CAIRN RESEARCH

Specialists in Fluorescence Imaging, Electrophysiology, and Microscopy

OptoSplit III

Instruction Manual

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Important Information
Please Read Before Installing and Operating Your OptoSplit III

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 multiple emission wavelengths. Traditionally such applications have been restricted by the speed of an electronic filter changer, or by the cost and complexity of adding extra detection hardware. 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 different wavelengths on the same camera chip. In conjunction with a research microscope and a suitable CCD camera the Cairn OptoSplit III allows the researcher to do exactly this.

The Cairn OptoSplit III is usually supplied with a rectangular aperture to define a ROI and includes controls to allow up to three 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.

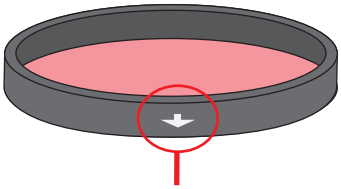
Installation of the OptoSplit III in the light path is straightforward and does not require any special tools. Should you wish to make use of the full input image without splitting (non-split mode), then exchanging the triplecube for the bypass cube, and a simple adjustment of the input diaphragm will permit this. We do however recommend removal of the OptoSplit from the light path when the splitting mode is not required to ensure the maximum light throughput to your detector.

2 Installation Guide

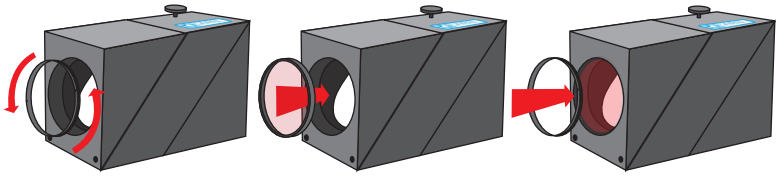
Before using the OptoSplit III image splitter you will first need to install the appropriate set of filters and mirror for your application into the Cairn Filter Cube. Instructions for these procedures are in sections 2.1 and 2.2. Always take extreme care when changing or adjusting the filter and mirror sets to avoid damage or soiling. If you purchased your OptoSplit III 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 three 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.



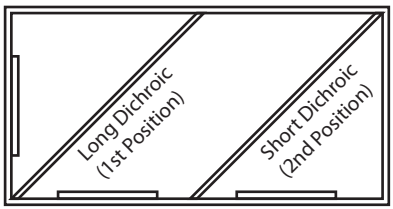
The arrow should be orientated into the filter cube and towards the light path.



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.

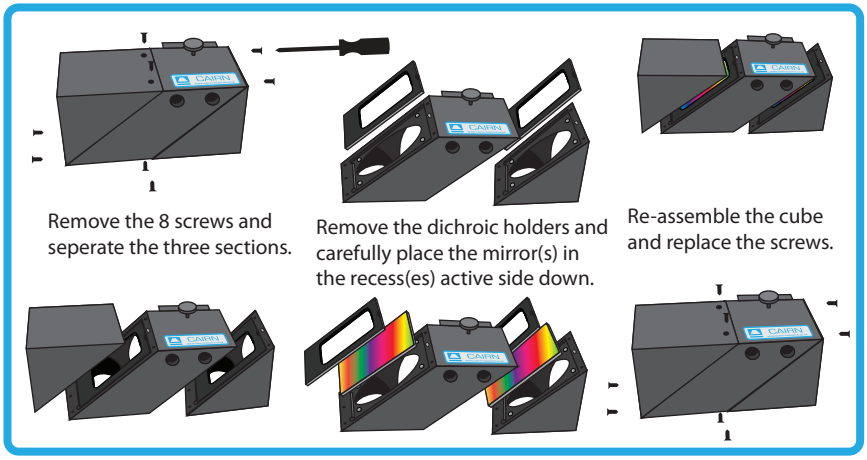


(View from bottom of cube)

In most applications, the dichroic beamsplitter(s) will have longpass characteristics, so the longer wavelength of the filters should be located in the straight through filter position, and the shorter wavelength filters in the second/third position.

