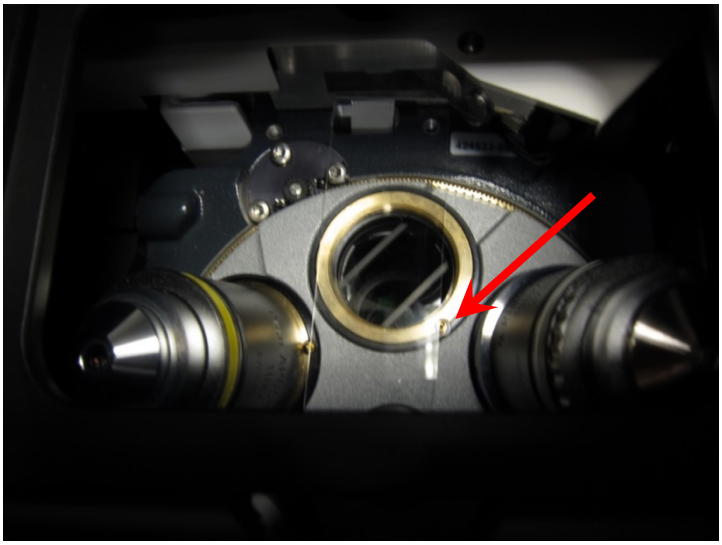
Stage Alignment for the Elyra

1) Select an empty objective position or remove an objective if all positions are taken

2) Remove the stage insert using the finger holes provided as shown below (do not remove using the alignment screws in the insert)

3) Remove the dust cover from the objective turret

4) Place a plain glass side over the empty objective position. Ensure that the slide sits flat by avoiding the attachment screw on the turret shown by the arrow in the below picture:



5) Place a second glass slide into the stage insert – do not use the retaining clip. Replace the insert into the stage using the finger holes provided (do not touch the alignment screws on the insert)

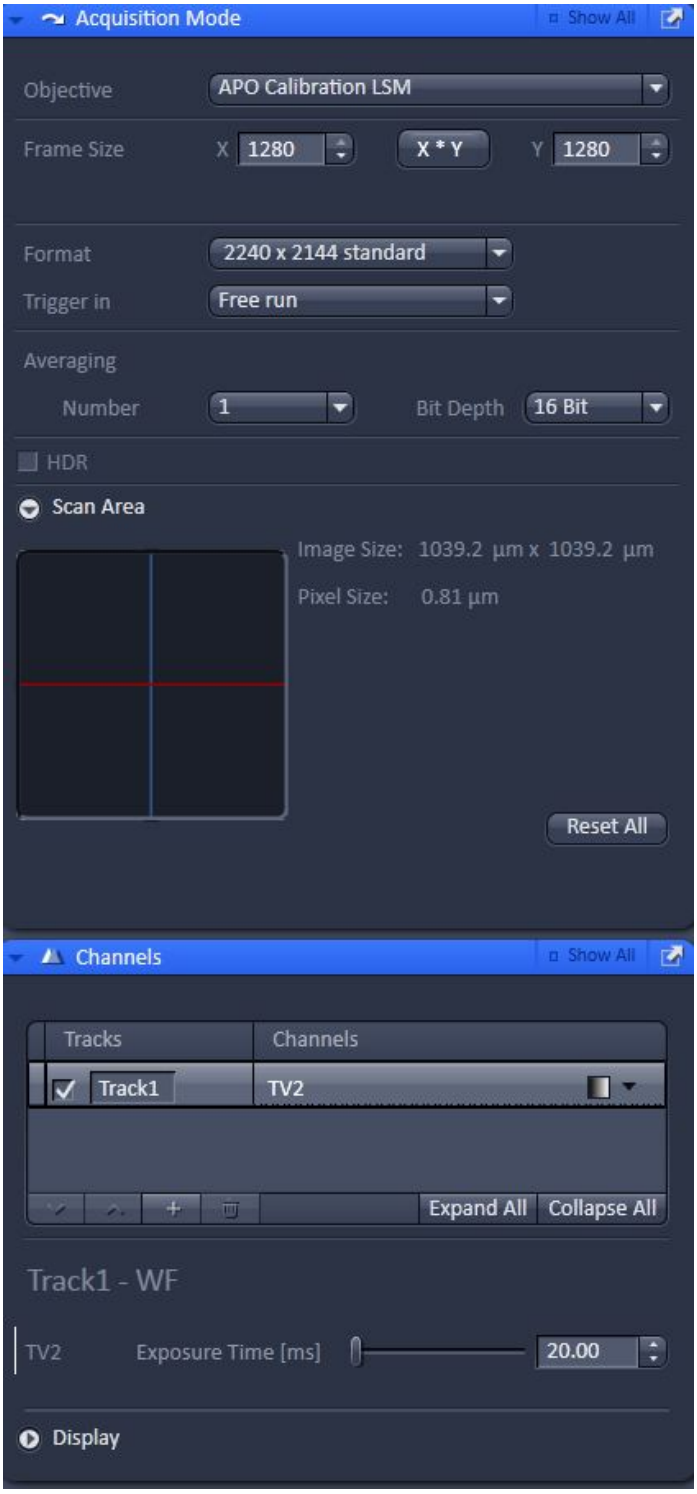
6) Ensure that the transmitted light arm is down

