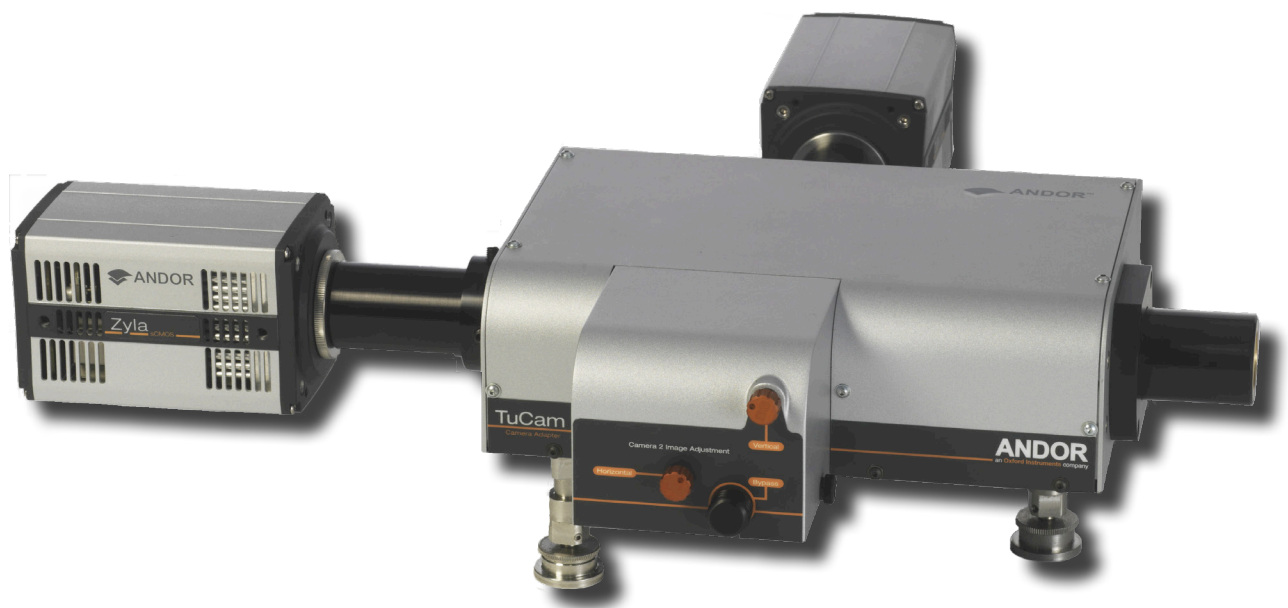


TuCam

Version 1.2 revised 01 June 2015



User Guide



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REVISION HISTORY

Version	Released	Description
1.0	03 Oct 2012	Initial Release
1.1	03 Oct 2014	Added weights information (Section 1.2.1) Updated company patent statement and note re access to new editions of the user guide (Section 1.7) Updated Accessories listing (Section 1.9) Translated content into new functional template, updated presentation and branding (All Sections) Updated product Images to current appearance.
1.2	01 Jun 2015	Product images updated to reflect latest branding and change to front porch and image adjustment.

SECTION 1: INTRODUCTION

Firstly thank you for choosing Andor's TuCam dual camera adapter. We are delighted you have selected our equipment to fulfill your requirements.

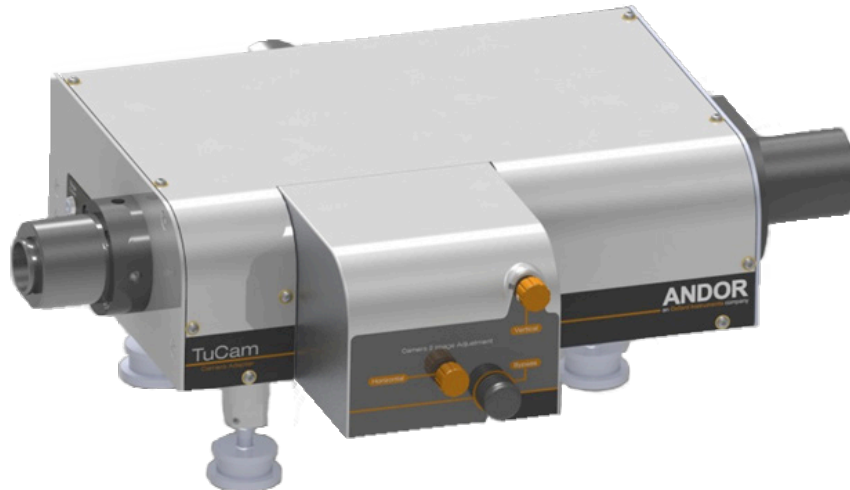


Figure 1: Tucam configured for C-mount.

Here are some of the feature and benefits available to you:

- **Unique design** - TuCam provides a unique level of flexibility for the end user. Computer optimized optics provide the highest image quality with minimized aberrations. Carefully selected AR coatings on the lenses together with broadband dielectric coatings maximize system throughput of your precious light
- **Interchangeable Filter Cassettes** - Our Filter Cassettes allow the user to quickly and simply exchange the filter cassette when the user wishes to observe a different wavelength range. Upon exchanging, minimal adjustment will be necessary to achieve highly accurate alignment of the two imaging paths
- An optional Polarizing Beam Splitter (PBS) may also be used to observe separate polarization states on the two imaging channels with a very high extinction ratio
- A standard mirror cassette is also available when direct image overlay is not necessary
- **Bypass Mode** - The cassette can also be fully retracted to allow transmitted light imaging. Upon return to image splitting, the cassette will magnetically lock into position with minimal need for realignment.
- **Robust, compact & accurate** - TuCam is delivered on a highly rigid platform supported on height adjustable legs to allow accurate leveling of the system. Once locked down on an Optical bench the robust design means that the image overlay in the two paths will remain consistent
- Folding optics reduce the overall footprint of the TuCam unit (CSU version) and by using dielectric mirrors the impact on system throughput is minimal
- Fine pitch adjustment screws on all mechanisms provide accurate motion control. Although we use achromatic lenses throughout, we have provided these accurate focusing mechanisms to allow the user to achieve best focus over the full wavelength range
- **Range of Filters available** - For best performance we recommend using Semrock's range of Image Splitting Dichroic Beamsplitters. These dichroic mirrors have an extremely flat surface that reduces the level of aberrations in the reflected beam path
- Our filter cassettes include positions for Emission filters to ensure that you only see the wavelength band that you want
- **Flexibility** - We provide a range of magnification options so as to make full use of the size of the sensor in your detector
- Available for use with a wide range of cameras: including CCD's, EMCCD's, ICCD's and sCMOS. Also available for third party detectors

NOTE: Before removing the TuCam from its packaging, please refer to Section 2.1.

1.1 TECHNICAL SUPPORT

If you have any questions regarding the use of this equipment, please contact the representative* from whom your system was purchased, or:

Europe

Andor Technology
7 Millennium Way
Springvale Business Park
Belfast
BT12 7AL
Northern Ireland
Tel. +44 (0) 28 9023 7126
Fax. +44 (0) 28 9031 0792

www.andor.com/contact_us/support_request

USA

Andor Technology
425 Sullivan Avenue
Suite # 3
South Windsor
CT 06074
USA
Tel. +1 (860) 290-9211
Fax. +1 (860) 290-9566

www.andor.com/contact_us/support_request

Asia-Pacific

Andor Technology (Japan)
IS Building
3-32-42 Higashi-Shinagawa
Shinagawa-ku,
Tokyo 140-0002
Japan
Tel: +81-3-6732-8968
Fax: +81-3-6732-8939

www.andor.com/contact_us/support_request

China

Andor Technology
Room 1213, Building B
Luo Ke Time Square
No. 103 Huizhongli
Chaoyang District
Beijing, 100101 P.R.
China
Tel: +86 (0)10 51294977
Fax. +86(0)10-6445-5401

www.andor.com/contact_us/support_request

* The latest contact details for your local representative can be found on our website.

1.2 SPECIFICATIONS

NOTE: The specifications quoted below are for the 1.2x CSU version:

PARAMETER	SPECIFICATION
General	Optimized for CSU-X
Focal plane size 1.2x (W x H)	12 x 8.4 mm
Uniformity	> 90 %
Wavelength range	400 - 750 nm
Typical throughput ¹	94 %
Distortion	< 0.5 %
RMS alignment error ²	< 15 μ m
Magnification options	1x, 1.2x, 1.5x, 2x
Optical axis height	110 mm
Focus range	\pm 5 mm

¹ Measured for Camera path 1 and with no other filtering in place

² Measured across sensor area (8.2 x 8.2 mm)

PARAMETER	SPECIFICATION
Focal plane size 1x	Up to 22 mm diameter
Uniformity	> 90 %
Wavelength range	400 - 750 nm
Typical throughput ³	94 %
Distortion	< 0.5 %
RMS alignment error ⁴	< 15 μ m
Magnification options	1x, 1.2x, 1.5x, 2x
Optical axis height	110 mm
Focus range	\pm 5 mm

³ Measured for Camera path 1 and with no other filtering in place (425 to 675 nm)

⁴ Measured across sensor area (8.2 x 8.2 mm) Specification

1.2.1 MECHANICAL DIMENSIONS

Weights for C-Mount Version:

Cassette = 0.35 Kg [12 oz]

Main unit = 4.6 Kg [10 lb 2 oz]

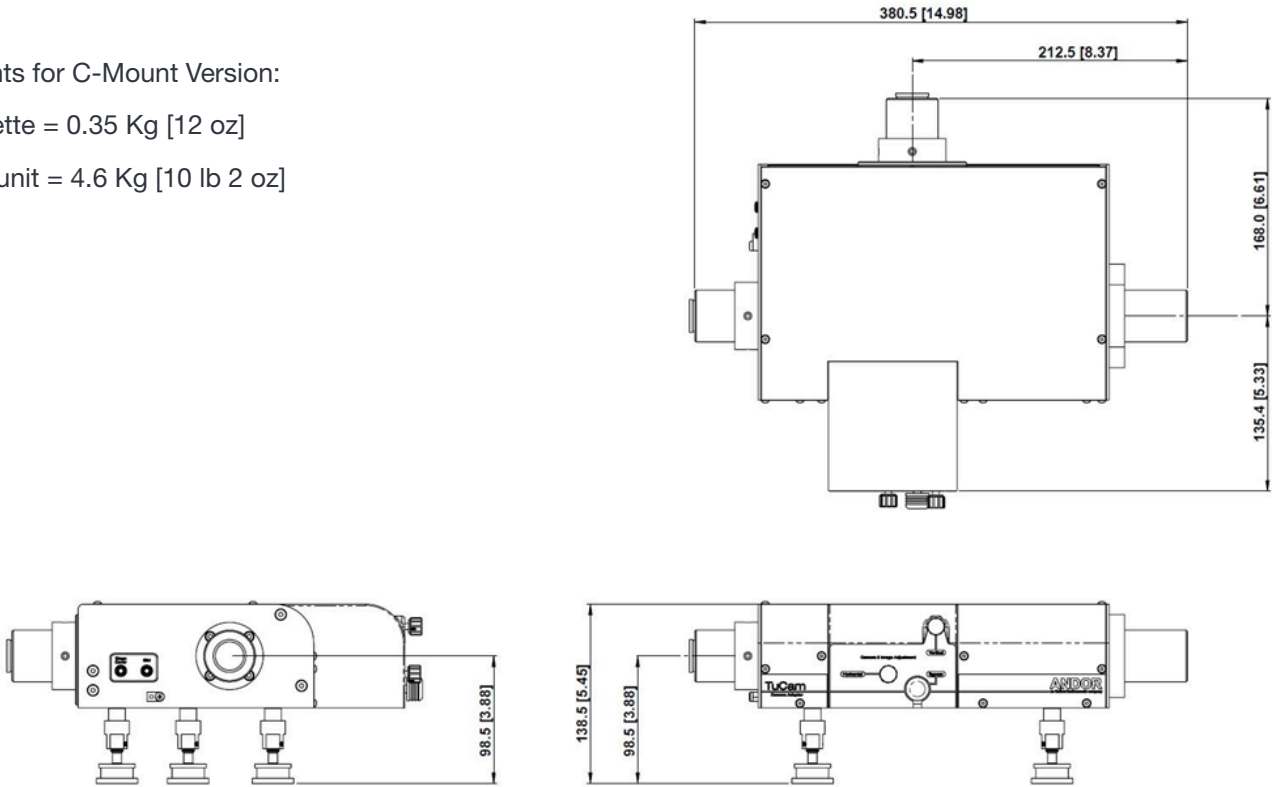


Figure 2: C-Mount Version

Weights for CSU-X Version:

