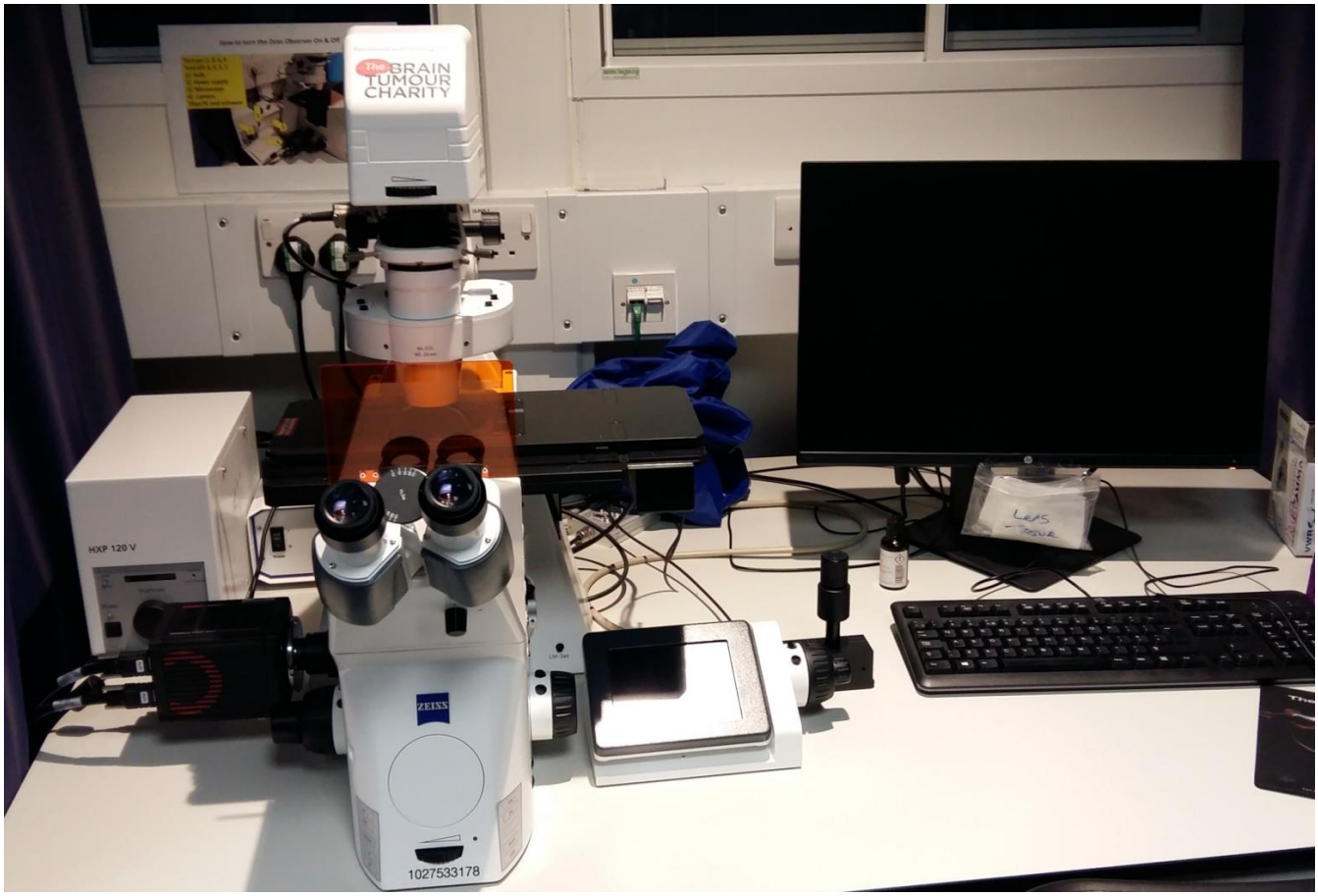
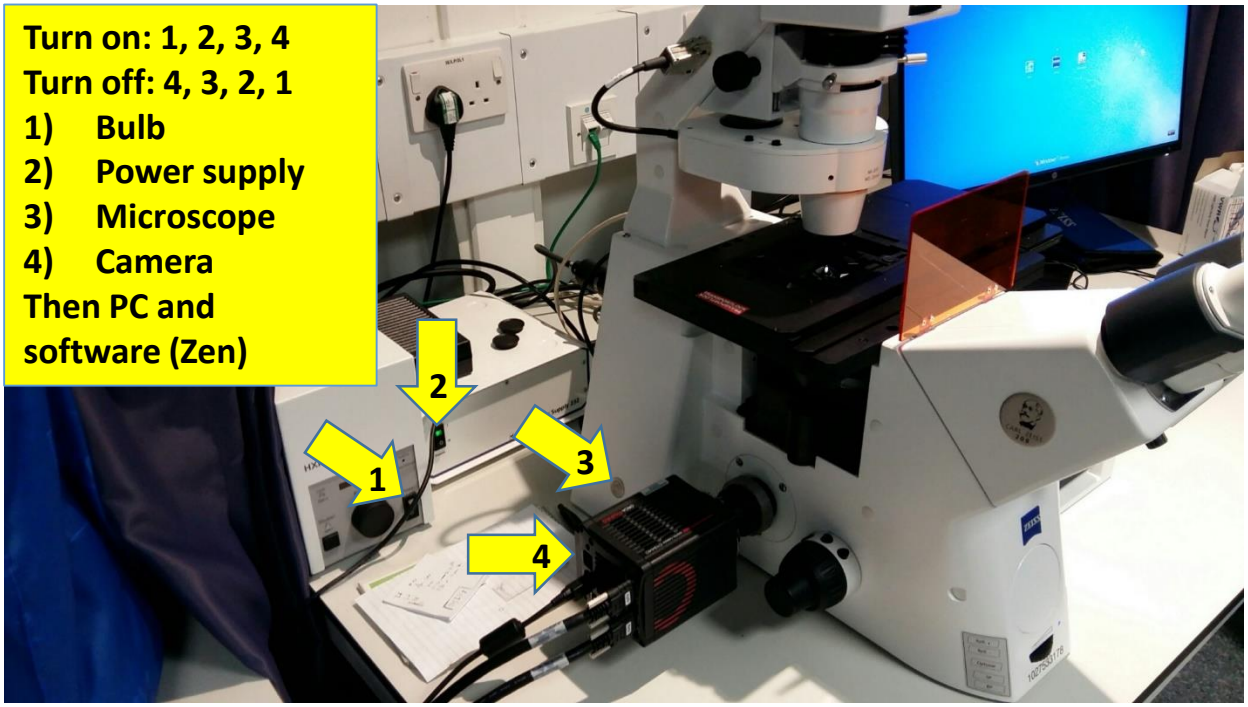
Zeiss Observer 4 Colour Fluorescence
Hamamatsu Flash 4.0v3 camera

