

MultiSplit V2 Manual

The MultiSplit is calibrated and QC'd prior to delivery, however some alignment is required upon initial setup using the calibration cubes provided (each containing a 50% mirror). This manual provides instructions for mounting the unit on your microscope and basic alignment to project four identical images onto your camera chip. A more detailed technical manual can also be provided upon request.

For the purposes of this manual, all text in **bold blue** is referenced on subsequent images.

1) Mounting to your microscope

1. The MultiSplit is supplied with four detached support feet, these should be screwed into the mounts on the base of the unit prior to setup. A blue locking ring allows each support leg to be locked off at the correct position.
2. Screw your microscope c-mount onto the input section of the MultiSplit and attach to your microscope frame.
3. Adjust the support feet until the unit is level (a spirit level is recommended).
4. Initial setup is completed without your imaging camera mounted on the unit, so for the time being, place your camera safely to one side.

2) Key Controls

The following images show the MultiSplit panels, which will be referenced during setup of the unit.

2.1) MultiSplit Panels

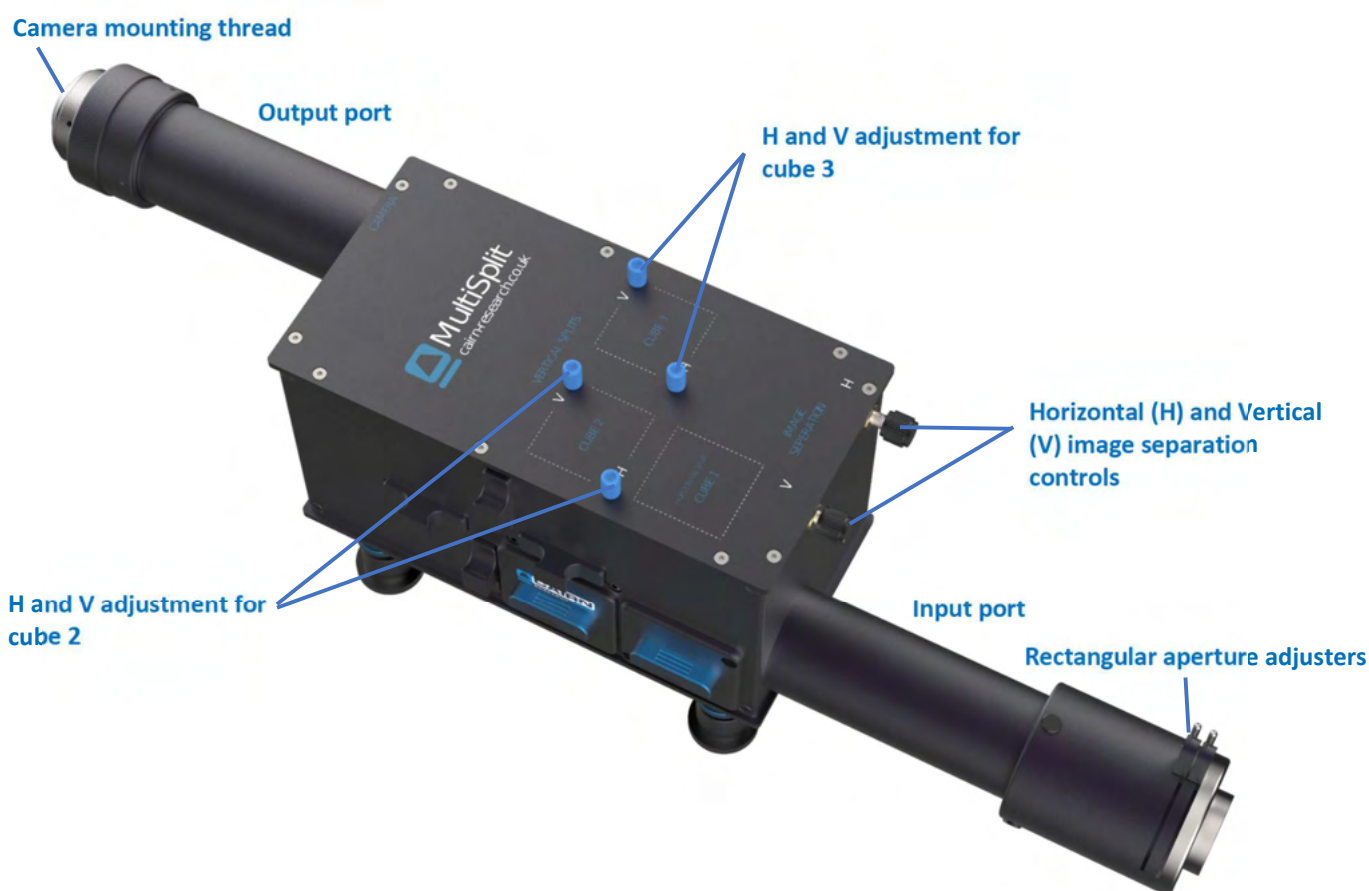


Figure 1: MultiSplit top panel



Figure 2: MultiSplit side panel 1

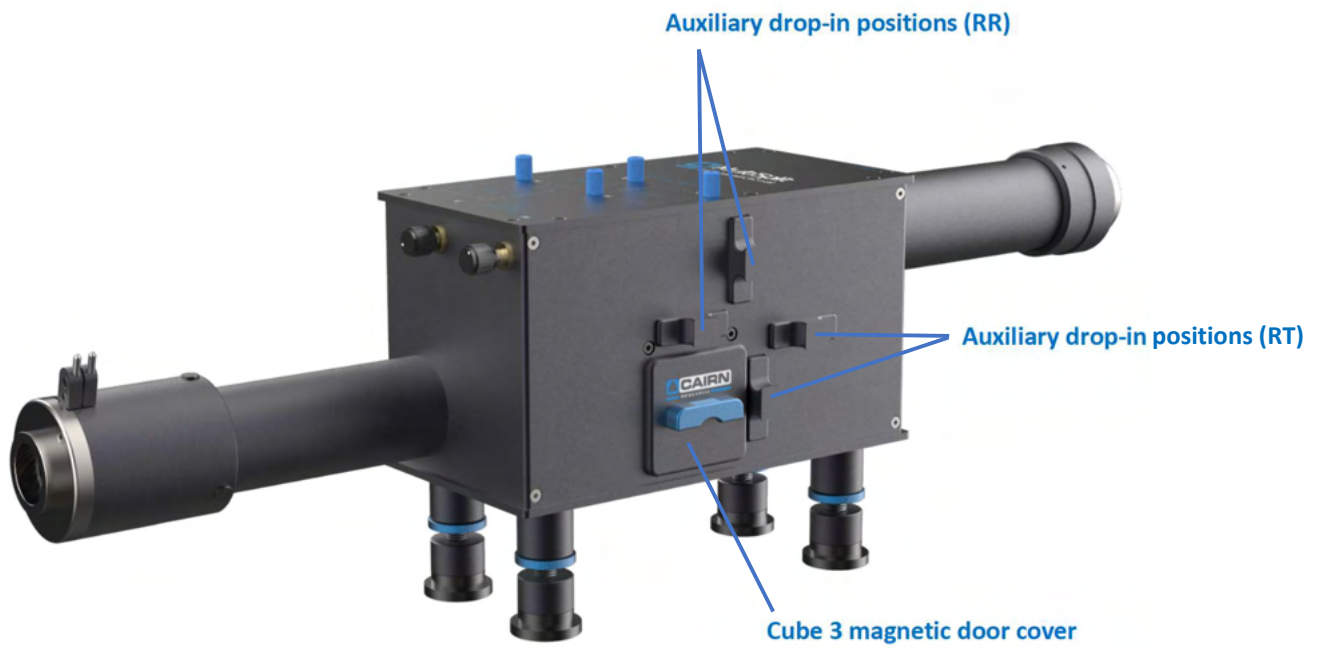


Figure 3: MultiSplit side panel 2

2.2) Defining Field of View

An adjustable **rectangular aperture** (or diaphragm) precisely defines the region of interest reaching the camera sensor. This region is then reflected by both internal mirrors within the unit and dichroic mirrors placed in the light paths to project this identical region into four quadrants. *

** Please note, the MultiSplit has been designed specifically to generate four images projected into quadrants – the internal mirrors are not designed to be used in any other format (e.g. for two images side by side, or a single full field 'bypass' image)*

1. Slide the two **aperture adjusters** (one horizontal, the other vertical) to define your region of interest.
2. This region can be locked in place once alignment is complete by turning the aperture handles.
3. Once your camera has been mounted following initial alignment, the aperture edges should be in sharp focus in your image when partially closed. If not, please contact us (tech@cairn-research.co.uk) for further advice and we will be happy to advise depending upon your hardware setup.