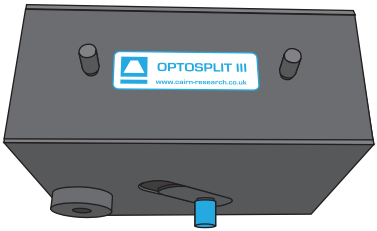
2 .2 Installing Dichroic Mirrors into the Cairn Filter Cube

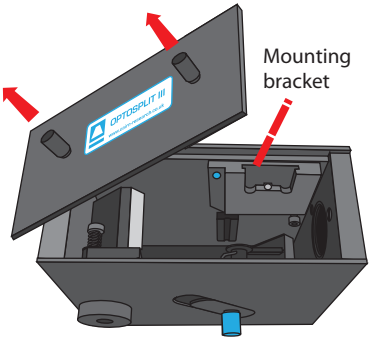
To fit your dichroic mirrors into the Cairn Filter Cube remove each of the eight screws that secure the three sections together. You will then be able to gently pull the cube apart. There is one steel locating rod in each part of the cube to ensure the correct alignment when it is reassembled. Once the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



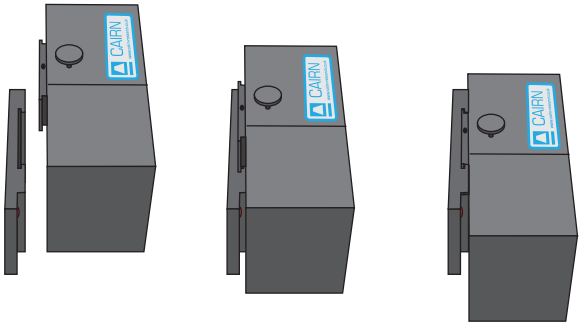
2.3 Inserting the Cairn Filter Cube into the OptoSplit III

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 III, 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.)

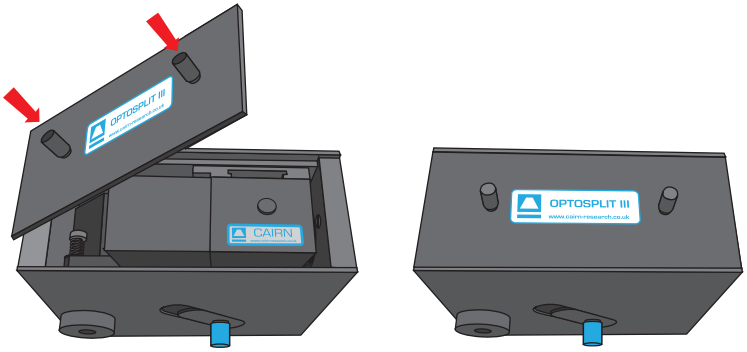




Remove the access panel using the two handles.



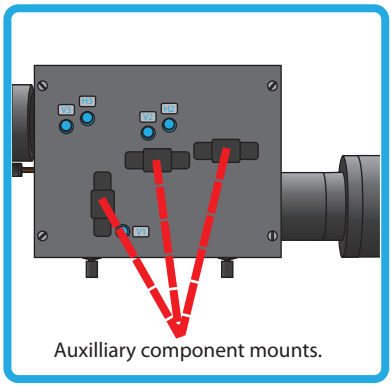
Mount the filter cube onto the brackect on the interior of the OptoSplit II.



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 there is a significant difference in brightness between the images.



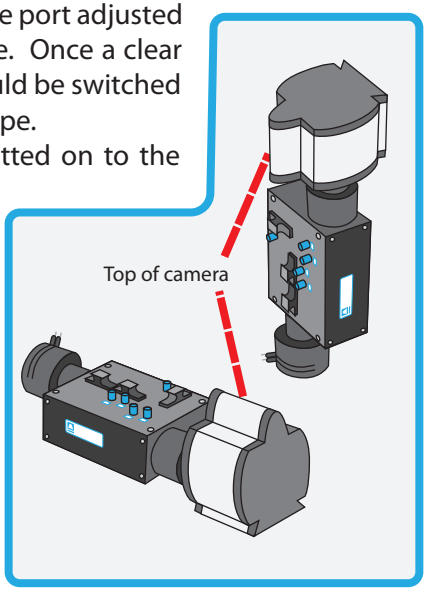
Auxilliary component mounts.