Cassette = 0.35 Kg [12 oz]

Main unit = 5.5 Kg [12 lb 2 oz]

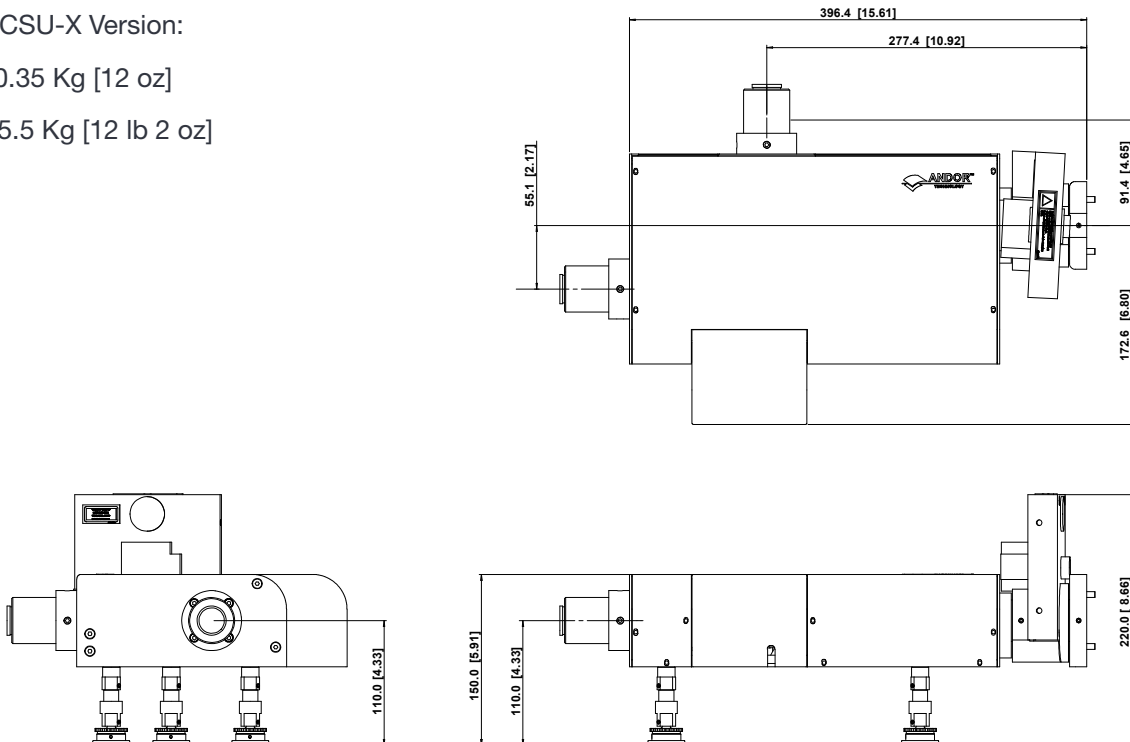


Figure 3: CSU-X Version

1.3 TRANSMISSION CURVES

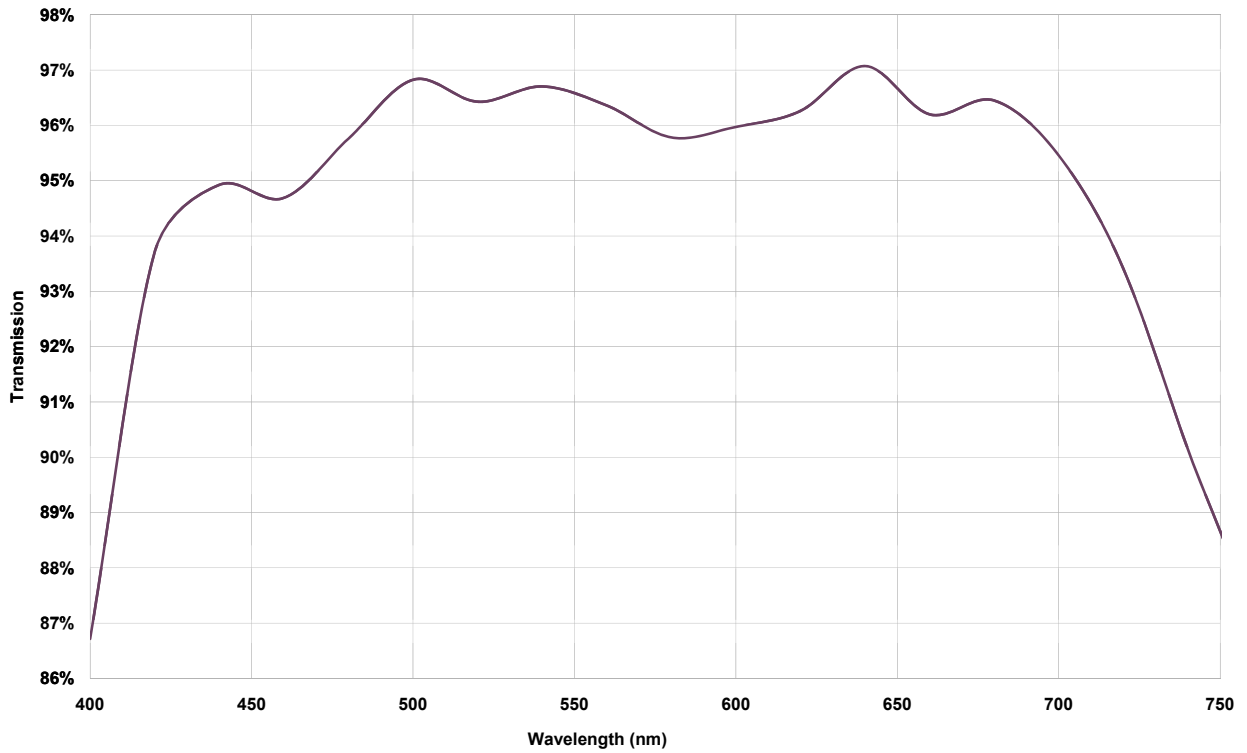


Figure 4: Transmission curve for CSU-X version

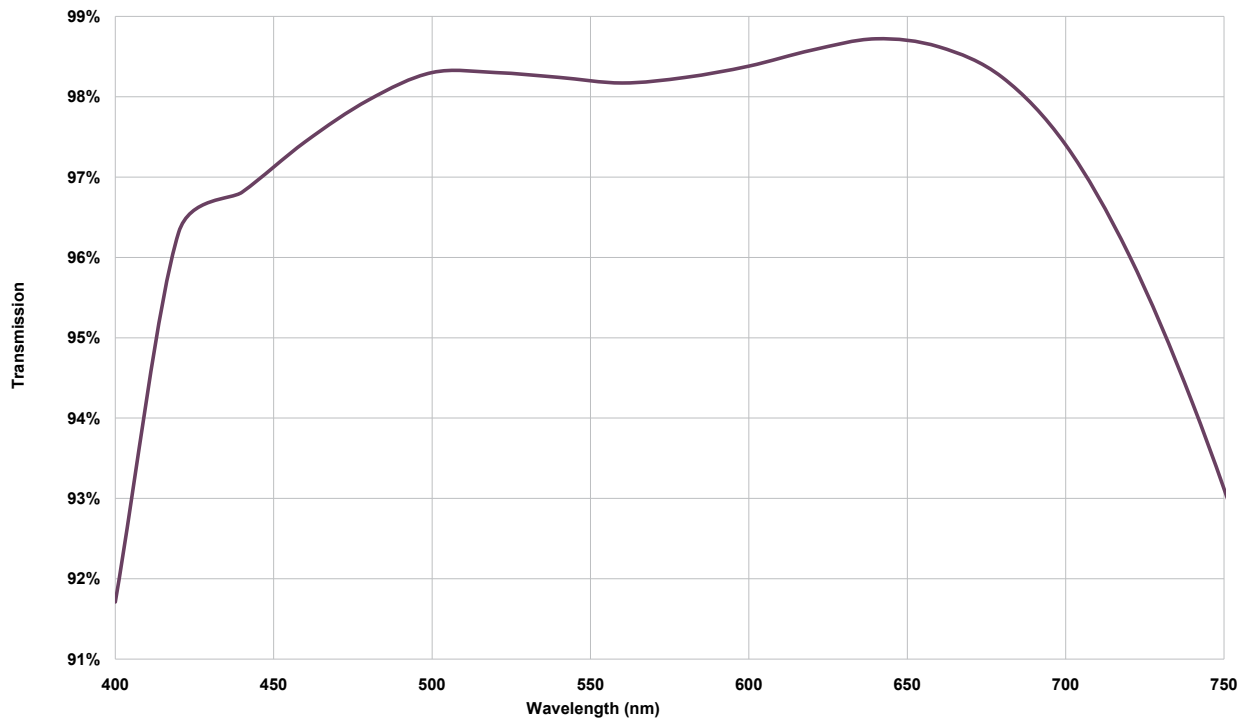


Figure 5: Transmission curve for C-mount version

1.4 TuCAM SERIES PRINCIPLES

The TuCam is based around our own unique design to afford the user a diversity of options. The TuCam is pre-aligned and optimized at our factory prior to shipping, so the user will only ever need to adjust the focus and minor adjustments to the cassette.

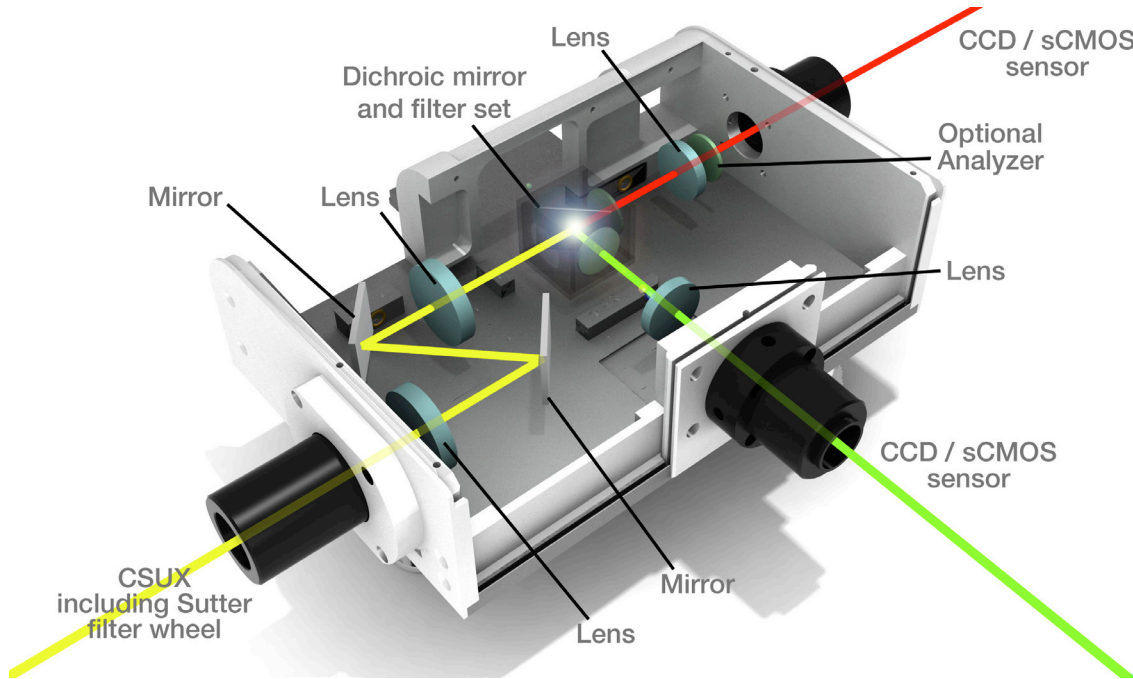


Figure 6: TuCam lightpath (CSU-X version)