Instructions for
Brightfield (black and white camera)
Fluorescence (4 colours),
Z stacks and
Tile scans.

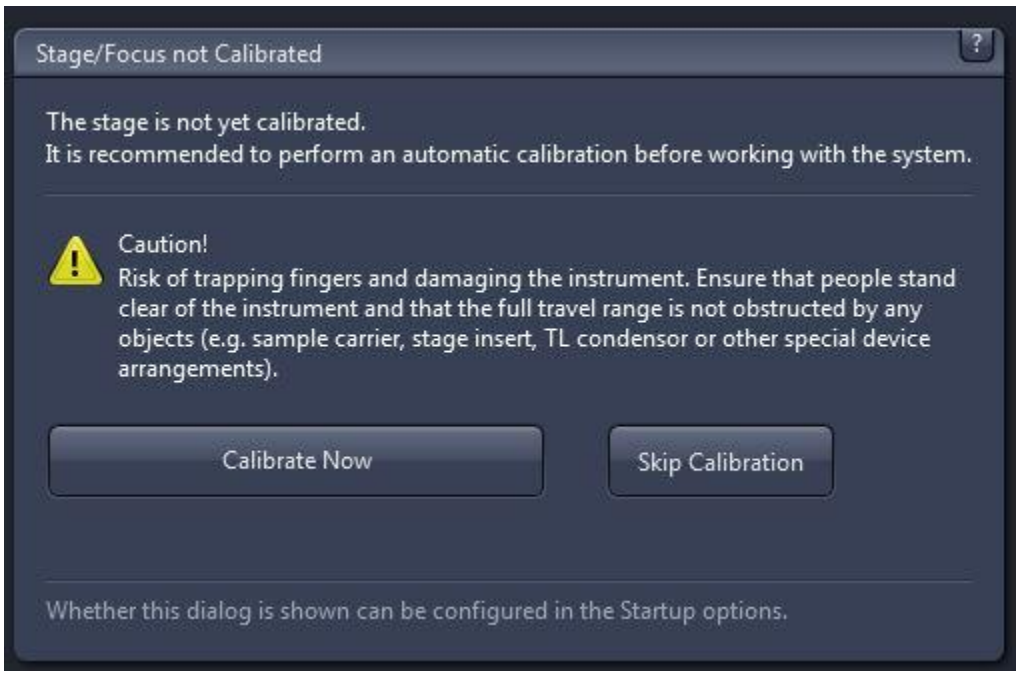
Other functions available, such as scanning multiwell TC plates, adjusting focus offset for different channels, time series, high speed imaging (100-1000+ fps), deconvolution. Ask Dale for details.



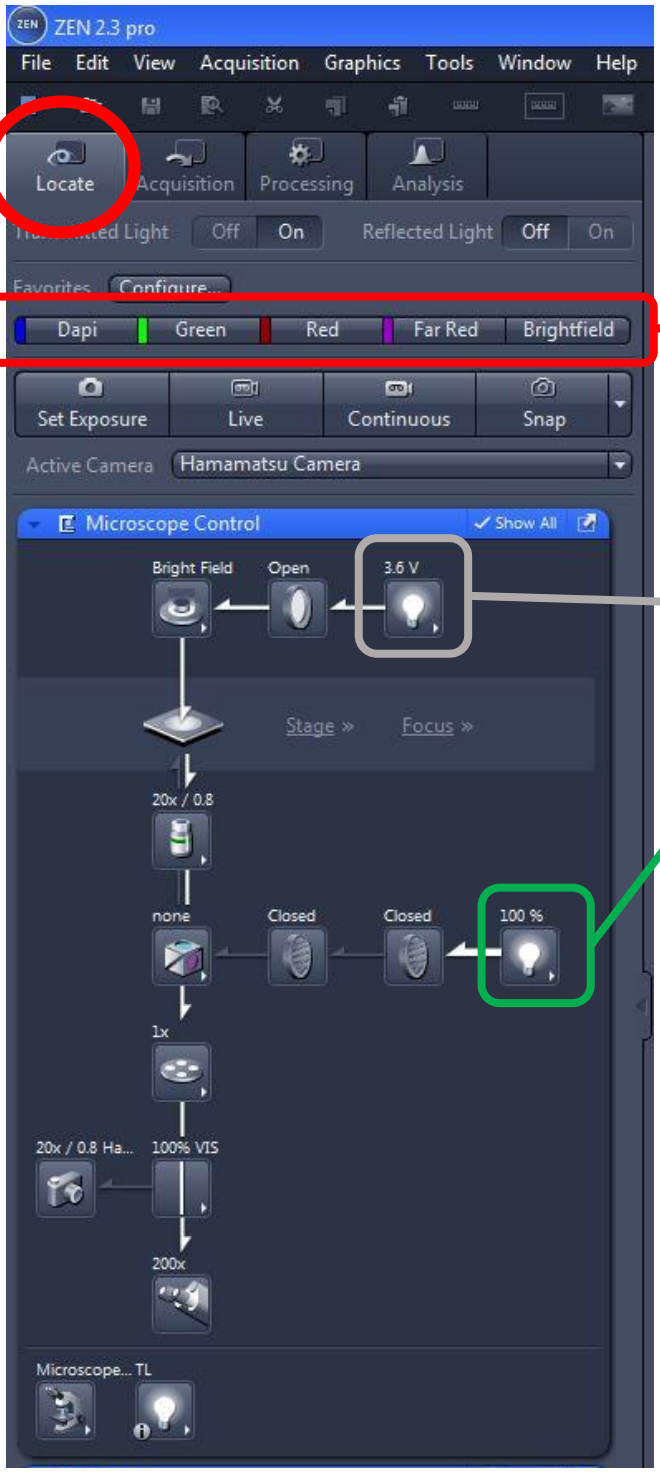
Turn on the system:



