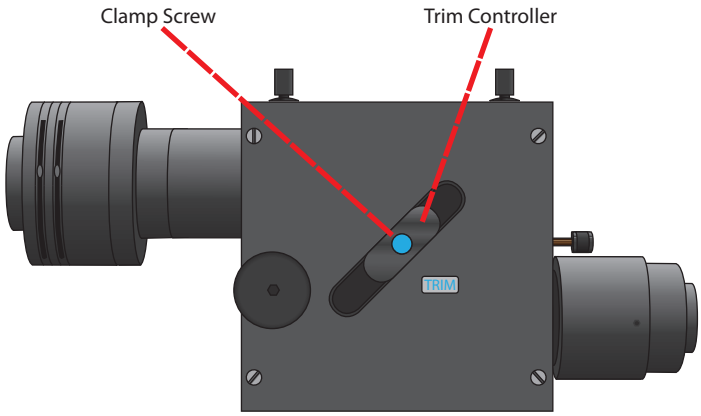
3.5 Image Optimisation (Trim Adjustment)

Vignetting is a darkening of the image towards one edge.



Vignetting is when the image appears darker towards one edge. This effect can be easily corrected by adjusting the trim control. To adjust the trim control, loosen the clamp screw on the underside of the Optosplit II by a quarter of a turn in order to free the slider which controls the trim control. Gently move the slider until the vignetting is eliminated. If the slider is moved too far then the vignetting will become apparent on the other image and will need to be adjusted in the opposite direction until both images are the same intensity. It is important to remember to re-tighten the clamp screw once the adjustment is complete.

If the Optosplit II has been configured into single channel (non-split mode) (see section 3.6) and Trim adjustment is required to compensate for vignetting being observed, it is likely the slider will need to be at the extreme of its range of movement to either end.



3.6 Single channel (non-split) mode

When dual splitting is not required, the OptoSplit II unit can be used in bypass mode, allowing the unit to remain in situ on the microscope whilst utilising the full camera chip to generate one image.

There are several ways this can be achieved:

A: Removing the filter cube

Suitable if no emission filters are required in the light path

1. Remove the dichroic mirror cube (see section 2.3)
2. Centre the image using the Split control
3. Open the aperture
4. Adjust the Trim control to remove any vignetting (see section 3.5)

B: Blocking the longer wavelength path

Suitable if only the shortest wavelength channel is required

1. Leave the dichroic mirror cube in place and block the long wavelength path using the shutter plate provided. Most dichroic mirror have long-pass characteristics, in which case the longest wavelength will be the transmitted channel.

   (See section 4 for help differentiating the channels).
2. Centre the image using the Split control
3. Open the aperture
4. Adjust the Trim control to remove any vignetting

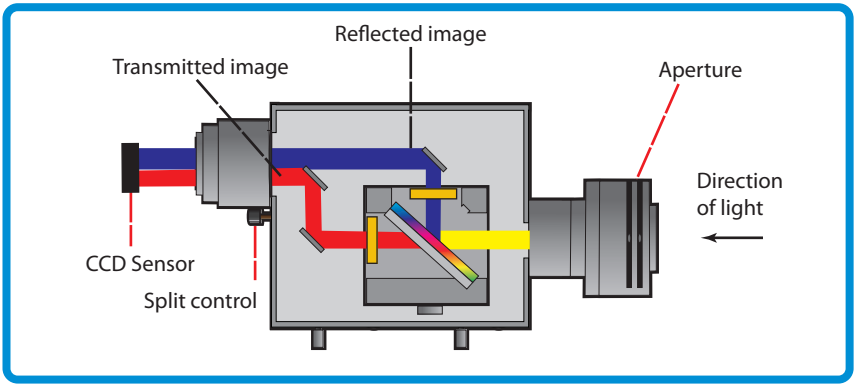
C: Blocking the shorter wavelength path

Suitable if only the longest wavelength channel is required

Follow the steps for option B, but block the short wavelength path (reflected channel) with the shutter plate instead.

4 Technical Summary

The Optosplit II uses a conventional dichroic mirror to seperate the light into two different spectral bands. This wavelength selection is usually augmented by the use of bandpass filters as shown below.



The design is loosely based on technology described by Kinosita et al. (J.Cell Biol. (1991) 115, 67-73), but includes several proprietary features, most notably:

A rotating mirror cradle to give precise symmetrical control of the degree of separation whilst maintaining identical path lengths.

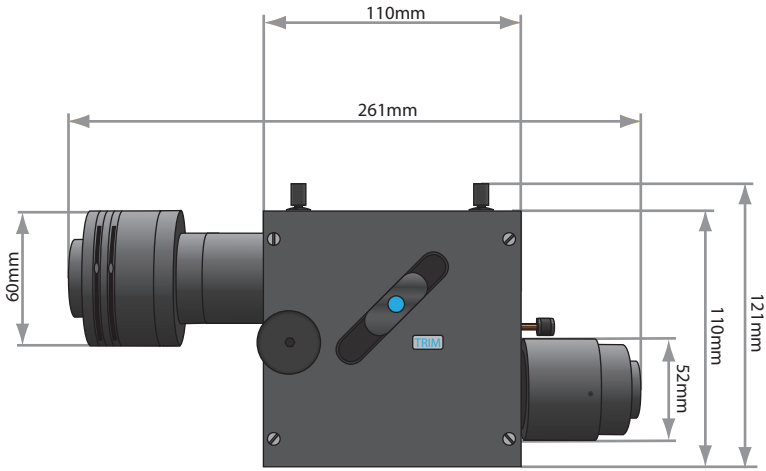
A half-size fully silvered mirror at the output port for recombining the images.

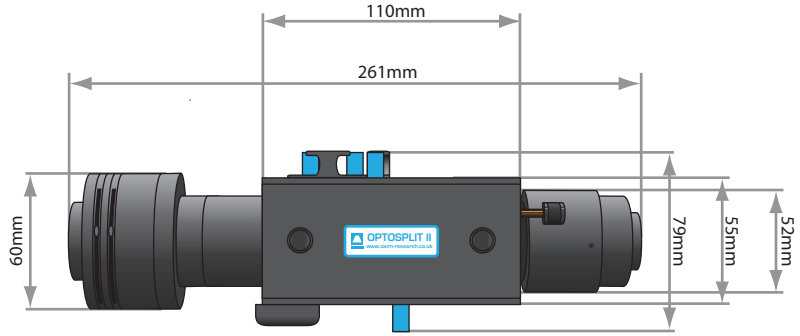
Fine adjustment controls for camera focus and vertical alignment.

Optional ROI definition using adjustabe rectangular aperture.

The system is supplied with high grade AR coated achromatic doublet lenses and dielectrically coated mirrors for maximum throughput.

Dimensions excluding couplings.	H55, W110, D110 (mm)
Dimensions including couplings.	H55, W261, D110 (mm)
Approximate weight.	1.26 kg
Power consumption.	0


110mm
261mm
60mm
TRIM
52mm
110mm
121mm


110mm
261mm
60mm
OPTOSPLIT II
79mm
55mm
52mm

5 Technical Support

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