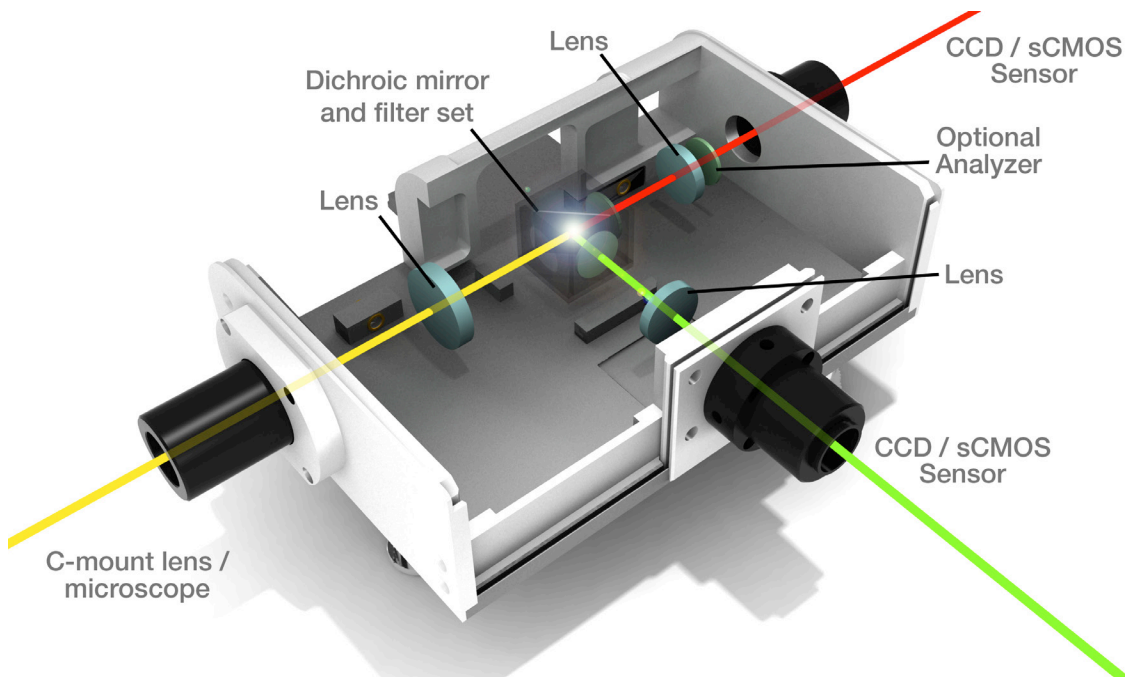


Figure 7: TuCam light path (C-mount version)

1.5 WORKING WITH THE USERS GUIDE

This user guide is aimed at experienced researchers who will be using their TuCam to overlay two separate imaging channels.

We hope you find using our product easy & beneficial. However, if you have any suggestions as to how our software, hardware or documentation could be improved, we would be pleased to hear from you. Contact details of your nearest representative can be obtained in Section 1.1.

1.6 DISCLAIMER

The information contained herein is provided “as is” without warranty, condition or representation of any kind, either express, implied, statutory or otherwise, including but not limited to, any warranty of merchantability, non-infringement or fitness for a particular purpose.

In no event shall Andor be liable for any loss or damage, whether direct, indirect, special, incidental, consequential or otherwise howsoever caused whether arising in contract or otherwise, arising out of or in connection with the use of the information provided herein.

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2. The publication of information in this documentation does not imply freedom from any patent or proprietary right of **Andor Technology** or any third party.

1.7 TRADEMARKS & PATENT INFORMATION

Andor and the Andor logo are trademarks of **Andor Technology**. Andor Technology is an Oxford Instruments Company. All other marks are property of their owners.

Changes are periodically made to the product and these will be incorporated into new editions of the manual. New releases of the manual are available through MyAndor: <http://my.andor.com/login.aspx>.

1.8 SAFETY PRECAUTIONS

- There are no user-serviceable parts inside the TuCam
- Care should be taken when working around the front surface optics

NOTE: It should normally not be necessary to remove the main cover. If the Camera 2 imaging path is out of alignment follow the instructions in Section 2.5

1.9 ACCESSORIES

A wide range of accessories are available to configure your TuCam as shown in the tables below:

Table 1: Standard filter sets

ANDOR PART NUMBER	SHORT DESCRIPTION	LONG DESCRIPTION
TR-EMFS-F01	GFP/RFP Dichroic Filter set	Semrock FF01-514/30-25, FF02-617/73, Dichroic FF580-FDi01-25x36
TR-EMFS-F02	CFP/YFP Dichroic Filter set	Semrock FF01-475/28, FF01-550/49-25, Dichroic FF509-FDi01-25x36
TR-EMFS-F03	Polarizing filter set (Moxtek)	Moxtek Flat Beam Splitter PBF02C 38x26mm, Moxtek High Contrast PPL04 C 25mm dia. Cairn P290/AUX/012 Holder - 25mm filters
TR-EMFS-F05	CAMELEONS-2 Dichroic Filter set	Semrock FF01-483/32-25, FF01-542/27-25, Dichroic FF506-Di02-25x36
TR-EMFS-F07	GFP/YFP Dichroic Filter set	Semrock FF01-497/16-25, FF01-550/32, Dichroic FF509-FDi01-25x36
TR-EMFS-F08	680/732 Dichroic Filter set	Semrock FF01-680/13-25, FF01-732/68-25, Dichroic FF700-Di01-25x36
TR-EMFS-F09	Cy3-Cy5 Dichroic Filter set	Semrock FF01-579/34-25, FF01-679/41-25, Dichroic FF640-FDi01-25x36
TR-EMFS-F12	Cy3/Cy5.5 Dichroic Filter set	Semrock FF01-579/34-25, FF01-692/40-25, Dichroic FF640-FDi01-25x36
TR-EMFS-F13	Fluo4/Fura Red Dichroic Filter set	Semrock FF01-530/43-25, Chroma HQ615LP, Dichroic FF580-FDi01-25x36
TR-EMFS-F14	GFP/Cy5 Dichroic Filter set	Semrock FF02-525/50, FF01-692/40-25, Dichroic FF580-FDi01-25x36
TR-EMFS-F15	50/50 BS Mirror	Chroma 50/50 beamsplitter, 25.2x35.6x1mm laser flat
TR-EMFS-F17	GFP/mCherry Dichroic Filter set	Semrock FF02-525/40-25, FF01-640/40-25, Dichroic FF580-FDi01-25x36
TR-EMFS-F20	GFP/Cy5 Dichroic Filter set	Semrock FF01-534/42-25, FF01-655/40-25, Dichroic FF580-FDi01-25x36
TR-EMFS-F21	GFP/mCherry:wide Dichroic Filter set	Semrock FF01-534/42-25, FF01-641/75-25, Dichroic FF580-FDi01-25x36

The following accessory is also available to enable Nomarski DIC imaging:

TR-DCIS-DIC-MNT Internal mounting for DIC polariser

Table 2: Mounting accessories for cameras, CSU and microscopes (110 mm optical axis height is recommended)

ANDOR PART NUMBER	DESCRIPTION
TR-IXON-MNT-110	Mounting feet for Clara, iXon3 or Neo sCMOS
CR-CSUX-MNT-110	CSUX 110 mm Opt Axis Mount Kit
TR-OLIX-MNT-110	Mounting feet for Olympus IX71/81
TR-NKTE-MNT-110	Mounting feet for Nikon TE-2000
TR-NKTI-MNT-110	Mounting feet for Nikon Eclipse Ti-E
TR-ZAXO-MNT-110	Mounting feet for Zeiss Axiovert 200 + Zeiss Axio Observer



Figure 8: (left) TR-DCIS-CA1-00: Blank cassette for mounting filter sets, (right) TR-DCIS-CA4-01: Cassette with first surface mirror

NOTE: All our standard filter sets use Semrock Imaging-Flat Dichroic Beamsplitters.

SECTION 2: INSTALLATION

2.1 UNPACKING

Before unpacking the instrument, allow the shipping box to fully acclimatize to room temperature.

Whilst the TuCam requires no special placement in a controlled environment, it should be noted that it is a precision instrument that will perform best when set up and operated properly. Please read this manual carefully before using and always handle with the care accorded to any precision instrument.

2.1.1 STANDARD SUPPLIED COMPONENTS

Carefully remove the TuCam from its packaging. The following should be included:

- **TuCam base unit** with feet and appropriate mounting screws and clamps for metric and imperial optical tables
- **Mounting flanges** for CSUX integration plus appropriate screws (if applicable)
- **Camera output tubes**
- **Cassettes** with pre-installed filters or mirror
- **User's guide** (this publication) and performance sheet
- **Allen key set**
- **Filter insertion tool**
- **Grid slide** for alignment
- **BNC Couplers** (for triggering if necessary)

2.1.2 OPTIONAL COMPONENTS

Depending on the options purchased the following may also be included:

- **Andor camera(s)**

If any items are missing, please contact your local sales outlet

The following pages show how to mount the TuCam and fit accessories.

2.2 INSTALLING SUPPORT LEGS

The TuCam base unit will have been supplied with three height adjustable support legs. Two support legs are attached to the TuCam at the camera end and one at the front.

The legs simply screw into the base unit in the locations shown below. The leg height is adjusted by rotating the knurled ring at the base; clockwise to increase height, anti-clockwise to reduce it. A locking bracket is also supplied with each leg to allow it to be firmly fixed to the optical table. The bracket slides over the 'foot' and can then be securely fixed to the optical table.

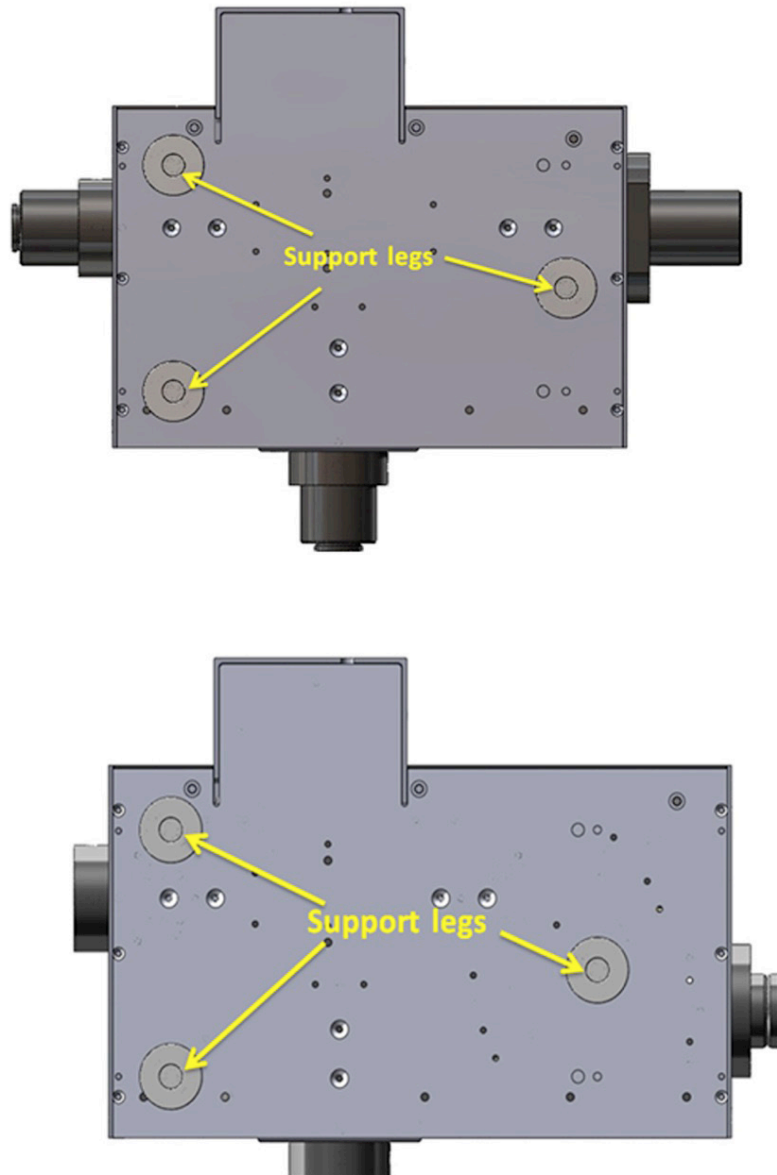


Figure 9: Underside views (A) = C-mount version, (B) = CSU-X version

Similar support legs can also be attached to the cameras to provide added support and rigidity. Andor's range of cameras have a number of mounting points to attach the legs. This not only supports the weight of the camera but can be used as an aid in correcting rotational misalignment during the setup of the TuCam unit.

2.3 MICROSCOPE & CSU MOUNTING ACCESSORIES

A number of accessories are available for raising the optical axis height of a range of microscopes and the CSU unit (see Section 1.9). The individual microscope mounts are designed to raise the optical axis of the LHS port to 110 mm to provide a consistent height for attaching other accessory units. They also provide a means of securely fixing the microscope to the optical table.