2.5 Installing the OptoSplit III in the Light Path

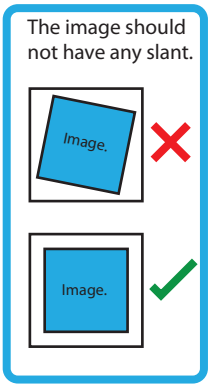
Before installing the OptoSplit III unit it is important to first set up the microscope, camera, and software to give a clear image of an object of less than half the size the camera frame. Ideally this should be a real sample with the appropriate optical properties for the installed filter set. Failing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

First 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 III 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 III, with the top orientated so that the top of the camera lies parallel with the top of the OptoSplit III. If you are fitting onto a vertical mount then the tops should still be orientated in the same direction.*



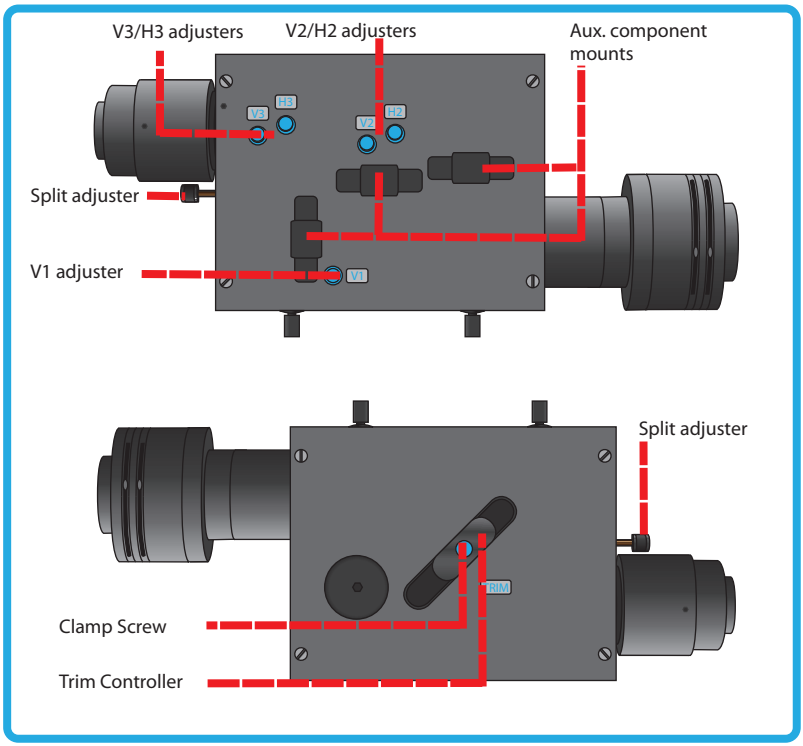
* A 1mm nylon spacer is also included with each unit. This may be required on the camera output mount, particularly when mounting a shutter in addition to the OptoSplit.



Having fitted the camera to the output port of the OptoSplit III 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.

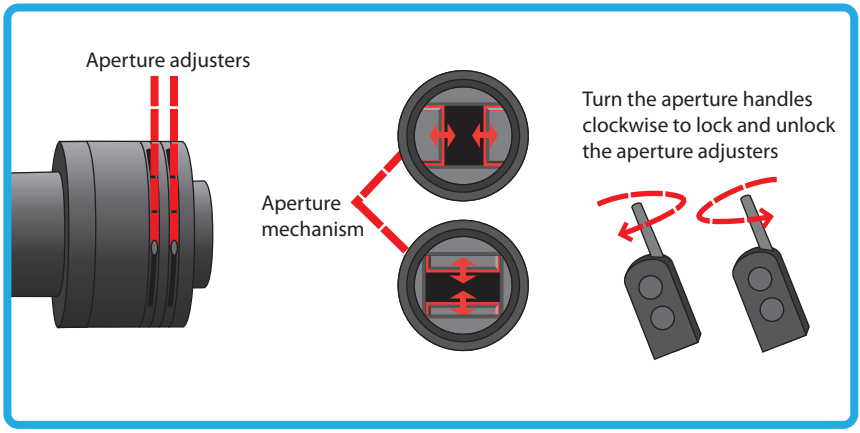
3 Operation of the OptoSplit III

The OptoSplit III uses a single control for adjusting image separation 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 III 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 III 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