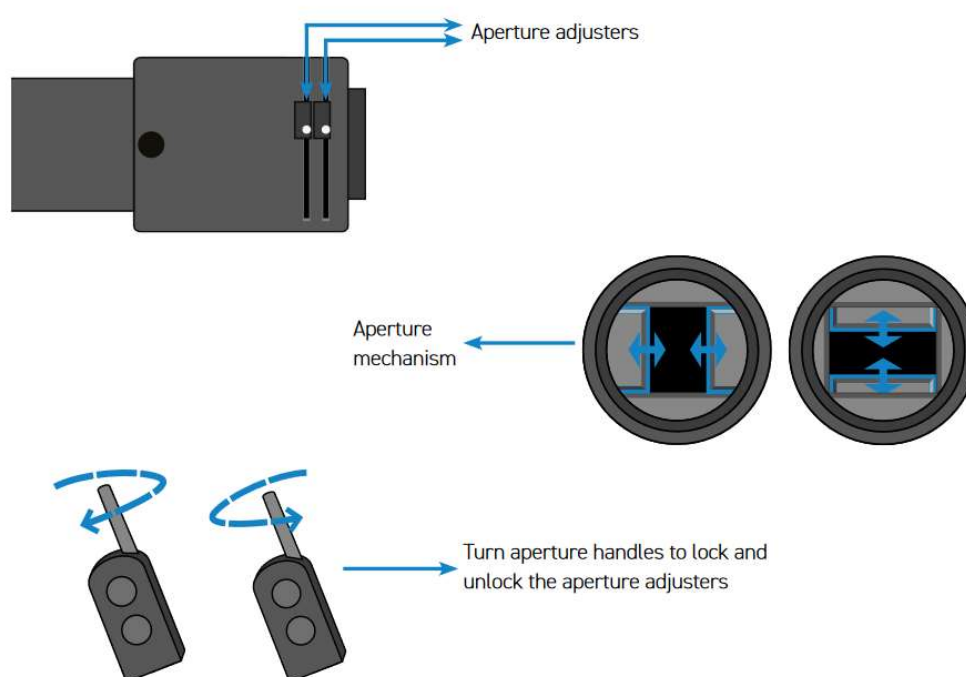


Figure 4: Rectangular Aperture

2.3) Filter cubes and cradles

The MultiSplit is supplied with three labelled calibration cubes to aid setup, each containing a 50% / 50% mirror. Two cube cradles are also supplied and are essential to house cubes 2 and 3 at a 90-degree angle within the unit.

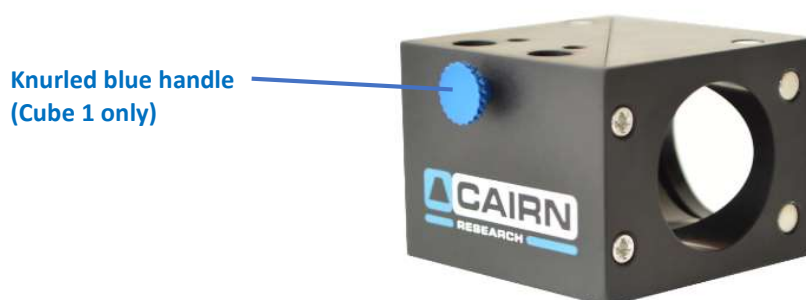


Figure 5: Cairn filter cube

Each cube will be labelled – Cube 1 includes a **knurled blue handle** and is inserted in the position closest to the **rectangular aperture**, once the **Cube 1 magnetic door cover** has been removed.

Cubes 2 and 3 (both the calibration cubes and Cairn filter cubes) will have this blue handle removed and once inserted into the cube cradles as shown in figure 6, the cradle can be inserted to positions 2 and 3 respectively. Please ensure the cube cradles sit flush within the unit and are not angled – internal magnets will ensure the cradle locates correctly.



Figure 6: Fitting cairn cubes 2 and 3 into the cube cradle



Figure 7: Cube cradle (containing cube 2) being inserted into the unit

3) Initial alignment

For initial setup, we suggest mounting the MultiSplit to your microscope without your imaging camera. The four quadrants can then be roughly aligned in the correct configuration, which avoids issues with some imaging cameras flipping the image in software. Final adjustments can then be made with the camera in place.

To aid this initial setup, a piece of paper held over the **camera mounting thread** is sufficient to observe the quadrants as each cube is added to the unit.



Figure 8: Camera mounting thread minus imaging camera

3.1) Light path diagram

It is pertinent at this point to consider the internal light path within the MultiSplit and familiarise yourself with the image nomenclature.

'T' refers to transmitted and similarly, 'R' refers to reflected.

Each of the four images is labelled accordingly:

Image reference	Related cube number	Nominal 'colour' of image
TT	No cubes in unit	Red
RT	Cube 1	Green
TR	Cube 2	Yellow
RR	Cube 3	Blue