After you start the Zen software, if you are the first user it will ask to calibrate the stage…
Press ‘Calibrate Now’



Use the Locate Tab for looking down the eyepieces…



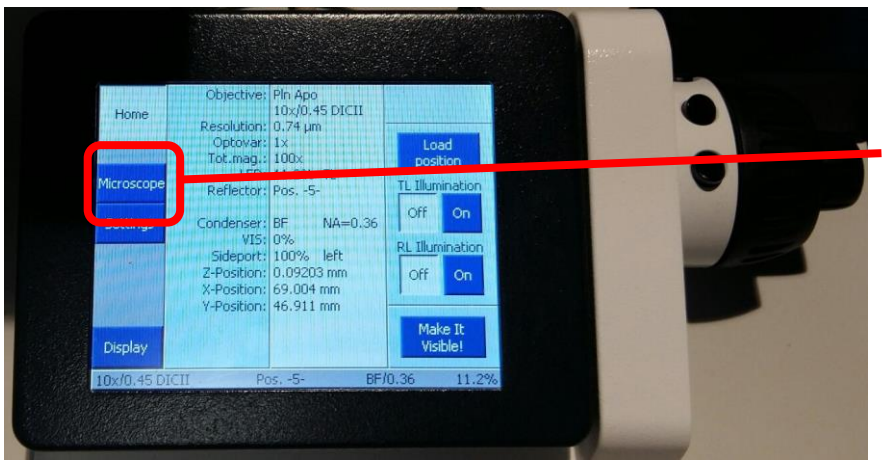
Choose the colour to see: Dapi, Green, Red, Far Red or Brightfield.

You can adjust bulb power
Brightfield
or
Fluorescence

Set magnification from the software or the touchscreen next to the scope

5x, 10x 20x & 40x dry objectives.
The last two (40x 1.3 & 63x 1.4) are oil objectives.

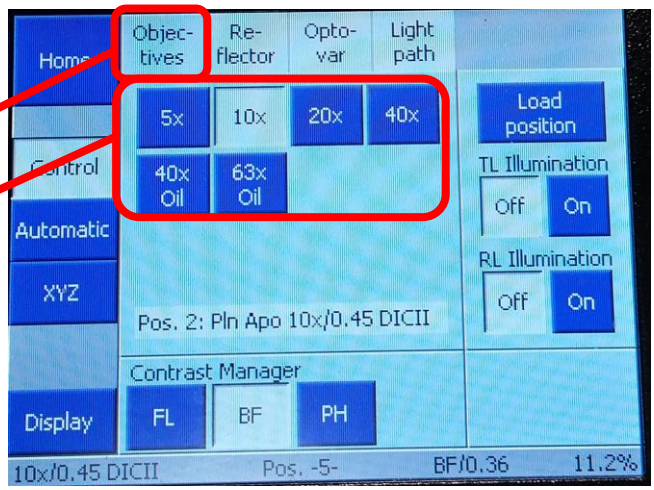
Additional magnification with the optovar.
Leave this on 1x, unless you plan to do deconvolution.