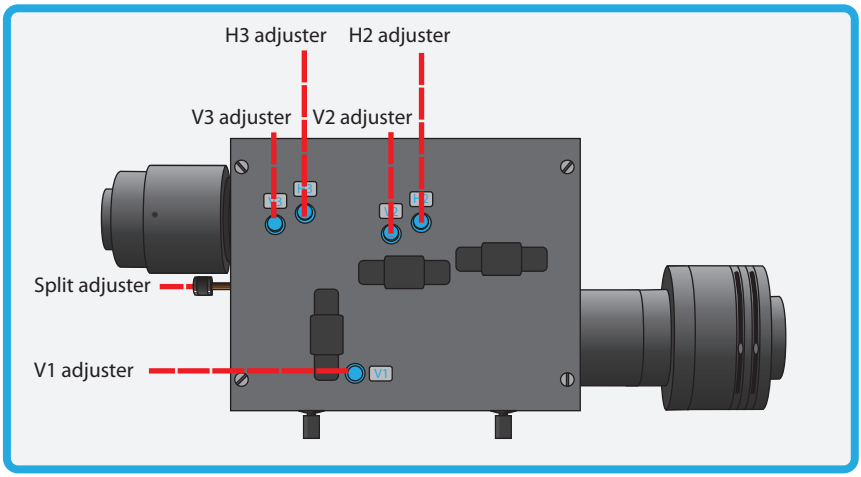
For successful image separation it is necessary to first define the field of view, and ensure the three optical pathways are overlaid.

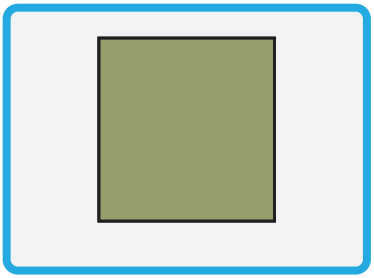
It is possible to separate the images by rotating the Split control in the clockwise sense, however image distortion will be evident when overlaying the two halves of the image.

The imaging procedure that follows assumes a Triple Filter Cube is installed and the full image is being monitored.

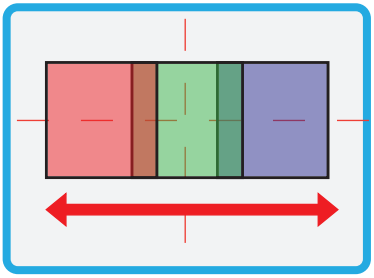
To begin, set a convenient sized aperture using the input aperture levers so the edges of the aperture are just visible within the field of view vertically, and restrict the field to just under 33% of the horizontal dimension.

It is also important to note that here the reflected image (that with the shortest wavelength), is depicted as being to the right of the screen. You will be able to identify this image easily after seperating all three as it will move with adjustments to the V1 control, but not adjustments to the V2 control.

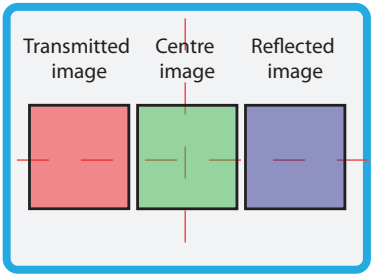




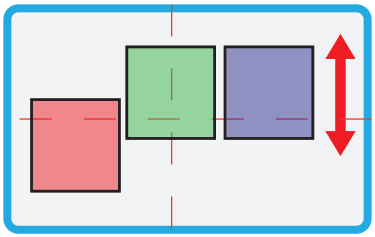
Here we see the three superimposed images. Although your image may be monochromatic, colours have been used here to define the three images.



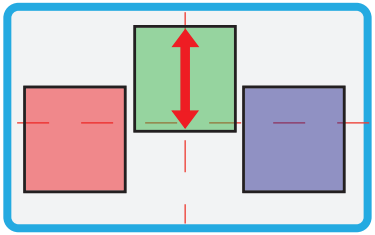
Turning the Split control anti-clockwise will seperate the reflected, and transmitted images symmetrically on the horizontal axis, whilst the Centre image will not deviate.