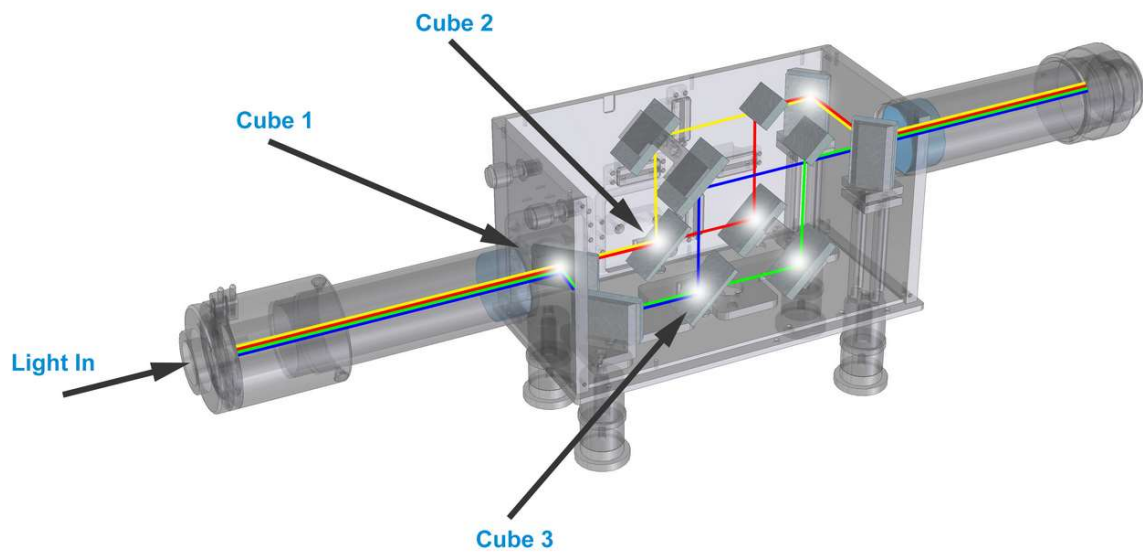


Figure 9: MultiSplit light path diagram

3.2) Positioning the primary image (TT)

1. Turn up your brightfield microscope light source to maximum and divert the light to the relevant side port.
2. Ensure no filter cubes are within the MultiSplit unit.
3. Hold a small piece of paper over the **camera mounting thread**.
4. Close the **rectangular aperture** down until one small square is visible through the piece of paper (~one quarter of the whole aperture).
5. Re-position this image using the **horizontal (H) and vertical (V) image separation controls** show in *Figure 1*.
6. When looking through the piece of paper towards the microscope from the output of the MultiSplit, move the TT image to the bottom left corner.

3.3) Positioning the reflected images (RT, TR and RR)

Once TT is in the correct quadrant, the remaining images can then be positioned in turn.

Cube 1: Image RT

1. Insert the calibration cube labelled Cube 1 into its correct position (note, this is the cube with the knurled blue handle) – this will cause image RT to appear.
2. Replace the **Cube 1 magnetic door cover**.
3. Using the 1.5mm Allen key provided, re-position this image using **Cube 1 H & V controls** (*Figure 2*) on the cube door. This mechanism tips and tilts the calibration cube (50% / 50% mirror), giving a smooth movement to reposition this image.
4. When looking through the paper towards the microscope from the output of the MultiSplit, move the RT image to the bottom right corner.

Cube 2: Image TR

1. Insert the calibration cube labelled Cube 2 into the cube cradle according to *Figure 6*.
2. Insert the cube and holder into cube position 2 – this will cause image TR to appear.
3. Replace the **Cube 2 magnetic door cover**.

- Using the blue thumb screws labelled **H and V adjustment for cube 2** in *Figure 1*, re-position this image to the correct quadrant.
- When looking through the piece of paper towards the microscope from the output of the MultiSplit, move the TR image to the top left corner.

Cube 3: Image RR

- Place the calibration cube labelled Cube 3 into the remaining cube cradle according to *Figure 6*.
- Insert the cube and holder into cube position 3 – this will cause image RR to appear.
- Replace the **Cube 3 magnetic door cover**.
- Using the blue thumb screws labelled **H and V adjustment for cube 3** in *Figure 1*, re-position this image to the correct quadrant.
- When looking through the piece of paper towards the microscope from the output of the MultiSplit, move the RR image to the top right corner.

With all three calibration cubes in place, the four quadrants projected onto the piece of paper should be:

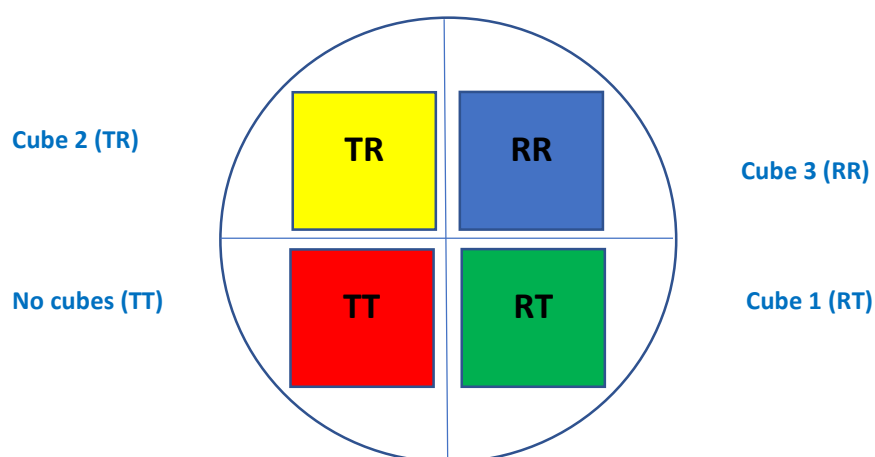


Figure 10: Images projected onto the paper over the camera aperture with all three cubes in place.