The CSU optical axis mounting kit (CR-CSUX-MNT-110) not only raises the optical height but provides a convenient method of tilting and raising the CSU relative to the microscope. This mechanism aids in the correction of misalignments in the optical axis of the microscope to ensure that the TuCam, or single camera adapter, can remain parcentric with view in the microscope eyepiece. Like the other Andor adapters, the CSU mounting kit can also be securely bolted to the optical table to maintain the alignment achieved at setup.

2.4 CASSETTE FILTER INSTALLATION

NOTE: For the best performance it is recommended that you have the filter set installed at our factory at the time of purchase. TuCam units are tested with each individual filter cassette prior to ship and we therefore cannot guarantee our specifications if filter sets are installed by the customer.

To achieve the ultimate imaging and alignment performance we recommend the use of the Semrock range of imaging grade dichroics. These dichroic mirrors are prepared using a specialist technique which prevents the substrate glass from warping during the deposition of the coating.

Each cassette has positions for installing a dichroic mirror (25 mm x 36 mm x 1 mm) and an emission filter (25 mm diameter) on each of the imaging paths. Filters and dichroic mirrors must be installed with the correct orientation. The reflective coating side of a dichroic mirror is normally identified by a mark or by the manufacturers' logo. It may also be identified by tilting the mirror under a light and determining which side of the glass the coating is on. If in any doubt contact the filter manufacturer for more information. The emission filters normally have a directional arrow to indicate the correct orientation (see **Figure 10** below for Semrock and Chroma filters). Likewise, if you are in any doubt contact the filter manufacturer for further information.

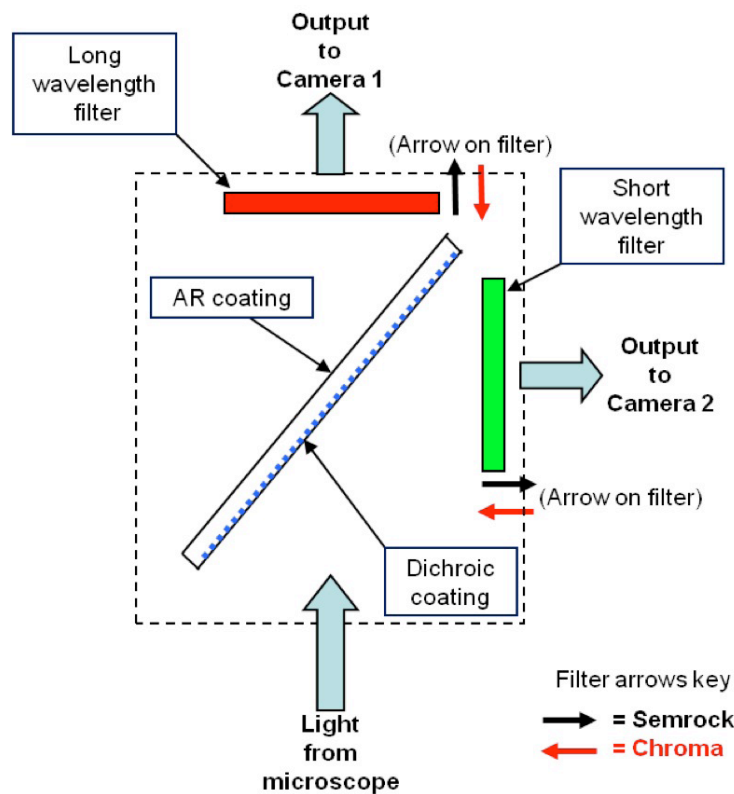


Figure 10: Filter orientation

NOTE: Chroma emission filters should be oriented opposite to that of Semrock with the arrow pointing away from the camera. However, the dichroic has the same orientation.

To install the dichroic mirror, carefully remove screws 1 and 2 that hold on the block for the Camera 2 emission filter (see photograph below). Remove the block and set it to the side. Next, remove screws 3 and 4 and the 'C'-shaped block that holds the dichroic mirror in position. Rotate the cassette around so as the holes for screws 3 and 4 are pointing upwards.

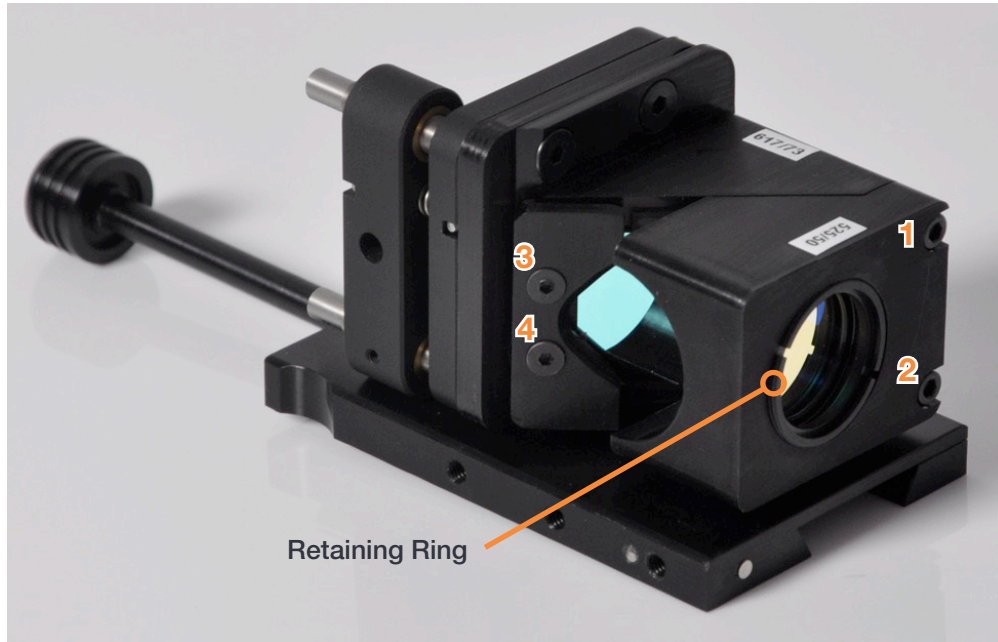


Figure 11: Mirror Retaining Ring

After you have determined the reflective side of the dichroic, carefully place the dichroic in between the locaters with the reflective side facing upwards (protective gloves should be worn to prevent finger marks on the filter surfaces). Ensure that the dichroic mirror is sitting flat then place the 'C' block back into position. Replace screws 3 and 4 and loosely tighten, just enough to hold the mirror in place. Over-tightening of these screws can cause the mirror to bend and degrade imaging performance. Replace the Camera 2 emission block.

The emission filters are housed in a 25 mm aperture, tilted at 3° to minimise back reflections and are held in place by a retaining ring. To install a filter, remove the retaining ring (SM1) using the supplied spanner wrench. In general, dichroic mirrors reflect the shorter wavelength and transmit the longer wavelength. You should therefore make sure that you install the emission filter in the correct path; Camera 1 for longer wavelength and Camera 2 for shorter wavelength. Place the filter into the holder in the orientation as detailed on the previous page. If your filter is from another manufacturer, contact them for advice on correct orientation. Replace the retaining ring and tighten.

2.5 MOUNTING AND REMOVING THE CASSETTE

The filter cassette(s) supplied with your system have been pre-aligned prior to shipping. To install the cassette firstly remove the bypass stop screw and then slide the cassette onto the dovetail rail. Replace the bypass stop screw as it acts as a mechanical stop for the cassette in the bypass position.

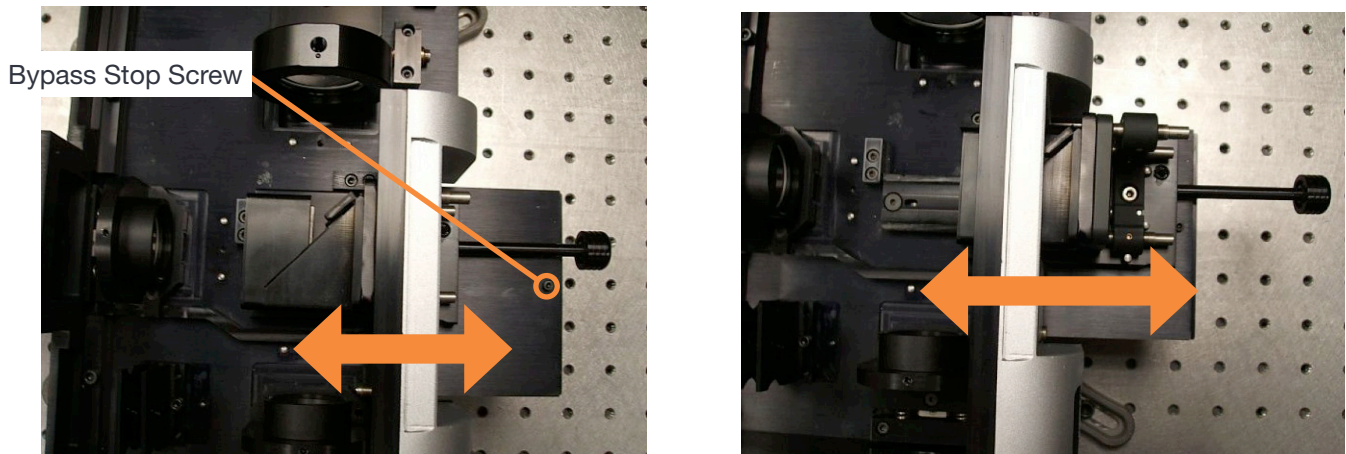


Figure 12: Bypass mode operation

The cassette locking screw should be used when highly accurate alignment of the two imaging paths is required and should be locked in position prior to commencement of alignment. Once aligned the cover should be placed over the cassette portal to ensure light tightness.

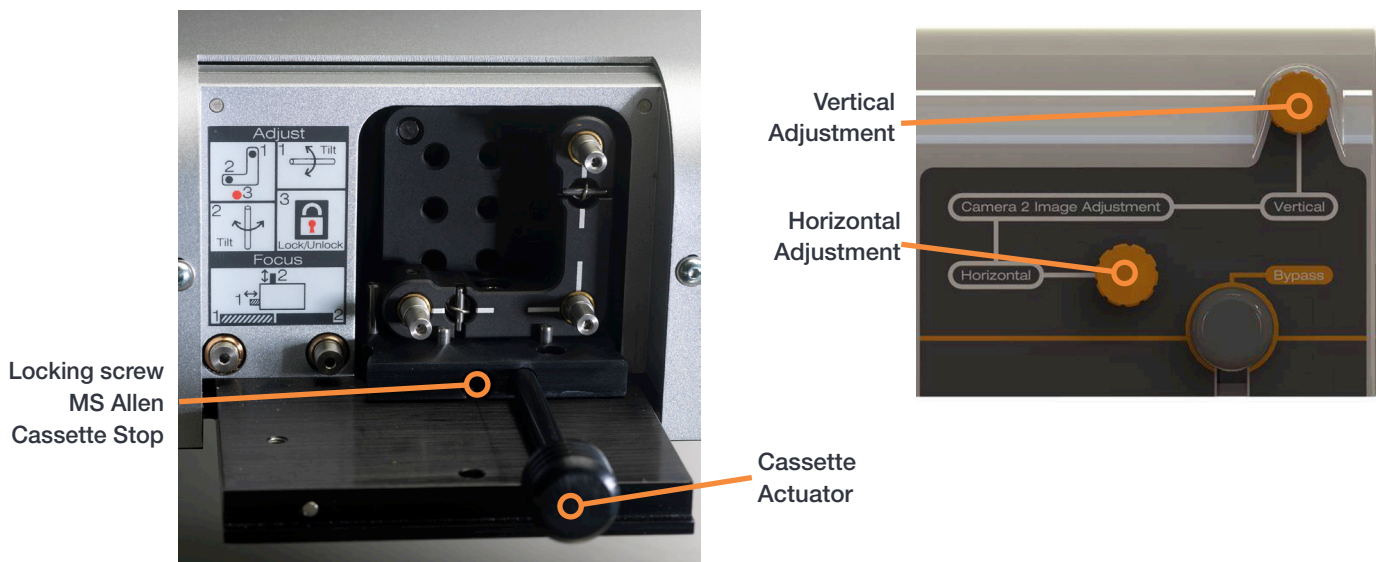


Figure 13: Internal view with cover removed (left) and external view of the front porch (right) - showing Bypass and Vertical and Horizontal adjustment knobs

The filter cassette has a useful bypass mode where it can be fully retracted to allow transmitted light viewing on one of the camera arms. To initiate the bypass mode you must firstly loosen the cassette locking screw and pull the plunger back until it hits the mechanical stop. The removable mechanical stop is there to indicate when the bypass position is reached. Upon returning from the bypass position the cassette will magnetically lock in position (for best alignment repeatability the cassette should be locked in position).