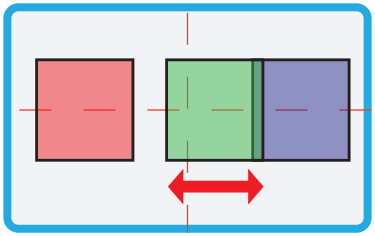
Note: To prevent calibration of the OptoSplit III off-axis, the Reflected (or short wavelength) image has no independent horizontal controls, so it is important that it is adjusted into position first, and the Centre, and Transmitted images are calibrated to it.



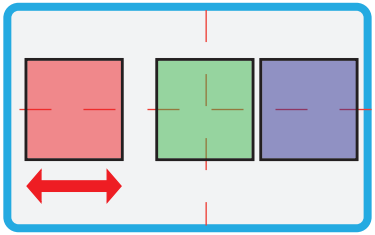
Turning the V1 controller will adjust the vertical position of the Reflected, and the Centre images, the remaining image is the Transmitted image which should be on the left side, if it is on the right, then adjust the Split control until the outside images are exchanged



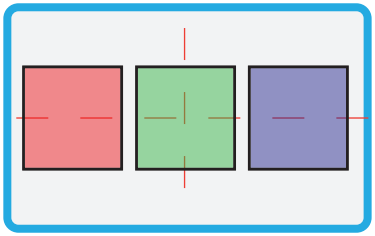
Adjustments to the V2 controller will move the Centre image along the vertical axis, and allow for adjustments to be made independently of the Reflected image.



Adjustments to the H2 controller will move the Centre image along the horizontal axis.

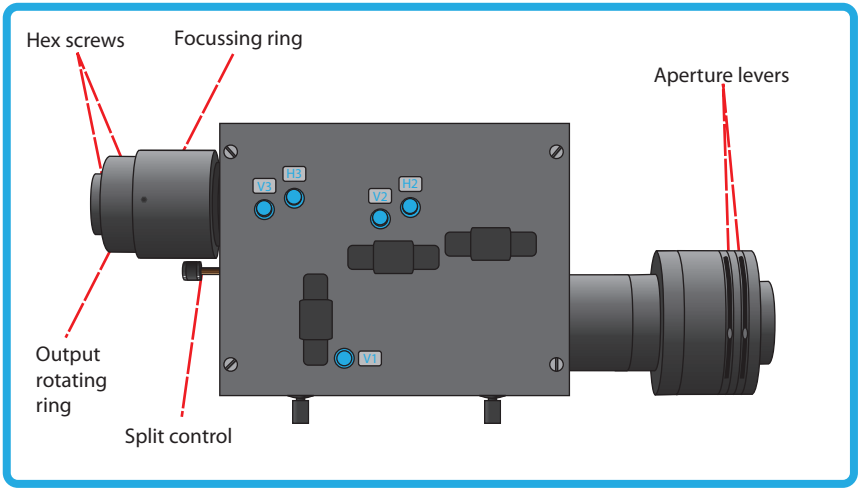


Adjustments to the H3 controller will move the Transmitted image along the horizontal axis.



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 images are located as closely as possible on the camera chip.

3.3 Focussing the Camera on the Aperture



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

1. Set a convenient sized aperture using the input aperture levers so the edges of the aperture are within the field of view. Block one light path by fitting the shutter plate provided into the one of the Aux mount positions.
2. Slacken the two hex screws that retain the output rotating ring to allow the camera to turn in the C-mount.
3. Slightly loosen the single hex screw, which locks the focussing ring.
4. Rotate the focussing ring between the camera and the OptoSplit III whilst holding both in position and until the aperture comes sharply into focus.
5. The camera rotating ring should be locked off with the camera chip orientated precisely in line with the OptoSplit III, as shown in the figure in the previous section.

6. The remaining hex screws should then be re-tightened & the shutter plate removed.

7. If the microscope image is in focus down the eye-pieces, but blurred when viewed through the OptoSplit III with the aperture is in focus, then the microscope C-mount is not parfocal with the eye-pieces. The side port should be adjusted as described by the microscope manufacturer if parfocality is required (recommended), or the microscope focus control can be used to independently bring the image into sharp focus on the OptoSplit III field aperture.

