



CAIRN

RESEARCH

Optosplit III Multi Image Splitter



Installation & Operating Instructions

*NOTE: The Triplesplit is supplied pre-aligned and centred.
Please refer to this manual before making any adjustments.*

I. Important - Please read before installation

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 (e-mail tech@cairn-research.co.uk).

The following points should be considered when using the Triplesplit for the first time:

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

Do not adjust splitter controls before reading this manual!

2. When changing the 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 lens tissue.

II. Package Contents

Optosplit III Image Splitter

- Triplesplit base unit
- Filter Cube(s) _____
- 3 Aux slot covers
- 2 Shutters to occlude optical paths
- Auxillary filter/lens holder(s) _____

Coupling

- Rectangular diaphragm with female c mount input
- Output focussing adjuster with male c mount output

Filters

- Dichroic filter _____
- Short emitter _____
- Long emitter _____

- Dichroic filter _____
- Short emitter _____
- Long emitter _____

- Dichroic filter _____
- Short emitter _____
- Long emitter _____

- Dichroic filter _____
- Short emitter _____
- Long emitter _____

- Auxillary Filter/Lens _____
- Auxillary Filter/Lens _____

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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 to a system. In many instances the region of interest does not require the full resolution of the camera so the ideal solution would be to simultaneously image at multiple wavelengths on the same camera chip. In conjunction with a research microscope and a suitable C-mount camera the Cairn Triplesplit allows the researcher to do exactly this.

The Image splitter is usually supplied with unity magnification and fitted with a rectangular aperture to define the ROI. It 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 on-line analysis tool such as the Splitview module in Universal Imaging's Meta series software; Field Split in Andor Bioimaging's iQ; or our Optosplit drop-in for ImageJ.

As supplied, the instrument is configured to attach to the c-mount output port of a research microscope, with a c-mount camera fitted to its output. The maximum detector size that can be completely filled in straight through mode is 8x8mm for the standard unity magnification unit. 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 Triplesplit 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

The Triplesplit comes supplied with a rectangular aperture, as shown below. This allows you to make the best use of the camera chip area, but there is the alternative of a circular iris available. The rectangular diaphragm has two levers that independently set the horizontal and vertical dimensions of your region of interest, whereas the circular iris has a single lever that adjusts the field diameter. Whichever device you have coupled to your system, it will be supplied pre-centred on the input C-mount and this centration cannot be adjusted.

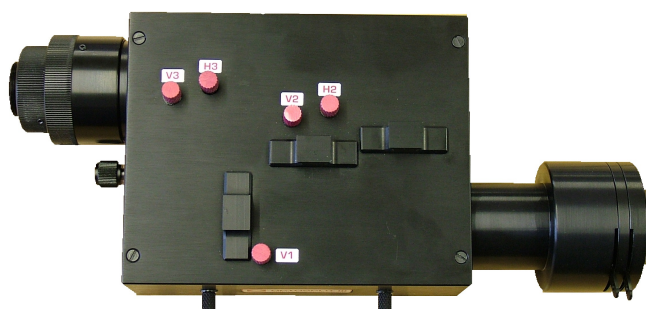


Figure 1 : Optosplit with adjustable rectangular diaphragm

Before using the Triplesplit for the first time you will need to install an appropriate filter set or beamsplitter into the device. If your Triplesplit has been purchased with a full filter set, then this will be pre-installed in an Optosplit or Triplesplit filter cube. The filter cubes are readily exchanged (See section 2.1) and so can be installed before or after fitting the Triplesplit to the system. Fitting the Triplesplit to the light path is simply a matter of coupling to the microscope C-mount output, and attaching a camera to the Triplesplit output port. Full instructions on fitting into the optical pathway and correct alignment are given below in section 2.2. Once installed in the light path, configuring the splitter for three (or two) channel operation is straightforward, but please note it is possible to mis-align the system which can result in image aberrations and vignetting (image shading). Correct alignment and operation is detailed in sections 2.3 and 3.

The Triplesplit is provided with a means of adjusting the internal optical pathways to allow optimisation of the light paths for coupling to a range of instruments with different optical requirements. This “Trim” adjustment is usually factory set for coupling to a standard research microscope (Operating at around $f/\#10$), assuming split operation. Depending on the camera chip size (and input optical pathway), a vertical shading pattern may be observed on one of the images. This indicates adjustment of the “Trim” control is required to reposition the internal light paths so they do not clip the optical components of the other light path. Full details are given in section 2.4.