Touchscreen at start up.
Press:
Microscope

Go to Objectives tab

Choose objective



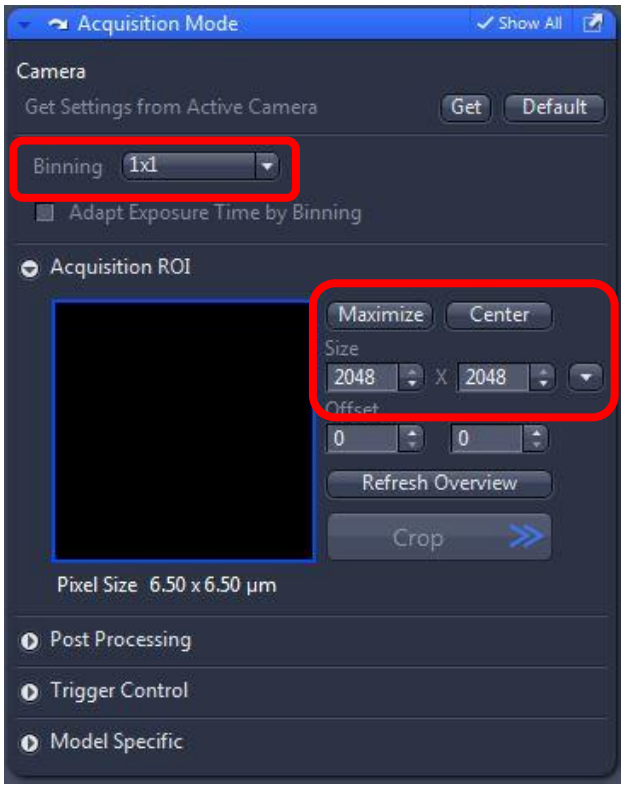
Acquisition tab for capturing images

Check the Acquisition mode, open the control window and check Binning is 1x1,
Acquisition ROI is 2048 x 2048, (Press Maximize).

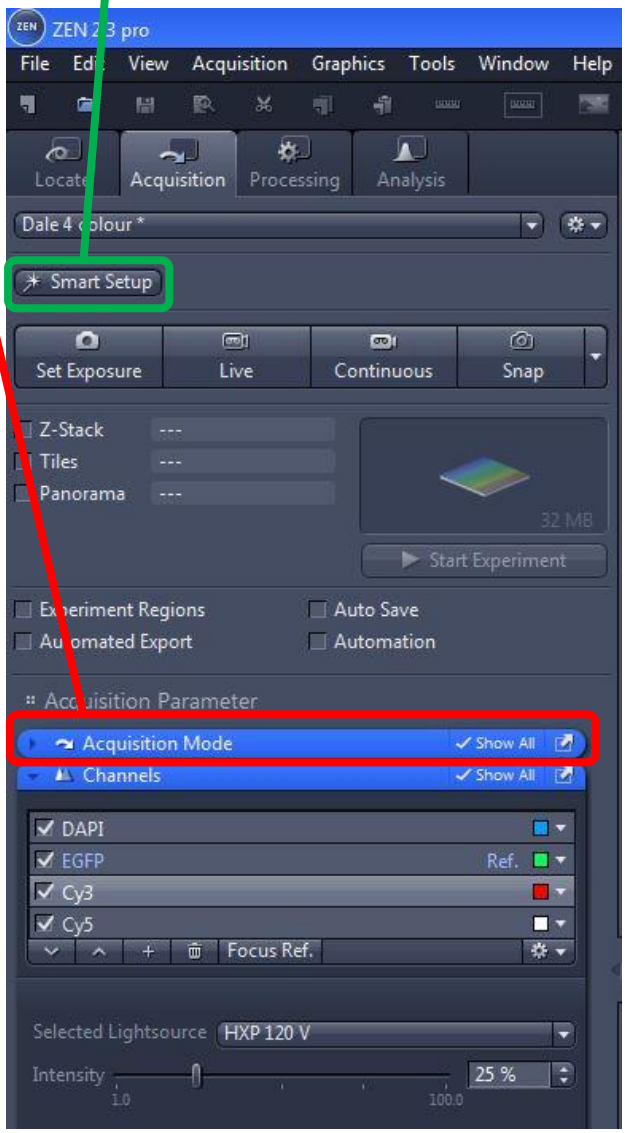
You only need to check this at the start of your session, to be sure it hasn't be changed by the previous user.

Binning at 2x2 (or more) increases sensitivity and speed for fast live imaging.