If it is necessary to fully remove the cassette from the TuCam you must first remove the mechanical stop for the bypass. Then simply slide the cassette out from its rail using the plunger and place the cassette in the storage box supplied. This will ensure that your filter set remains clean.

2.6 CONNECTING A TuCAM TO A MICROSCOPE IMAGING PORT

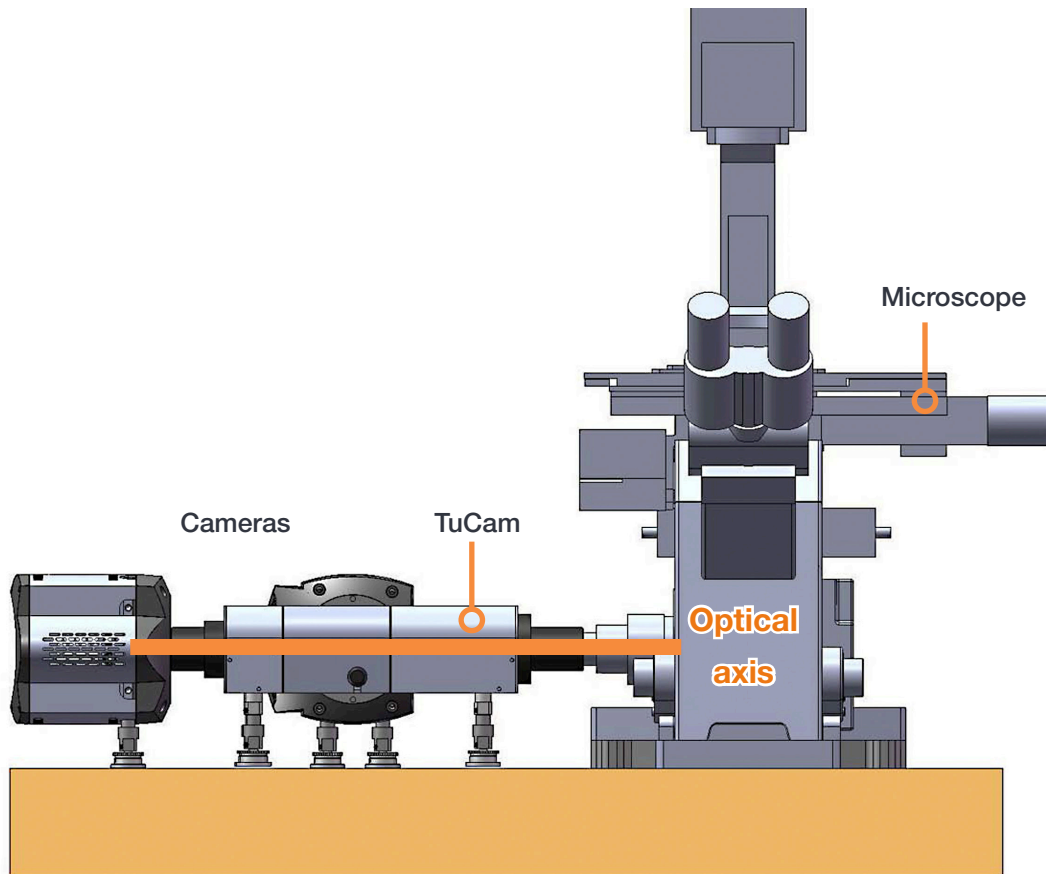


Figure 14: Example C-mount setup showing optical axis

The c-mount version of the TuCam has been designed to be attached to one of the imaging ports of a microscope. The main microscope manufacturers are supported: namely Olympus, Nikon, Zeiss and Leica. If you purchased your cameras from Andor along with the TuCam system they will have been pre-aligned prior to ship and should only need minor adjustment. The cameras will be labelled to indicate which image path they had been mounted to during testing.

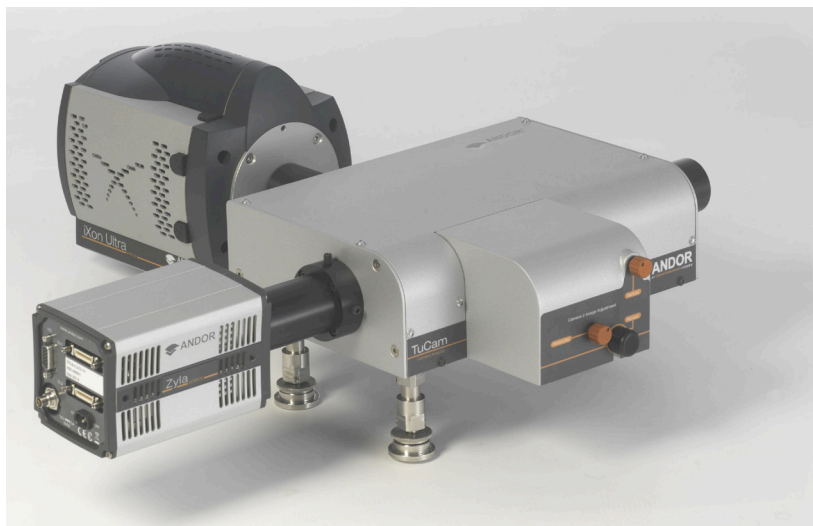


Figure 15: Typical C-mount input

Attach the microscope c-mount adapter to the entrance port of the TuCam and then to the port on the microscope body ensuring that it is securely tightened. Using a spirit level, adjust the height of the rear support legs until the TuCam unit is level.

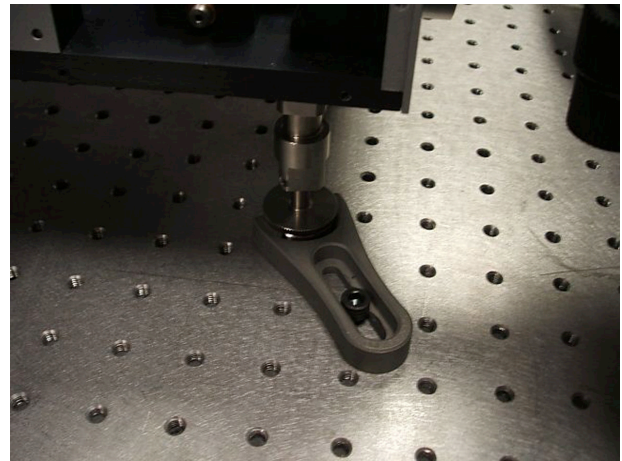
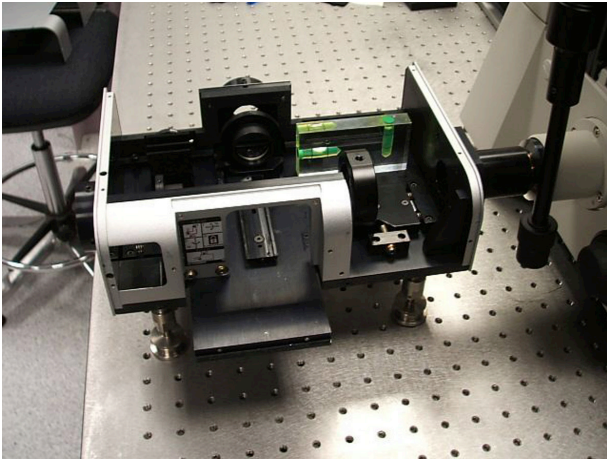


Figure 16: (left) FlexiPort system allows adjustment of the horizontal and vertical axes relative to the scope (right) Support leg locking mechanism

Our unique FlexiPort attachment system and oversized collector lens allow the user to correct for the misalignment of the microscope's optical axis (note that the position of this lens should never be adjusted). This is a common problem with microscopes and our system negates the need to have an expensive service of your microscope. This correction is particularly useful to prevent vignetting when using a large area sensor such as Neo or Zyla sCMOS.

The position of the TuCam can be altered to centre the optical axis of the scope on the collection lens. To do this, firstly place the alignment pinhole in the imaging path of camera 1. Set the scope to illuminate through the port that the TuCam is attached to using the microscopes lamp source. A bright spot should now be visible on the alignment pinhole. Adjust the rear support legs of the TuCam to vertically centre the bright spot on the pinhole. Horizontal alignment is achieved by laterally sliding the TuCam relative to the microscope.

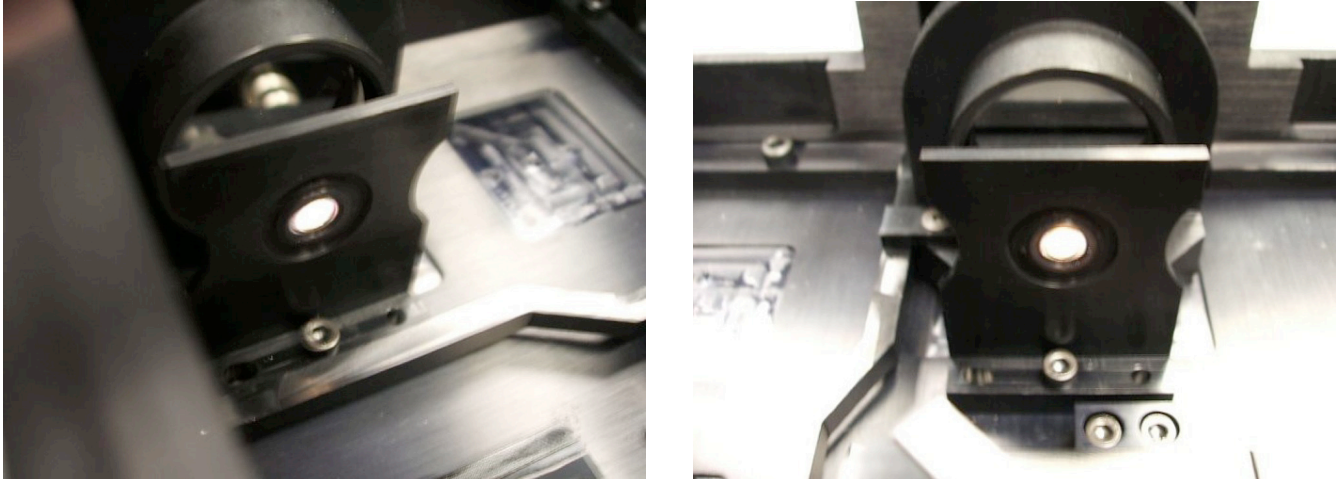


Figure 17: (left) Beam output from microscope projected onto alignment pinhole for Camera 1, (right) Beam output from microscope projected onto alignment pinhole for Camera 2

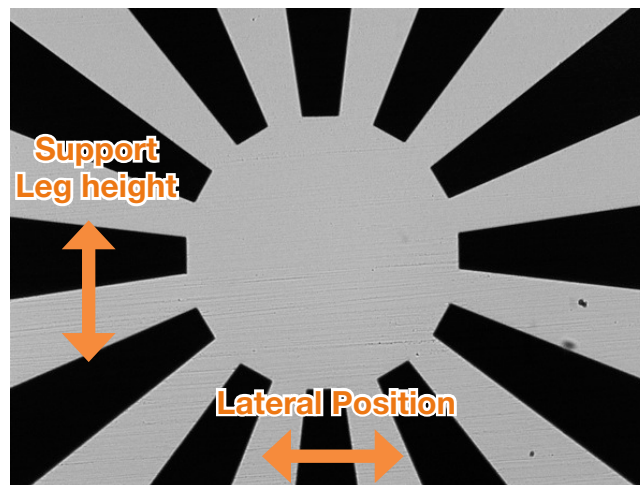


Figure 18: Image of 'Sun' pattern on camera as support leg height and lateral position are changed. Note insure that the horizontal and vertical image orientation on your camera are set correctly

After performing the above steps you may still find that the image obtained by the camera is still not parcentric with the microscope. Camera sensors are often slightly offset relative to their c-mount due to manufacturing tolerances. This can be corrected by firstly centring the image in the microscope eyepiece (use the 'Sun' pattern on the supplied alignment grid and a crosshair eyepiece if available). Run your camera 'Live' and adjust the support leg height and lateral position on the table until the image is centred. Once centred the system should be locked in position by fixing the support legs onto the optical table using the brackets provided.

Remove the alignment pinhole and place it in the Camera 2 path. Slide the cassette onto the dovetail and lock in position. Observe the bright spot on the alignment pinhole and use the vertical and horizontal adjustment controls on the front porch to centre the spot on the pinhole to centre the spot on the pinhole. These initial steps only provide a gross alignment of the system to make it easier to locate features on the cameras. The camera sensors tend to be slightly offset relative to the c-mount so a second alignment step may be necessary.