Note the quadrant colours are for illustrative purposes only and indicate the image wavelength from high to low (Red > Yellow > Green > Blue).

4) Obtaining a split image on your camera

As the four quadrants are now in the correct position, your imaging camera can now be attached to the MultiSplit.

The silver knurled **camera mounting thread** on the output tube can be rotated to secure the camera, whilst also ensuring any cables connected do not get tangled. Once the camera is fully screwed onto the c-mount, ensure it is level and stable. The four small nylon tipped grub screws on this silver section can be tightened using the 1.5mm Allen key supplied to give extra rigidity.

Our **support jack** is highly recommended as an additional accessory to ensure the camera is fully supported on the output port.

It's useful at this stage to familiarise yourself with each of the four channels on your imaging camera. This can be easily achieved using the shutter blanking plate supplied with each unit. This tool will block the light path in the corresponding channel (see figure 14 and 15 for the auxiliary positions for each channel). The shutter blanking plate can also be used during operation should one or more of the channels not be required.

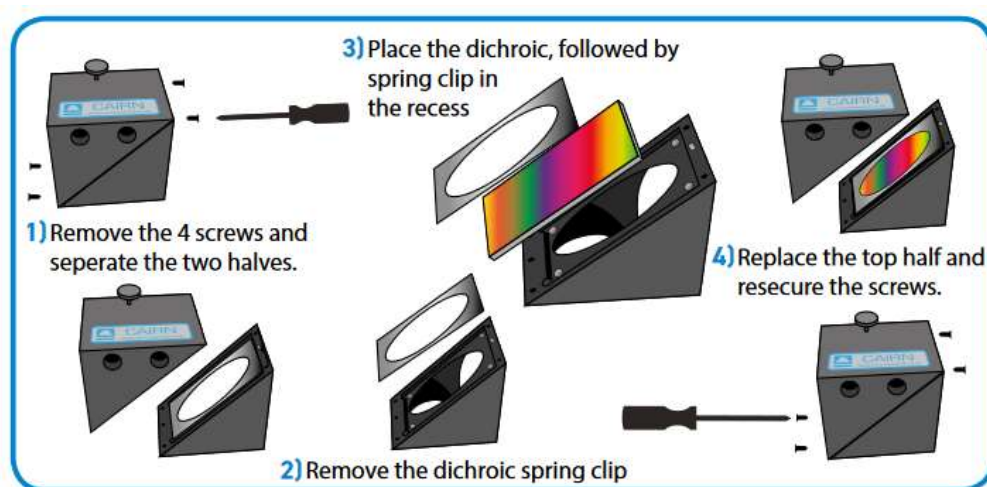
5) Filter cube assembly

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

5.1) Installing dichroic mirrors and emission filters

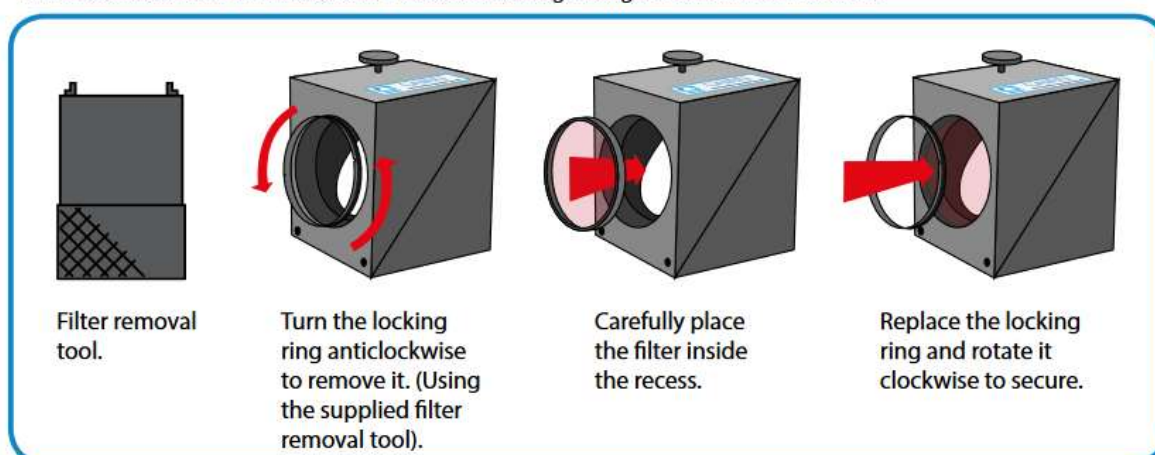
As UK distributors, we have worked very closely with Chroma to develop a dichroic mirror range for use in our image splitters with minimal image distortion. As a result, we only recommend the Chroma Ultra-Flat (UF-2) 2mm thick dichroic mirror range.