2.1 Installing the filter cube

The first stage in configuring the Triplesplit is to install the filter set. If you wish to change the configuration after installation then the instructions here should be followed, but it is not necessary to remove the Triplesplit from the light path. The filter cube position can be accessed by removal of the side access panel, which is held in place by a set of magnets. The access panel is removed by pulling the two handles labelled A, shown in figure 2.

Figure 2 : Accessing the filter set



The Optosplit filter cubes accept 25mm filters and 38x26mm dichroic mirrors. If the cube has not been supplied prefitted with your filter set, take care to ensure the filter components are orientated as indicated by the manufacturer. For our standard filters this is with the arrow pointing towards the sample (i.e. Towards the microscope). The filters are held in place using the 25mm locking rings supplied, and the dichroics accessed by removing the four screws holding the two halves of the cube together. The filter cube is fitted to the slider as indicated in figure 3 below.

Figure 3 : Fixing the filters in position



Note: When fixed correctly in the cube the filters sit at a small angle to the optical axis to prevent reflected light from reaching the detector.

In most applications the dichroic beamsplitter will have long-pass characteristics. For a dual split cube this means the longer wavelength of the two filters should be located in the straight through filter position, and the shorter wavelength filter in the reflected position. For triplesplit cubes where there are two dichroics, the first reflected wavelength will be the shortest, the second reflected wavelength the midband filter, and the straight through transmitted wavelength the longest (please refer to the technical summary for details of the light path). Removal of the dichroic cube is the reverse of the fitting, with the cube sliding out of the mount when the handle on the cube is pulled towards you. The Triplesplit cube mount is designed to allow interchange of the regular Optosplit cubes as well as the Triplesplit filter sets. Together with the bypass cube, this allows easy and rapid switching between either one, two or three way operation.

In multi-wavelength operation it is likely that the images will have markedly different intensities. To allow the intensities to be balanced, we have included the provision for mounting independent neutral density filters in each light path. This enables the acquired image to have a comparable dynamic range in each channel.

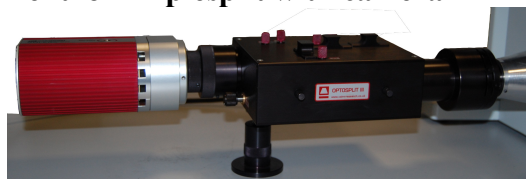
Note: To control the relative intensity of the two pathways we recommend using neutral density filtering in the brighter pathway using the auxiliary component mounts (M in fig 2). This is necessary only when one image is disproportionately brighter than the other.

2.2 Installing the Triplesplit in the light path

Before installing the Triplesplit system it is important to first set up the microscope, camera and software to give a clear image of an object on the imaging port to be used for the image splitter. If configuring the system with a fluorescence filter set fitted, then the object should ideally be a real sample with optical properties appropriate for the installed filter set. Failing this a standard brightfield image could be used, but this may lead to arbitrary intensity differences between the spectrally resolved images. To familiarise yourself with the operation of the various controls, it is recommended that the image splitter is first configured using the supplied calibration cube and brightfield illumination.

First the C-mount camera should be mounted on the microscope C-mount output and the port adjusted to give the sharpest image. Once a clear image can be seen the camera should be switched off and removed from the microscope. The Triplesplit should be fitted onto the microscope with the field aperture orientated towards the C mount port, as shown below in figure 5. The CCD camera can then be fitted onto the exit port of the Triplesplit. The exit port of the Triplesplit has a rotating ring (green arrow in fig.6 below) to allow the camera to be fitted in the same orientation as the Triplesplit. This is important to allow the Triplesplit to operate predictably. The correct orientation is for the top of the Triplesplit to lie parallel with the one edge of the camera sensor. If your Triplesplit is fitted with a rectangular diaphragm then the image of the aperture should be upright with no slant.