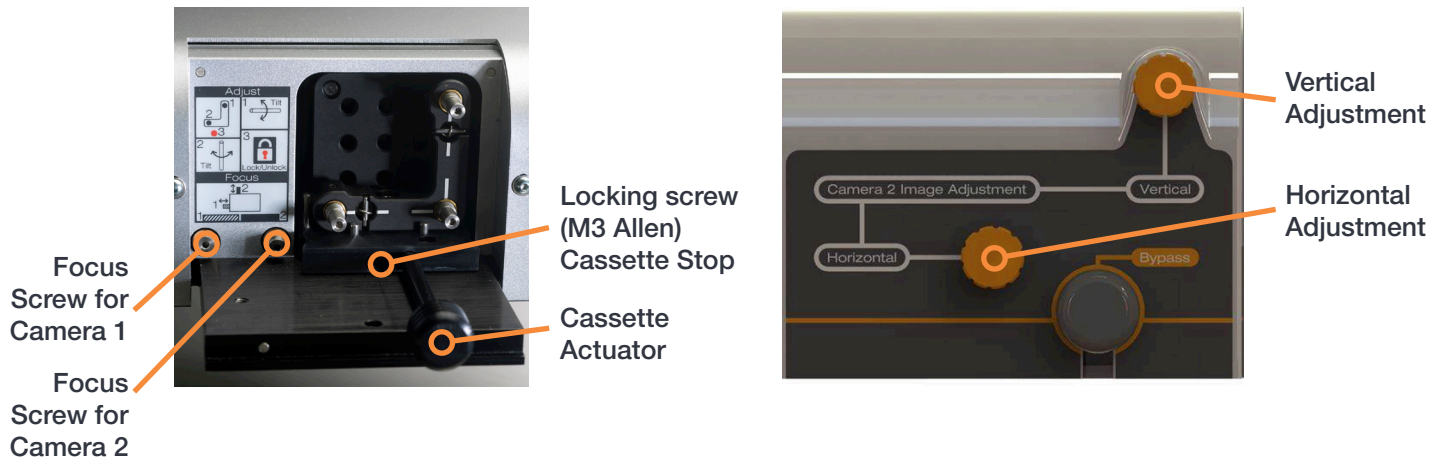


Figure 19: Cassette and camera focus mechanisms, internal view (left) and external view (right)

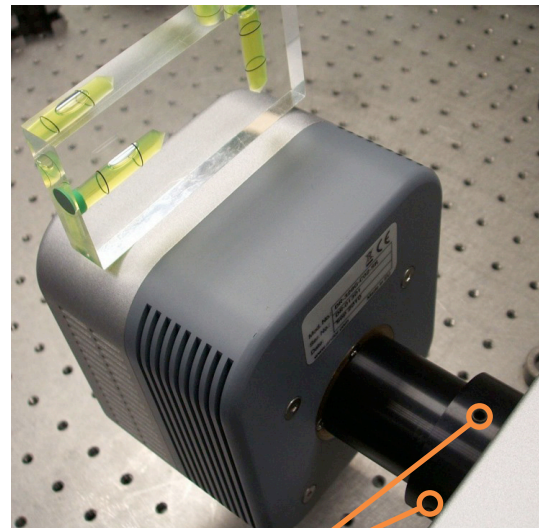
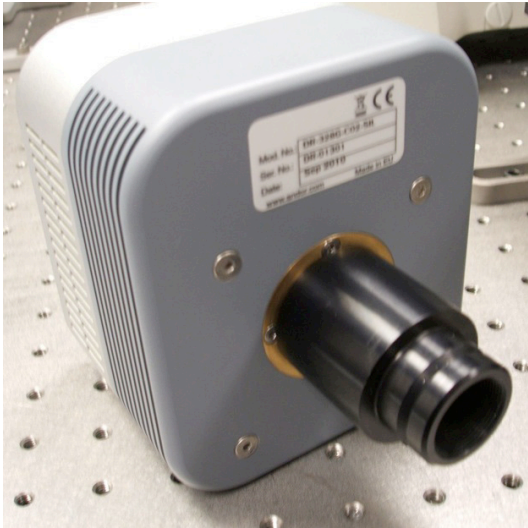
Once the unit is locked down you can begin to align the two imaging paths. It is recommended that you use the alignment target (XS-SSMS-GRID, shown below in **Figure 20**) supplied and a low/medium power microscope objective (20x to 40x). Grid patterns are preferable as these facilitate the angular alignment of the two imaging paths.



Figure 20: Alignment target (XS-SSMS-GRID)

The appropriate c-mount adapter should be attached to the camera that is to be used on the particular imaging path. The length of the adapter is magnification dependent (shorter lengths are for lower magnification).

The adapters and cameras should then be slid into the camera mounts on the TuCam unit and locked in position. A spirit level should be used to ensure the cameras are level. Keep Camera 2 slightly loose as it may be necessary to rotate it during alignment. Software alignment of the system can now commence.



Locking Screws

Figure 21: Levelling and locking the camera during alignment

2.7 ALIGNING THE TUCAM USING IQ

The following section indicates how to align the TuCam using Andor iQ. Other image overlay software is available and you should follow the same procedure to achieve similar results.

Connect the power supplies to the cameras and USB/Controller card Link cables to the PC and cameras. Power up the cameras and start iQ with a configuration that contains the 2 cameras as a minimum. A camera selection window will appear with a drop down menu that has the serial numbers of the 2 cameras in use. Select the camera in the Camera 1 path of the TuCam as **Camera 1** in the software. When iQ completes its start-up, go to the camera selection tab and activate the second camera as **Camera 2**

The user should then accurately focus an alignment target in the eyepiece of the microscope as this will ensure that the TuCam is parfocal with the microscope (we recommend using the supplied Multi-Grid Standard Stage Micrometer, XS-SSMS-GRID). We also recommend that you ensure the TuCam unit is parcentric with the microscope by comparing the image in the eyepiece to that obtained at the camera. Users should ensure that eyepiece focus is properly set beforehand according to manufacturer's instructions.

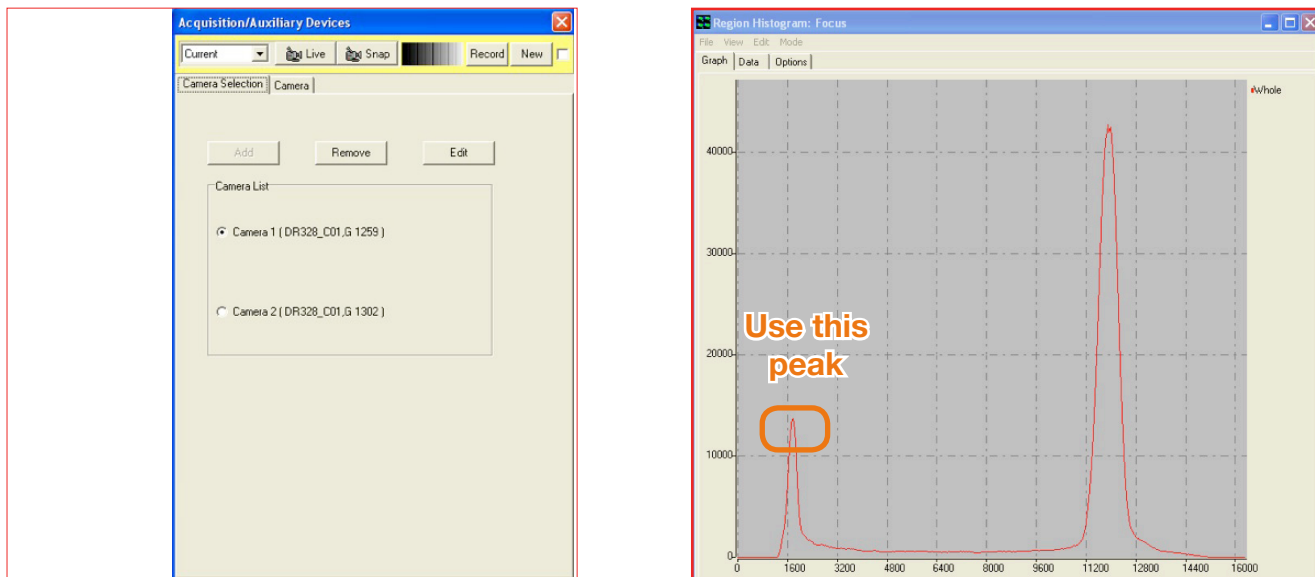


Figure 22: Aligning the TuCam in iQ

Select **Camera 1** in the software and set the camera acquisition parameters to achieve a suitably intense image with a peak intensity of approximately 75% of the full camera range. Where possible, position the alignment target so that an easily identifiable feature is present and use the **focusing screw for Camera 1** to obtain an accurately focussed image (see Figure 19 on page 22). To aid with focussing use the Region Histogram tool on the Analysis menu. When using this tool it is best to have the grid pattern covering the entire image area of the camera. Two peaks will be visible in the histogram; the one at a lower count value indicates the dark areas of the image. Best focus is achieved by maximizing the height of this peak.

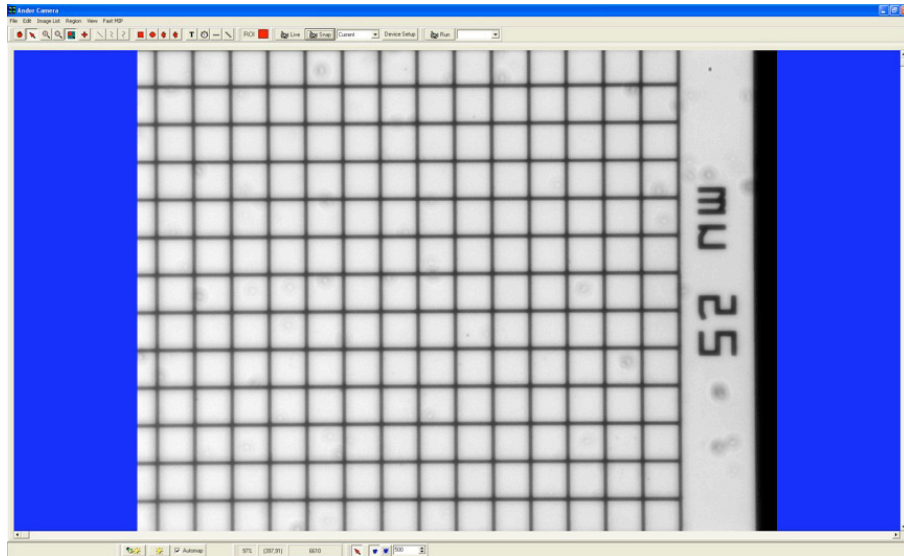


Figure 23: Alignment target for CSU version

Once focused, snap the image and save it as Camera1 Grid. At this point you should make sure **not to move the microscope stage or adjust focus** as you will end up aligning camera 2 to an image that is no longer there. Switch to Camera 2 in the Acquisition/Auxiliary Devices **window**.

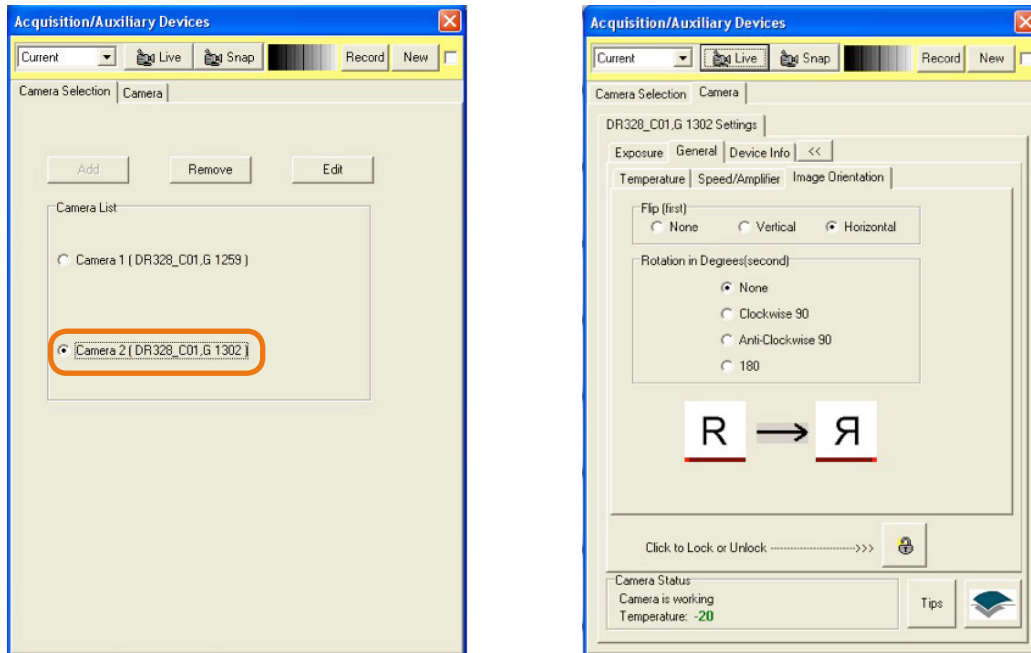


Figure 24: The Acquisition/Auxiliary Devices window

On the **Camera tab**, select **General-Image Orientation** and click on **Flip Horizontal or Flip Vertical**. The direction of flip will depend on how you have the cameras mounted and needs to be done as the image is reflected towards Camera 2 so therefore has the opposite orientation to that of Camera 1. Set the acquisition parameters for Camera 2 to give a similar intensity level as the image from Camera 1 and obtain accurate focus of this image also. Obtaining accurate focus on both imaging channels is very important as this will have an influence the relative magnifications.