3.4 Dual and Single Image Modes of Use

The OptoSplit III can be setup in single channel or two channel (OptoSplit) modes, by simply exchanging the filter cube & adjusting the field aperture. Single image or "bypass" mode is the simplest of these, only requiring the field diaphragm to be fully opened to enable operation in full frame mode. When using the OptoSplit III with the regular OptoSplit II cubes for dual image splitting, the setup is similar to that described above for three channel operation, but the second reflected image is not present. To fully align an OptoSplit II cube in the OptoSplit III, the adjustment sequence is given below:

1. Set a convenient sized aperture using the input aperture levers so the edges of the aperture are just visible within the field of view vertically, and restrict the field to just under 50% of the horizontal dimension.

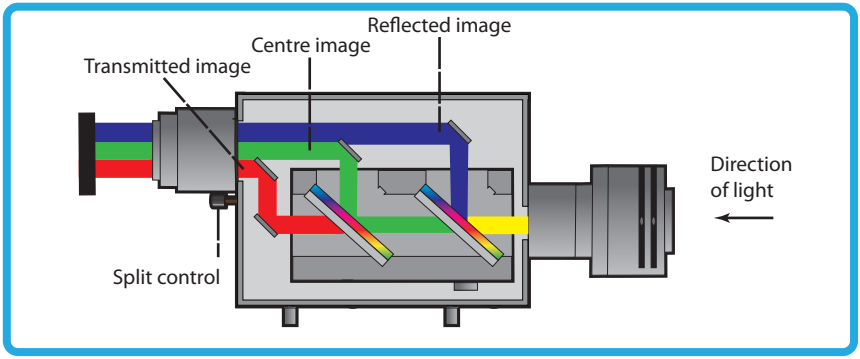
2. Identify the image that is reflected by the cube dichroic mirror by adjustment of the V1 vertical control.

3. Rotate the separation control counter-clockwise until the reflected image outer edge is located just inside the edge of the sensor.

4. Adjust the vertical position of the reflected image using V1 to centre the image. The vertical field aperture size can be adjusted at this point to optimise the sensor use.
5. Adjust the vertical height of the transmitted image using V3 to centre.
6. Adjust the horizontal position of the transmitted image using H3 so the outer edge of the image is just inside the sensor edge. If the images overlap, or there is a large gap between them, then adjust the horizontal field aperture size to optimise the sensor use, then readjustment of the split control will allow the outer edges of the images to be repositioned.

3.5 Locating the OptoSplit images on the camera

If the OptoSplit III controls have been badly misaligned, the images can show significant aberrations, or in extreme cases only a single image may be observable for any setting of the 'Split' control. In these cases the following procedure will enable the device setup to be reconfigured.



Locating the Reflected Image

1. Fit the system calibration cube & shutter the transmitted and centre illumination pathways using the shutter

plates provided.

2. Define a small area using the OptoSplit input aperture diaphragm and ensure the input to the OptoSplit is illuminated.
3. Attempt to centre the reflected image pathway horizontally using the 'Split' control to scan the position range.
4. If no aperture edges can be located for any position of the 'Split'control, enlarge the input aperture and repeat step 3.
5. Once the image of the input aperture has been located and centred horizontally, the vertical alignment of the aperture can be set using the V1 control.
6. Adjust the input aperture size so that it just underfills the camera field of view vertically, and just under one third of the camera field horizontally.