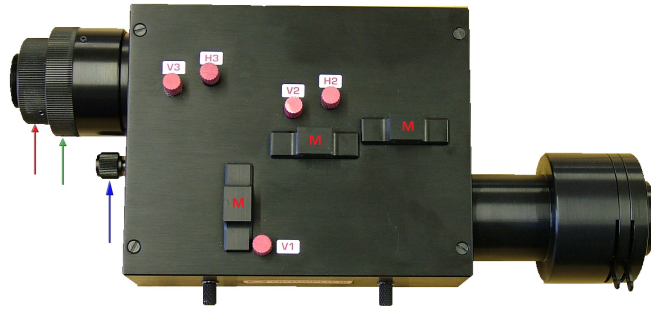
Figure 4 : Alignment of Triplesplit with camera



Having fitted the camera onto the Triplesplit it should be possible to see a sharp image of the aperture on the computer monitor, with the sample in sharp focus within the aperture. Please note that the diaphragm blades are usually closed for shipping! After coupling the Triplesplit to the system, the next step is to adjust the aperture to just fill the frame vertically, and to fill a third of the frame horizontally. During factory set up the Triplesplit is configured with the three images approximately superimposed, so when it is first installed a single central image should be observed. Image separation is achieved by a **counter-clockwise** rotation of the “split” control located on the Triplesplit face nearest the camera (figure 6 blue arrow). Separation of the images by a clockwise rotation of the control will result in increased image distortions, which will be readily apparent when overlaying the images from the different sides of the camera chip.

2.3 Focussing the camera on the Triplesplit aperture.

Figure 5 : Adjustments to focus the image on the camera



If the Triplesplit aperture blades are not in sharp focus on the camera, then adjust the fine focus on the camera using the following procedure. Refer to fig.6 above for screw locations:

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 (M).
2. Slacken the two hex screws that retain the output rotating ring (red arrow) to allow the camera to turn in the C-mount.
3. Slightly loosen the single hex screw, which locks the focussing ring (green arrow).
4. Rotate the focussing ring between the camera and the Triplesplit whilst holding the Triplesplit and camera in position 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 Triplesplit, 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 eyepieces, but blurred when viewed through the Triplesplit with the aperture is in focus, then the microscope C-mount is not parfocal with the eyepieces. 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 Triplesplit field aperture.

2.4 Image Separation in Triplesplit mode

For successful image separation it is necessary to first define the field of view, and ensure the three optical pathways are overlaid. Separating the three images is then a simple case of rotating the “split” control in a counter-clockwise direction, which will cause the shortest and longest wavelengths to move symmetrically away from the centre wavelength image which remains undeviated. Fine adjustment of the image locations may be required to fully optimise the use of the camera sensor.

Note: 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 image image separation procedure follows, and assumes a triple filter cube is installed and the full image is being monitored:

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 33% of the horizontal dimension.
2. Identify the image that is reflected by the first dichroic mirror (short wavelength channel) by making small adjustments of the V1 and V2 vertical controls.
3. Rotate the separation control counter-clockwise to separate the images horizontally until the first reflected image outer edge is located just inside the edge of the sensor. Note the long wavelength channel (light not reflected by either dichroic) will be displaced to the opposite side of the image, but it is the position of the short channel that is configured first.

4. Adjust the vertical position of the short channel image using V1 to vertically 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 second reflected and transmitted images using V2 and V3 respectively.
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 (Step 3) will allow the outer edges of the images to be repositioned.
7. Finally, horizontally centre the second reflected image (centre channel) using H2 so this image sits in the middle of the sensor.

Note: There is no independent H1 control to ensure the splitter cannot be configured off axis. This would introduce unpredictable image aberrations whilst giving the impression of correct setup. To adjust the horizontal position of the reflected image, use the “split” control and subsequently correct the position of the transmitted image using the H2 control.

2.5 Dual and single image modes of use

The Optosplit 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 Triplesplit with the regular “Optosplit” 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 cube in the Triplesplit, 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.