On the **Wizards** menu select **Camera Alignment**. This tool allows the user to overlay a live image on top of a previously acquired image.

On the first **window** of the Wizard select **Use Reference Image** and click **Next >>**

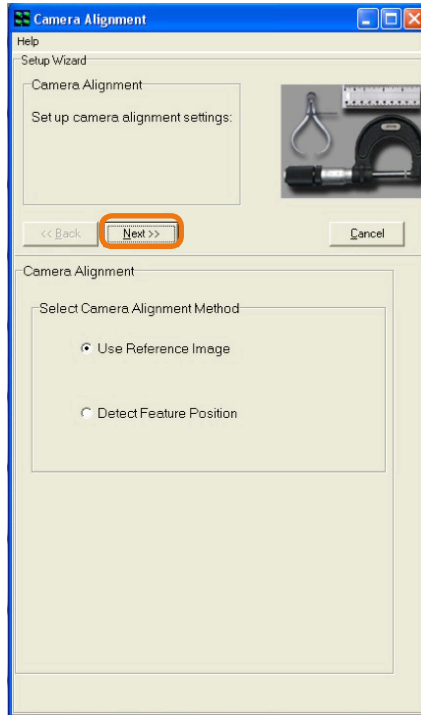


Figure 25: Camera Alignment Window

This will bring up the image list, e.g.:

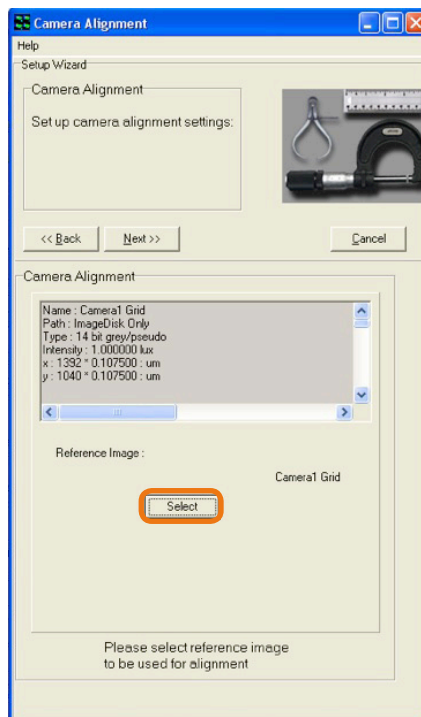


Figure 26: Image List

Choose the image that had been taken previously using **Camera 1** and click **Select** to advance to the next window.

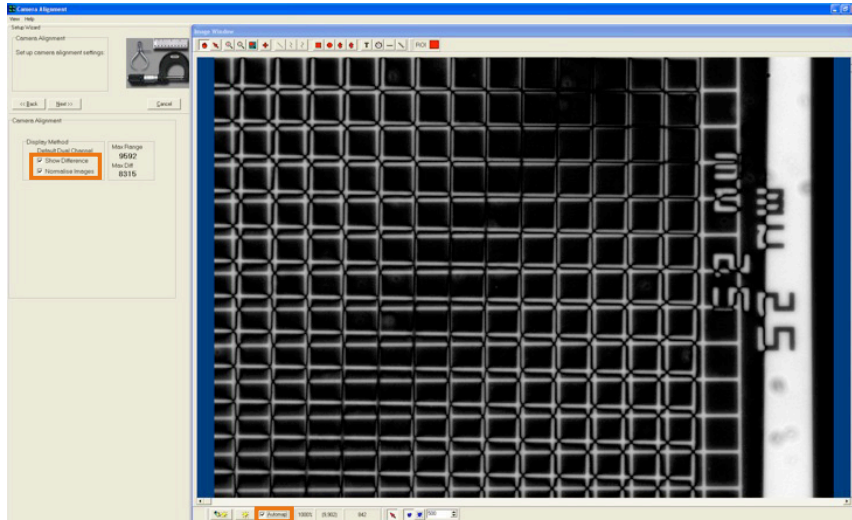


Figure 27: Selection of Camera Alignment options

On this window tick on the **Show Difference**, **Normalize Images** and **Automap** checkboxes. An overlay of the two imaging paths will now be visible with the image from Camera 2 running live.

Use the x and y adjustment screws on the cassette to approximately align the image from Camera 2 with that from Camera 1. At this point you can correct the angular misalignment by rotating Camera 2 until the lines of the grid are parallel.

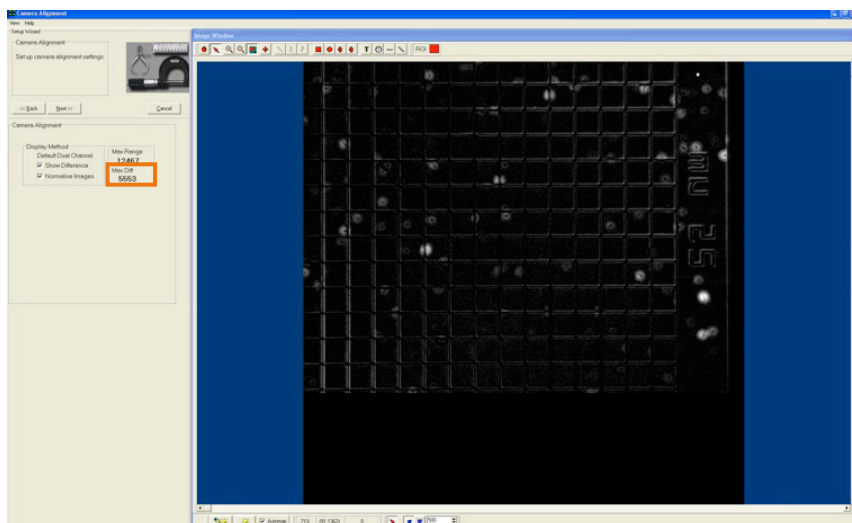


Figure 28: Setting the lowest Max Diff value

Once the angular misalignment is corrected, the images can then be accurately aligned by using the x and y adjustment screws on the cassette to obtain the lowest value of **Max Diff** (highlighted).

Sometimes you may find that there is a slight difference in the magnification of the two imaging paths. This is caused by the two imaging paths not being accurately focussed and can easily be corrected for by adjusting the focus of the imaging path for camera 2. Turning anti-clockwise will increase the magnification and vice versa. Note that only small changes are necessary, if at all, and if you find that the image from camera 2 is no longer in focus you should start the setup procedure again. The alignment can be further refined but the method will be dependent on your current setup.

The CSU pinholes are the recommended method as they allow accurate focus to be achieved and present a wealth of alignment points. However, you may find that the CSU is not parfocal with the microscope thus causing a slight shift in alignment relative to the scope. If this is the case you may prefer to use fluorescent beads or some other high resolution sample that you have available. In doing so you must also ensure that your sample remains in a fixed position and that you do not have any focus drift. Set-up is the same up to this point, but please follow the guidelines below:

- When using the CSU pinholes, remove the alignment target from the scope and switch off the CSU to stop the disk spinning

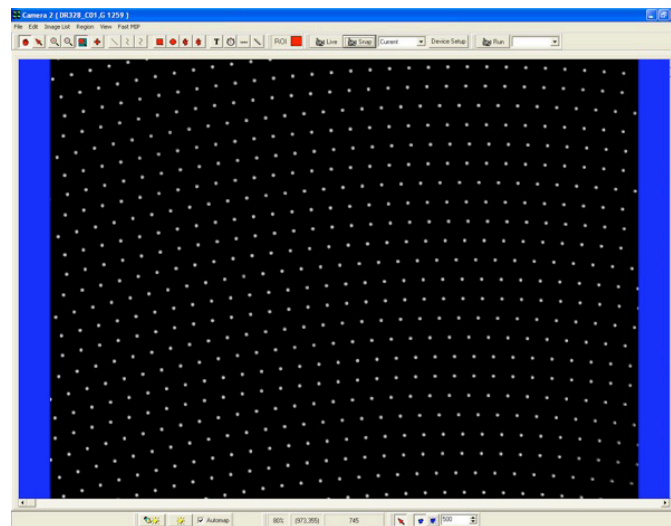


Figure 29: Example of CSU pinhole image

- When using Fluorescent beads, replace the target with a bead sample suitable for use with your particular system set-up. For alignment purposes ensure there is an adequate distribution of beads spread across the image, e.g.:

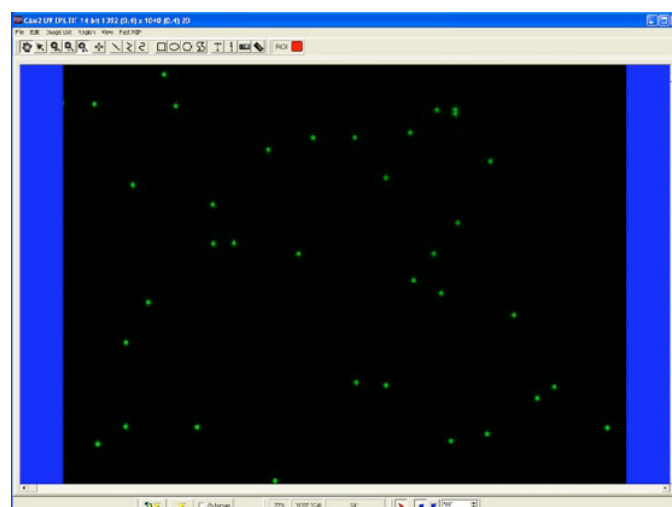


Figure 30: Example of Fluorescent bead image

On Camera1 set the camera acquisition parameters so as to obtain an image similar to that above, making sure that none of the pixels are saturating. The pinholes also allow a highly accurate focus to be obtained. In the iQ software select the Region Histogram from the Analysis menu. Two peaks will be visible, one at the image background level and the other at the average intensity level of the pinholes. Best focus is obtained by maximising the intensity of this peak.

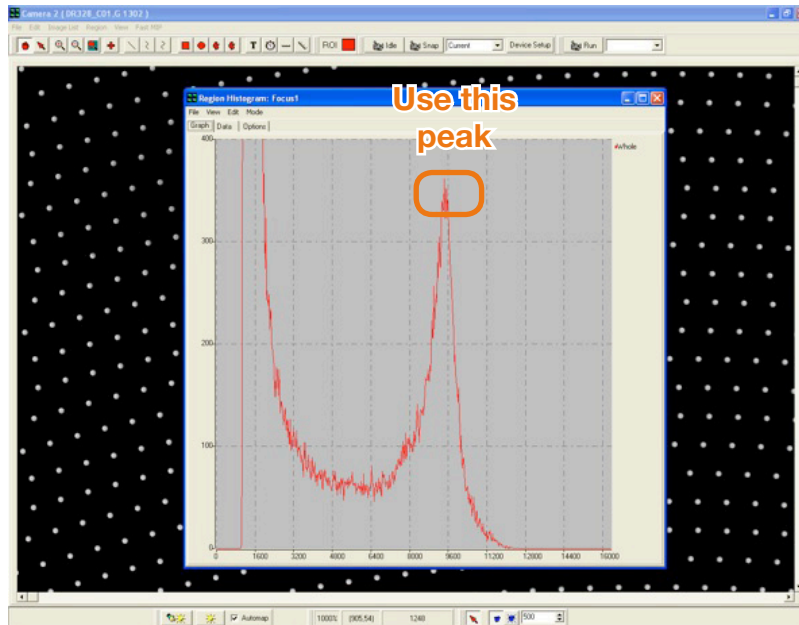


Figure 31: Using the peak average intensity of pinholes to obtain best focus

Snap this image as before and rename it Cam1 CSU Pinholes. Switch to Camera 2 and repeat the histogram focusing method above.