7) Select the ‘Acquisition’ tab within ZEN and open the Light Path menu

8) Choose WF (widefield) within the Light Path menu and configure the system with the BP 420-480 emission filter imaging to the SIM camera. Use the 1.6 Optivar.

9) In the ‘Channels’ tab choose an exposure time of 20ms (see below)

10) In the ‘Acquisition Mode’ tab, ensure that the full frame of the camera is being used (see below)



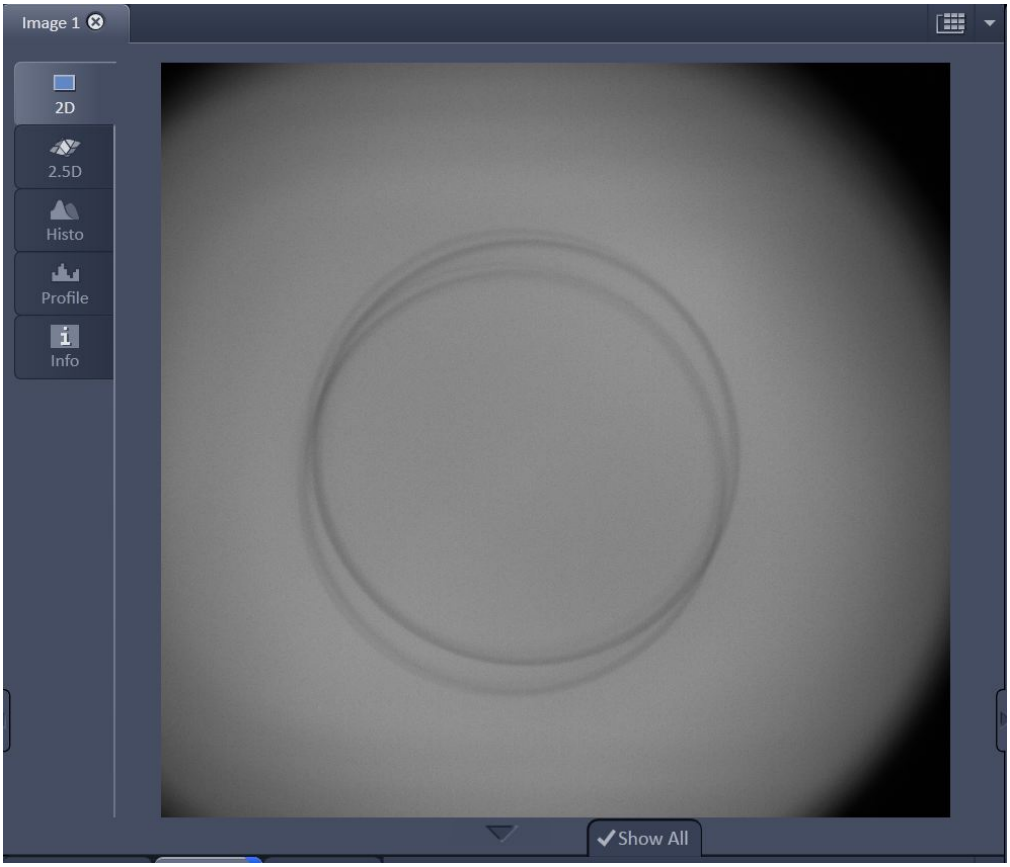
11) If using an X-Cite LED system turn on the BDX LED and change the intensity to 5%

12) If using the X-Cite metal halide system, ensure that this is switched on and the intensity set to minimum