Note: Images should still be separated by rotating the “split” control counter-clockwise

2.6 Image Optimisation (Trim Adjustment)

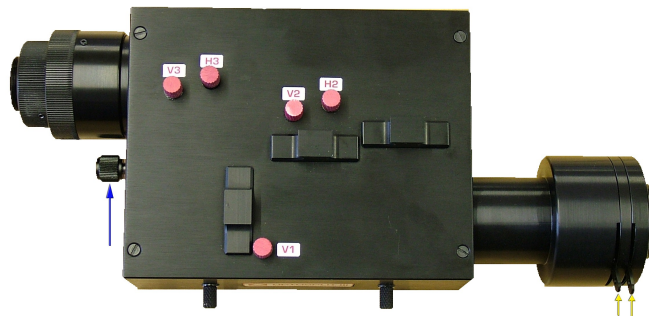
If image shading (vignetting) is observed in the image after the procedure in section 2.4 has been carried out, then the “Trim” adjustment may need to be altered. The vignetting is caused by the light path for one of the pathways clipping the optics of the other, and is easily adjusted by using the “Trim” control. The typical shading pattern for this is shown below:

To adjust the trim bar, loosen the clamp screw by a quarter turn, then move the slider so any shading just disappears from the field of view. If the slider is moved too far then vignetting will usually be observed on one of the other images.

3 Operation of the Triplesplit

The Triplesplit is an intuitive device, with a single control for adjusting image separation (blue arrow in fig 7). This allows different field sizes to be defined, allowing speed optimisation for fast imaging applications. There are additional controls for defining both the field of view and image centration which depend on the type of aperture fitted to the Triplesplit. The figure below shows the Triplesplit fitted with the rectangular aperture, and its size is adjusted using the pair of levers indicated by the yellow arrows. If an iris diaphragm is supplied, this is adjusted by rotation of a single lever in the same location.

Figure 6 : Control positions for aligning the two images



3.1 Adjusting the position of the images.

Once the camera is set up it is useful to familiarise yourself with the operation of the alignment adjustments. Please note that the diagrams below indicate the images for a system using an appropriately aligned and centred rectangular diaphragm, if your system has an iris diaphragm then the images will be shaped accordingly. An image similar to that shown below will be observed when the Triplesplit is first used, with the three images superimposed.

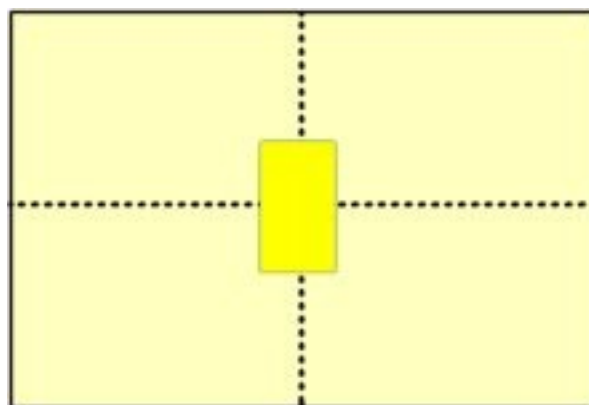


Figure 7 : Superimposed images on aligned system

When the camera is mounted correctly (as described in section 2 above) the “Split” control will control the horizontal image separation of the short and long wavelength images either side of the mid wavelength image (blue arrow in fig.7 above), and together with the aperture levers these are the only controls that will be routinely adjusted during experiments. The remaining controls on the top-plate all adjust the alignment and centring of the two images and should not need to be adjusted unless the filter set has become mis-aligned.

Note: Do not adjust any top plate controls until you are familiar with the basic operation of the Optosplit and you have referred to section 3.2 as complete realignment is not trivial.

1. Separating the Images

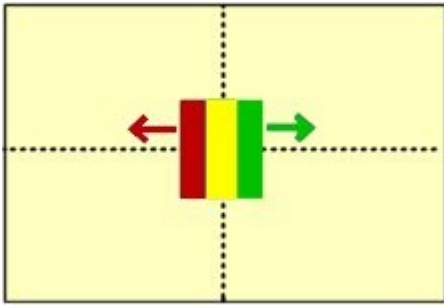


Figure 8 : Image separation

Turning the “Split” control counter-clockwise will horizontally separate the spectrally resolved images (fig.7 blue arrow).

Note: Only small adjustments are necessary to separate the images.