The Cairn Filter Cube accepts 26mm x 38mm x 2mm dichroic mirrors and 25mm ring mounted emission filters. Other dichroic mirrors will fit; however, they 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.



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

Figure 11: Installing dichroic mirrors and emission filters in the Cairn Filter Cube

5.2) Filter cube positioning

When using the MultiSplit to image four colours simultaneously, the example below provides guidance for dichroic mirror and filter cube positioning according to the internal light path as shown in *Figure 9*.

In this example, four laser lines are used for excitation, however, other light sources (such as LED's) can be used. Please contact us (tech@cairn-research.co.uk) if you would like specific filter advice for your requirements.

Laser lines:

405nm

488nm

561nm

640nm

Cube number	Dichroic mirror	Emission filter (transmitted)	Emission filter (reflected)
1	T565lpxr-UF2	None	None
2	T635lpxr-UF2	ET 700/75m	ET 600/50m
3	T495lpxr-UF2	ET 525/50m	ET 445/58m

5.3) 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 18mm polariser is available to act as a 'clean-up' for the reflected S wave.

The MultiSplit can therefore be configured for a mixture of experiments, such as two-colours in two different polarisation states.

* Please note the polarising beamsplitter cube is very fragile!



Figure 12: Polarisation components available as additional accessories

5.4) Accessory kits: Neutral Density and Corrector Lenses

Additional accessories are available upon request, which are housed in auxiliary drop-in holders and inserted into each of the four light paths where required. Blank covers are provided as standard in the absence of these drop-ins.



Figure 13: Auxiliary drop-in holder and retaining ring

Neutral Density kit

A series of four neutral density filters are useful if one (or more) of the channels is disproportionately brighter than another.

03GN25: 50% Transmittance

05GN25: 32% Transmittance

06GN25: 25% Transmittance

10GN25: 10% Transmittance

Corrector lens kit

If one or more of the channels looks out of focus in comparison to others, it may be due to chromatic aberration. This results when the focal length changes depending upon the emission wavelength and is predominantly introduced by the objective lens. When imaging multiple wavelengths simultaneously, the phenomenon is *observed* using the MultiSplit, rather than being caused by it.

Our corrector lens kit provides a series of weak crown glass lenses, often used by trial and error for correcting the unwanted effect. We recommend starting with the weakest lens (longest focal length) in the out of focus channel.

IO137: Focal length 1346mm

IO138: Focal length 2019mm

IO139: Focal length 4034mm

IO140: Focal length 5000mm (1" holder required)

IO141: Focal length 10,000mm (1" holder required)

6) Multi-depth Imaging

The MultiSplit has two auxiliary drop-in positions for each channel, accessible from the outer panels of the unit. The corrector lens kit described in section 5.4 can be used in these positions for two purposes: a) chromatic correction or b) to deliberately shift the focal plane for multi-depth imaging.

For precise focal plane shifting, we have a spreadsheet available to estimate the best focal length lens (if available). Please contact us (tech@cairm-research.co.uk) for this further detail and we will be happy to advise for your application.

The optimum positions for shifting the focal plane in each channel is indicated below.