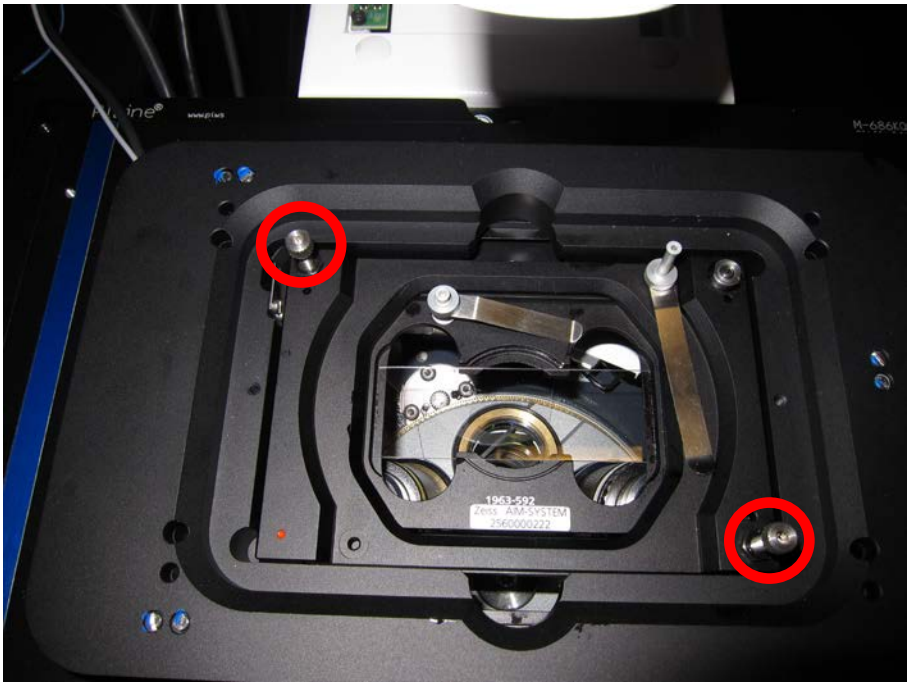
13) If using a X-Cite metal halide system, pull the bulb end of the light-guide slightly out of the unit to reduce the intensity of the image

14) Press ‘Live’ to view a live refresh in the imaging window. Change the display settings until you can see an image

15) For both LED and Metal halide systems, gently pull the microscope end of the light-guide out of the Elyra head having loosened the locking nut (back left hand side of the system). You will see two reflection rings on the screen that will become sharper as you pull the light-guide. Select the correct position by choosing the ‘sharpest’ image



16) The two circles represent reflection rings from the slide on the reflector turret and the slide on the stage insert. Move the stage using the adjustment screws (circled in red on the below picture) until the two circles are overlayed



17) Remove the slide from the stage insert

18) Remove the stage insert from the stage using the finger holes provided (do not touch the alignment screws on the insert)

19) Remove the slide from the objective turret and replace the objective dust cover

20) Push light-guide back into the Elyra and also the X-Cite metal halide unit (if used)

21) The stage insert is now aligned. Ensure that the stage insert is always removed and replaced using the finger holes provided – if the alignment screws are used for taking the insert in and out then the alignment will be compromised which will lead to issues during image acquisition and subsequent processing

Channel Alignment Procedure for the Elyra

1) Set up a 4µm z-stack of beads using SIM(Tool for Calibration Multispec)
2) Acquire the SIM z-stack using the tracks required in the sample (e.g. for GFP and RFP sample collect a bead stack in green and red) ensuring that the smallest/optimal interval is used
3) Process the stack using SIM. Select Manual processing and untick Auto Noise Filer. Choose a noise filter of -6.
4) Go to the channel alignment tool in the processing tab. Choose 'Affine'. Ensure that the 'fit' box is ticked. Select the SIM processed bead data image and apply the channel alignment. This will create a data table that can be used to align SIM data. Save the table using the 'Save' button below the table. Save the channel aligned image.
5) Acquire a multicolour SIM image and process as usual.
6) Go to the Channel Alignment tool in the Processing tab and select the SIM image you wish to align.
7) Untick the 'fit' box. Select Affine.
8) Load the Channel Alignment table that you have created in 1-4 using the 'Load' button under the data table
9) Apply this table to the image using the Apply button at the top of the Processing tab.
10) A new image will be created with the channel alignment applied.