2. Adjust horizontal alignment

3. Adjust vertical position of images

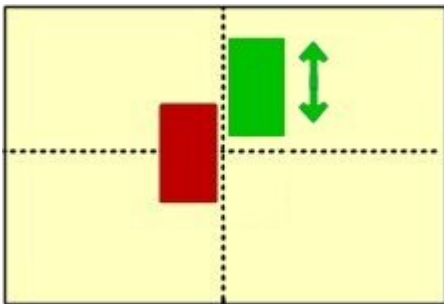


Figure 9 : Vertical alignment

Should the images be at different vertical heights to each other, this can be readily corrected by adjusting the three V controls. Turning the vertical alignment adjustment V1 will alter the vertical position of the short and centre wavelength image together, so first adjust V1 so the outer two images are vertically aligned. V2 operates on the centre image alone, so once the outer images are aligned the centre image can be corrected if required. As for the separation control, only very small adjustments are necessary.

4. Ready to record

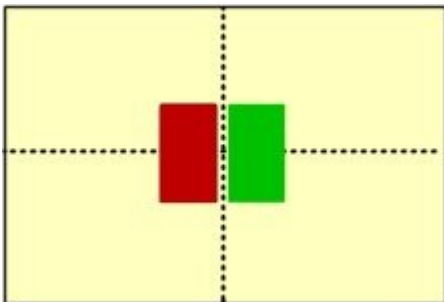


Figure 10 : Correct alignment

Once the spectrally resolved images are located side by side on the camera chip you are ready to record. When carrying out experiments the aperture should be set to mask the ROI tightly so that the two images can be located as closely as possible on the camera chip, allowing the camera to be configured for the fastest possible image capture.

3.2 Locating the Optosplit images on the camera

If the Optosplit controls have been badly misaligned, the Optosplit images can show significant 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 device setup to be reconfigured. Control locations are shown in figure 7.

Locating the reflected image

1. Fit the system calibration cube & shutter the transmitted and centre illumination pathways (M_c & M_i).
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 separation control to scan the position range (blue arrow, fig.7).
4. If no aperture edges can be located for any position of the separation 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.

The reflected image is now correctly aligned

Locating the transmitted image

1. Move the shutter from the transmitted (M_t) to the reflected illumination pathway (M_r), 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 H2 and V2.

The transmitted image is now correctly aligned.

Locating the centre image

1. Move the shutter from the centre channel (M_c) to the transmitted illumination pathway (M_t), 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.

The transmitted image is now correctly aligned.

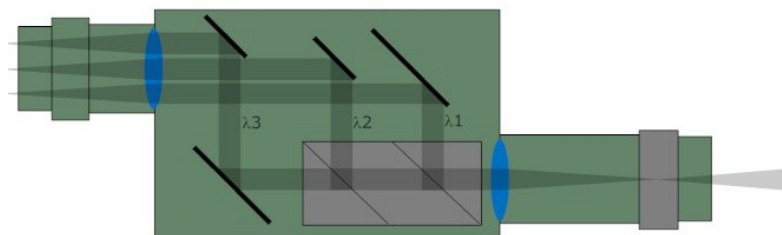
Aligning the split images

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 sections 2.4 and 2.5

The image splitter is now correctly aligned and ready for use

4 Technical summary

The Optosplit is designed to integrate into the optical pathway of a standard optical microscope using regular C-mount input and output couplings. Spectral separation is achieved using a conventional dichroic mirror to separate the light into two different wavelength bands, and is usually augmented by the use of bandpass filters (as shown in the schematic below). Image separation is achieved by a symmetric displacement of the spectrally separated beams in a central infinity space. The displaced beams are then refocused to form two images on the CCD sensor, corresponding to each of the light paths navigated. The magnification within the Optosplit is set to unity, but other ratios can be provided on request.



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

- Rotating mirror cradle to give precise symmetrical control of the degree of separation whilst maintaining identical path lengths.
- Half-size fully silvered mirrors at the output port for recombining the images.
- Fine adjustment controls for camera focus and vertical alignment.
- Optional ROI definition using adjustable rectangular aperture or iris diaphragm.

Version 2 of the Optosplit saw the introduction of our proprietary trim adjustment, this enables coupling of the image splitter to a wider range of input devices and maintaining device optimisation.

Version 3 saw the creation of the centre channel for “Triplesplit” operation

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

Dimensions (H x W x D), in mm, excluding couplings	55 x 110 x 110
Dimensions (H x W x D), in mm, including couplings	55 x 240 x 110
Approximate weight (kg)	1.26



5 Technical Support

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