Figure 14: Corrector lens positions for TR and TT

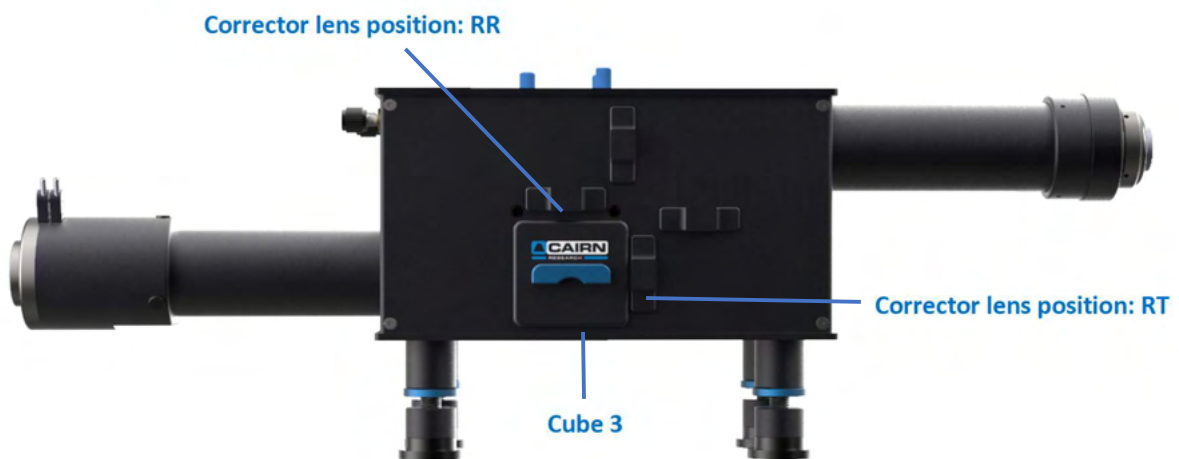
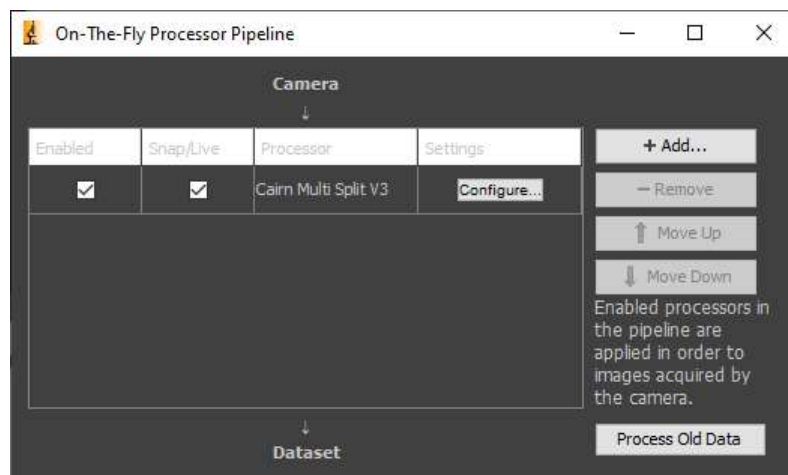


Figure 15: Corrector lens positions for RT and RR

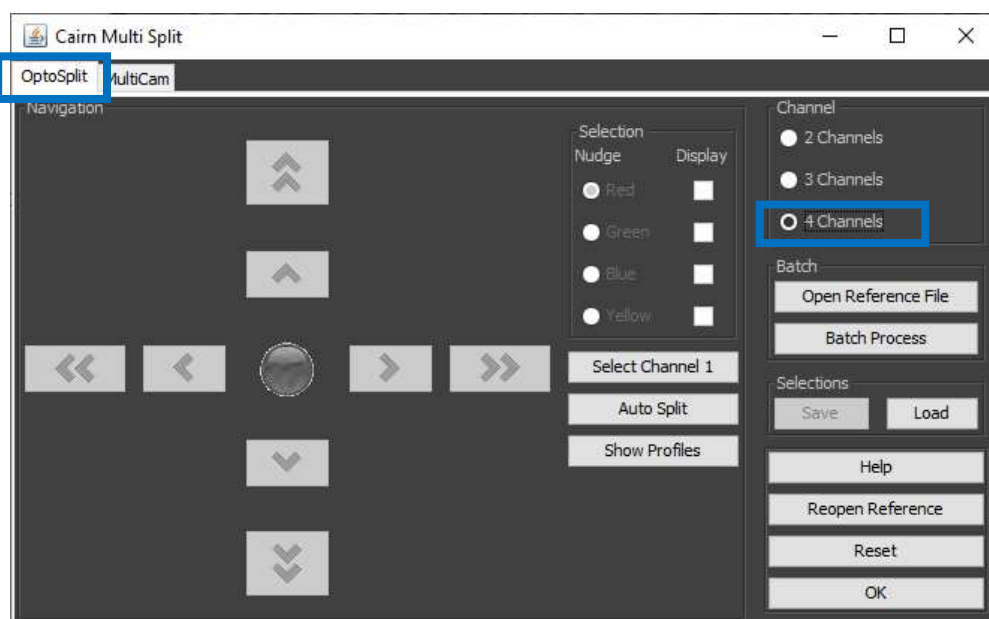
7) Analysing multi-channel data

For overlaying up to four channels 'live' or with a saved image, we have written a Micro-Manager plugin, available via our Software page on our website: (<https://www.cairn-research.co.uk/support/software/>).