Locating the Transmitted Image

1. Move the shutter from the transmitted to the reflected illumination pathway, the calibration cube should remain in place.
2. With the reflected image located, it is likely the transmitted image will be seen somewhere in the field of view. If this is the case, small adjustments of the H3 and V3 controls should be used to adjust this image to mirror the location of the reflected image on the camera chip.
3. If no image is seen in the field, we will again scan the image horizontally. For the transmitted image this is achieved using the H3 control.
4. If after scanning the horizontal axis no image can be found, again open the input aperture slightly & repeat step 3.
5. Once the edges of the aperture have been found, reduce the input aperture as for step 6 above, and centre the image in the camera field using H3 and V3

Locating the Centre Image

1. Move the shutter from the centre channel to the transmitted illumination pathway, the calibration cube should remain in place.
2. With the reflected & transmitted images located, it is likely the centre image will be seen somewhere in the field of view. If this is the case, small adjustments of the H2 and V2 controls should be used to adjust this image to mirror the location of the reflected image on the camera chip.
3. If no image is seen in the field, we will again scan the image horizontally. For the centre channel image this is achieved using the H2 control.
4. If after scanning the horizontal axis no image can be found, again open the input aperture slightly & repeat step 3.
5. Once the edges of the aperture have been found, reduce the input aperture as previously, and centre the image using H2 and V2.

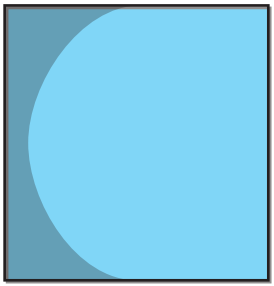
Aligning the Split Images

1. Remove the shutters from all pathways, keeping the calibration cube in place. The images should be approximately centred and overlaid at this point, so the image splitting will occur centred on the system optical axis.
2. Separate the images by rotating the "split" control counterclockwise until there are sited adjacent to each other and separated by a narrow dark band.
3. Fine adjustment of the images should be carried out as described in section 3.2

   The image splitter is now correctly aligned and ready for use

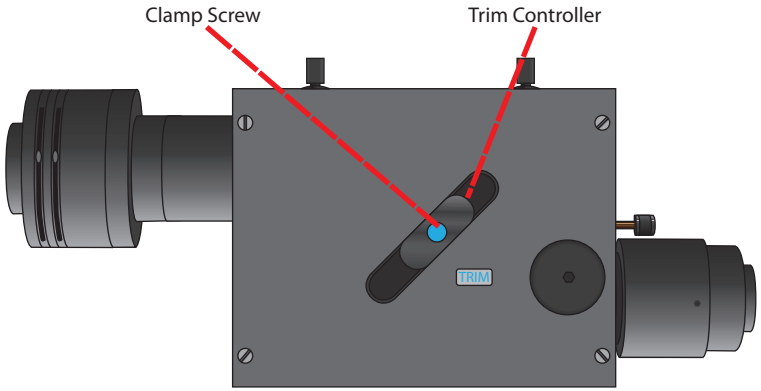
3.6 Image Optimisation (Trim Adjustment)

Vignetting is a darkening of the image towards one edge.



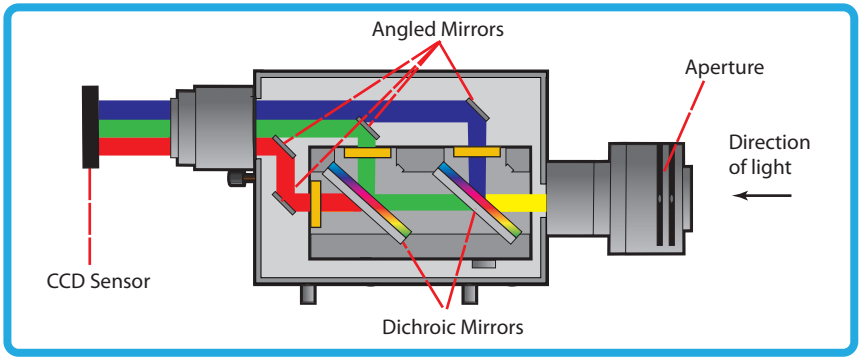
Vignetting is when the image appears darker towards one edge. This effect can be easily corrected by adjusting the trim control. To adjust the trim control, loosen the clamp screw on the underside of the OptoSplit III by a quarter of a turn in order to free the slider which controls the trim control. 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 III has been configured into single channel (non-split) mode 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.



4 Technical Summary

The OptoSplit III uses two conventional dichroic mirrors to seperate the light into three 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 including couplings.	H150, W380, D70 (mm)
Approximate weight.	1.80 kg
Power consumption.	0

5 Technical Support

E-mail: tech@cairn-research.co.uk

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