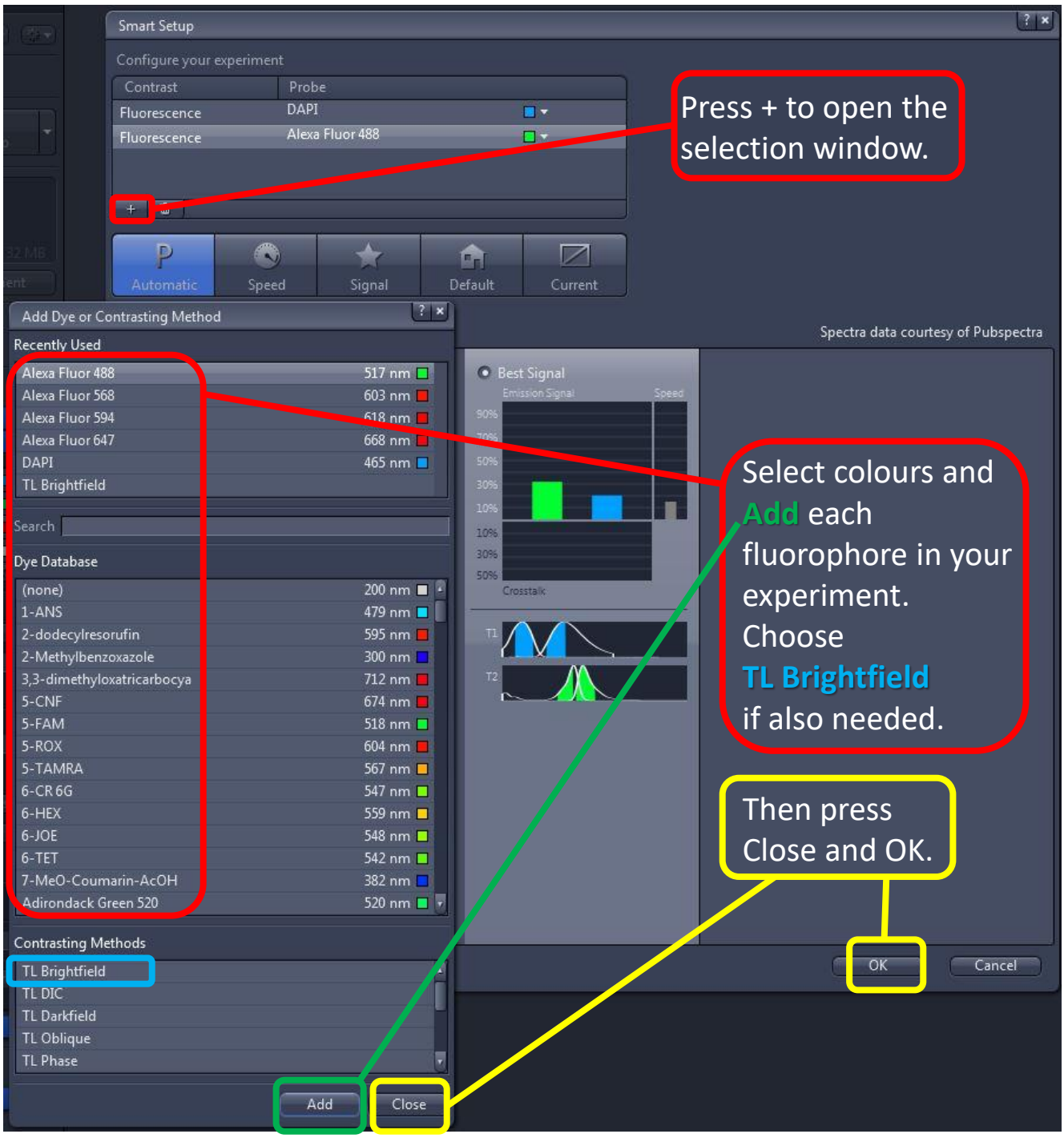
Reducing the acquisition ROI can help with producing an evenly illuminated tiled image.