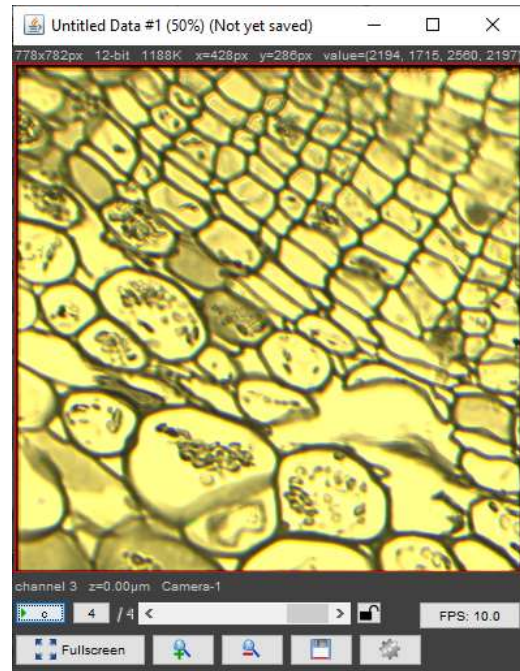
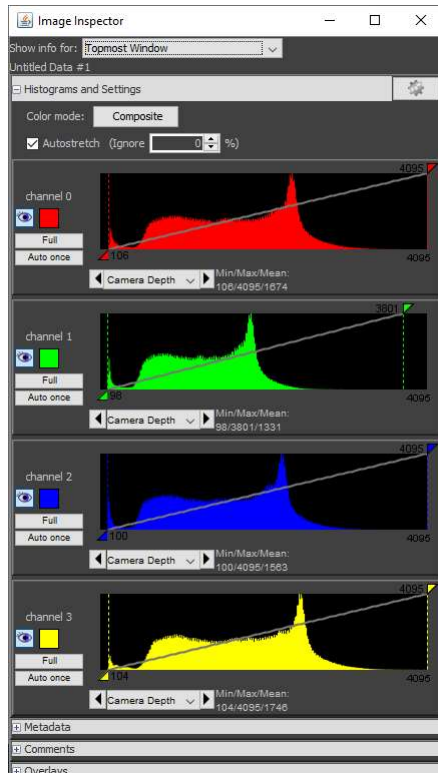
1. Ensure you have a compatible version of Micro-Manager installed (Beta or Gamma nightly builds are compatible).
2. Download the Cairn_MultiSplit_3.jar file and paste into the mmplugins folder in the root Micro-Manager directory on the C drive.
For example: C:\Program Files\Micro-Manager-2.0.0-gamma1_20190722\mmplugins
3. Obtain a live camera image of your four split channels, or alternatively open your saved Tiff file in Micro-Manager.
Please note, when overlaying the channels post acquisition, the original image must have been saved in Micro-Manager, the overlay plugin will not work if the file was saved in another software package.
4. Click on Plugins > On-The-Fly Image Processing > Cairn Multi Split V3
5. Ensure the Plugin is ticked as 'Enabled' and 'Snap/Live' in the On-The-Fly Processing Pipeline dialogue box.



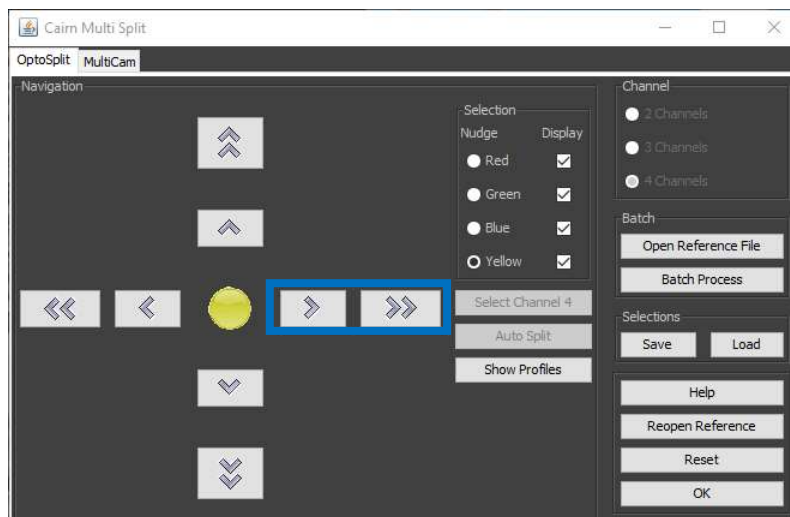
6. Within the 'Cairn Multi Split' dialogue box, ensure the tab is set to 'OptoSplit' and the channel option is set to '4 Channels'



7. Draw a rectangular region of interest (using ImageJ) around your longest (red) wavelength channel. Click on 'Select Channel 1'.
8. Move the same region of interest to the 'green' channel and click 'Select Channel 2'.
9. Similarly, repeat for the Blue (Channel 3) and Yellow (Channel 4). A four-colour composite overlay will now be displayed, with individual intensity histograms for each channel.



10. Each individual image can be nudged using the arrows within the Multi Split window, then saved once complete.



If you have any questions during installation or use of the MultiSplit, please do not hesitate to contact our Tech Support Team (tech@cairn-research.co.uk)