Open the Camera alignment Wizard and select the image from Camera 1, e.g.:

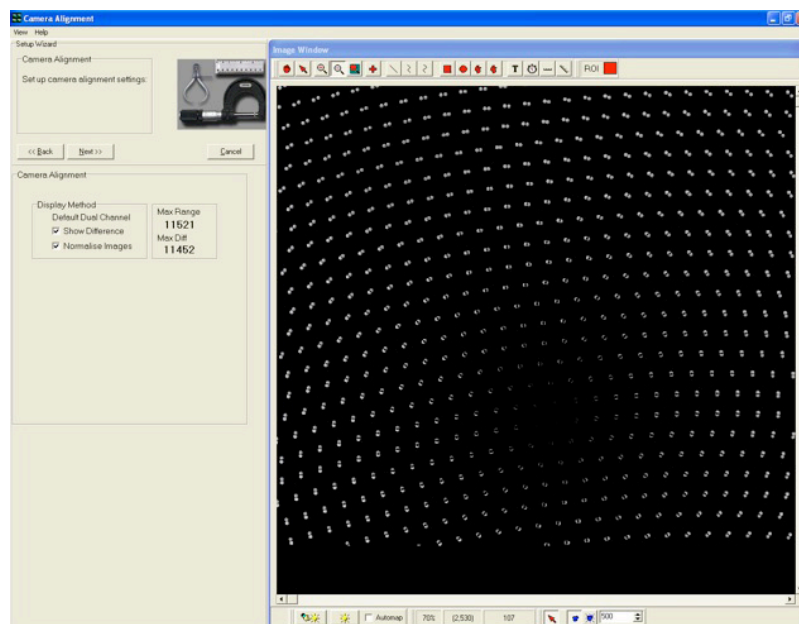


Figure 32: Example image from Camera 1

Click on the checkboxes as before and correct any angular misalignment by rotating Camera 2. Use the x and y adjusters to obtain the best alignment of the image by minimizing the Max Diff score. Take care not to move too far away from your current position as it is easy to get lost due to the lack of a reference point. You should be able to achieve alignment across the entire field to within a few pixels.

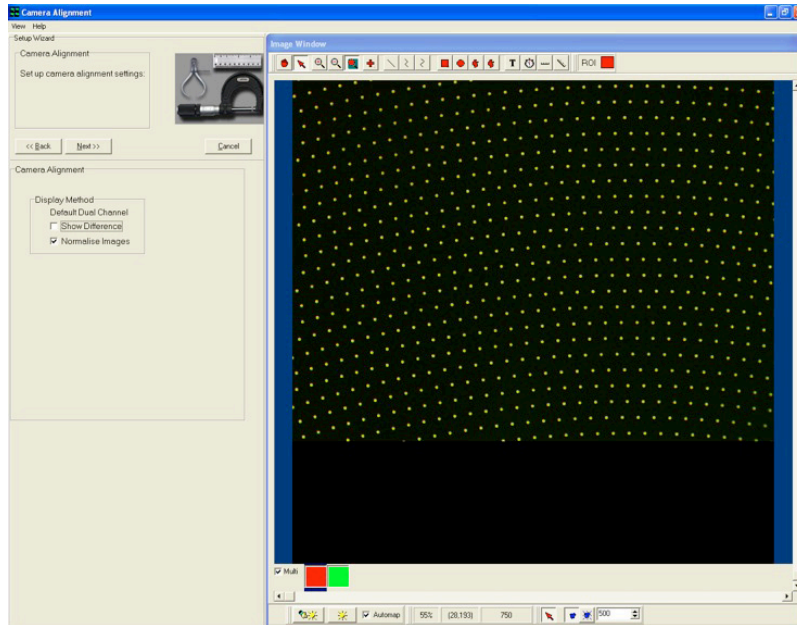


Figure 33: Overlay of the CSU pinholes from the two imaging paths

2.8 CONNECTING A TUCAM TO THE CSU

The TuCam has been designed to integrate with the CSU and Sutter filter wheels. For best results it is recommended that the system be mounted on a level optical table or breadboard and that the microscope, CSU and filter wheel are level and aligned on the optical axis of the microscope port. The TuCam will normally have been installed by one of our engineers and it is not recommended that you remove it from your system. However, should you need to remove your cameras, you should try to remember which camera was attached to which TuCam port as this will make setup simpler.

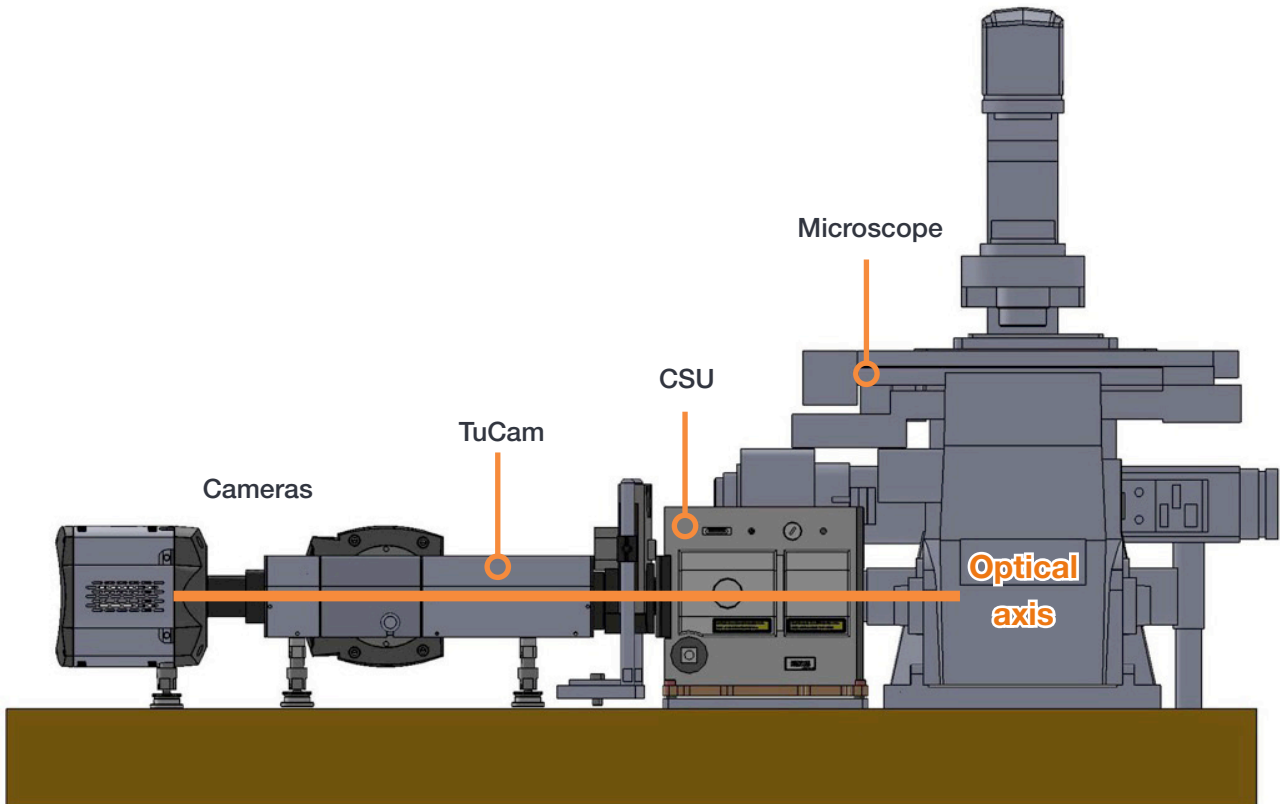


Figure 34: Example CSU setup showing optical axis

If the TuCam is removed from your system, care should be taken to reinstall it as it had been previously. You should ensure that the TuCam follows on the optical axis of your confocal system. This will not always be perpendicular to the microscope port as these often have a slight angular deviation that is exaggerated by the long optical path. Upon reattaching, check that the image on Camera 1 is parcentric with that observed in the microscope eyepiece. To correct any misalignment you will have to slide the TuCam laterally on the table or adjust the height of the support legs. Once you are satisfied that the image on Camera 1 is parcentric securely fasten the TuCam unit onto the optical table and follow the normal camera alignment procedure in the previous section.

SECTION 3: MAINTENANCE

There are no user serviceable parts inside the unit. If the equipment is used in a manner not stated by Andor, the warranty may be impaired.

3.1 CLEANING (EXTERNAL)

Only use a dry, clean, lint free cloth to clean all external painted surfaces. If necessary, use a water diluted detergent to lightly dampen the cloth - do not use Isopropyl alcohol, solvents or aerosols.

SECTION 4: TROUBLESHOOTING

4.1 IMAGE NOT PARCENTRIC WITH MICROSCOPE

TuCam unit is not properly aligned with optical axis of microscope. Slide the TuCam laterally on the table and adjust the support leg heights until the image obtained in Camera 1 is parcentric with the image in the microscope eyepiece. Once you are satisfied that the image on Camera 1 is parcentric securely fasten the TuCam unit onto the optical table.

4.2 IMAGE NOT PARFOCAL WITH MICROSCOPE

Microscope focus has shifted before TuCam was focused. Accurately focus the image in the microscope eyepiece then proceed to focus both cameras using their independent focusing screws.

4.3 NON-UNIFORM IMAGE INTENSITY

Caused by a poorly aligned system. Check that the image from camera 1 is parcentric with the image in the microscope eyepiece and re-centre as necessary.

4.4 CAMERA IMAGES OUT OF ALIGNMENT

Image misalignment can be caused number of reasons:

1. **Cassette not tightened:** To maintain accurate alignment of the 2 imaging paths the cassette should be held in position using the locking screw both during and after camera alignment
2. **Microscope stage/alignment slide moved:** When aligning the 2 camera images, care must be taken to ensure that the stage or slide does not move after the image on camera 1 has been taken. This will result in camera 2 being aligned to a false position
3. **TuCam has moved:** If the TuCam unit has moved relative to the microscope the 2 camera images will no longer be aligned. Follow the normal camera alignment procedure to correct this and ensure that the TuCam and any other equipment in the optical path are securely fastened to the optical table
4. **Camera has moved:** Make sure that both cameras are securely tightened to the TuCam when camera alignment is complete
5. **Dichroic/PBS loose:** Check that the dichroic mirror or Polarizing Beam Splitter is not moving around in the cassette. If loose, gently tighten the retaining screws. Be careful not to over tighten as this will cause the mirror to bend slightly therefore compromising image quality

4.5 MAGNIFICATION DIFFERENCE

Slight differences in the magnification of the 2 imaging paths can occur due to the manufacturing tolerances in the lens focal lengths and if the two images are not accurately focused. Small differences may be corrected by slightly adjusting the focus of camera 2. Turning the focusing screw anticlockwise will increase the magnification and vice versa. Note that only small changes should be necessary and the 2 camera images should both remain in good focus.

4.6 CAMERA 2 IMAGE APPEARS SKEWED OR WARPED

Dichroic mirror/PBS retaining screws are too tight or you are using a non-imaging grade dichroic.

1. To achieve the best alignment of the 2 camera images we only recommend using the Semrock range of imaging dichroics. Standard dichroics tend to become slightly warped during the deposition of the mirror coatings resulting in reduced imaging performance
2. Over tightening of the dichroic/PBS retaining screws will cause the mirror to warp thus degrading image quality. Loosen the screws to the point where they are just about holding the mirror in place

4.7 BLURRED IMAGE

This can be caused by a poorly focussed image or poorly aligned system.

Check microscope focus and TuCam focus using calibration slide and refocus as necessary ensuring that the TuCam and Microscope are parfocal. If the image appears blurred at one side suspect that the unit is out of alignment so follow the alignment procedures as outlined in **Section 2.7**.

SECTION 5: OTHER INFORMATION

TERMS AND CONDITIONS OF SALE AND WARRANTY INFORMATION

The terms and conditions of sale, including warranty conditions, will have been made available during the ordering process. The current version may be viewed at: http://www.andor.com/pdfs/literature/Andor_Standard_Warranty.pdf

WASTE ELECTRONIC AND ELECTRICAL EQUIPMENT REGULATIONS 2006 (WEEE)

The company's statement on the disposal of WEEE can be found in the Terms and Conditions