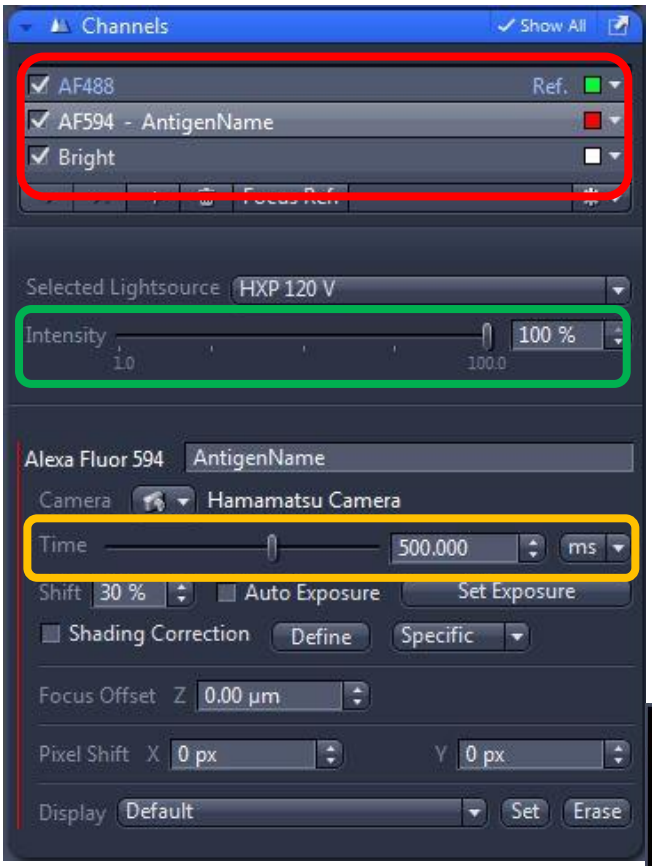
Set up the fluorescence channels (and Brightfield if used) by pressing Smart Setup.



Smart Setup – What colours are you imaging?



Channels set up – Adjust bulb intensity and exposure time for each channel. You can also give each channel a specific name.



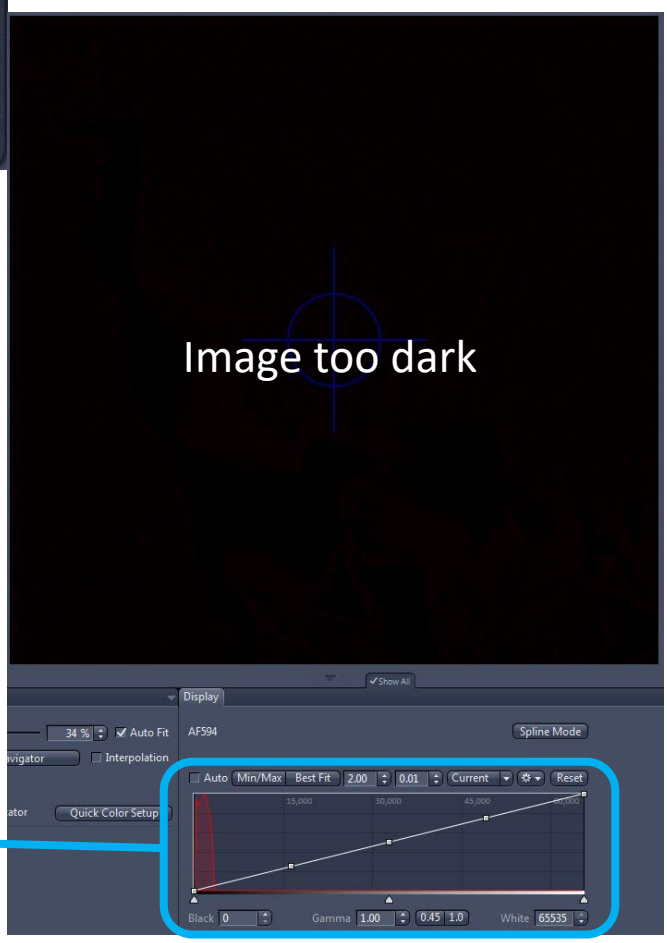
Channels. Highlight to adjust, or see it live, TICK to include in the captured image

Bulb power (Intensity). I typically use
<50% for Dapi,
> 50% for all other colours

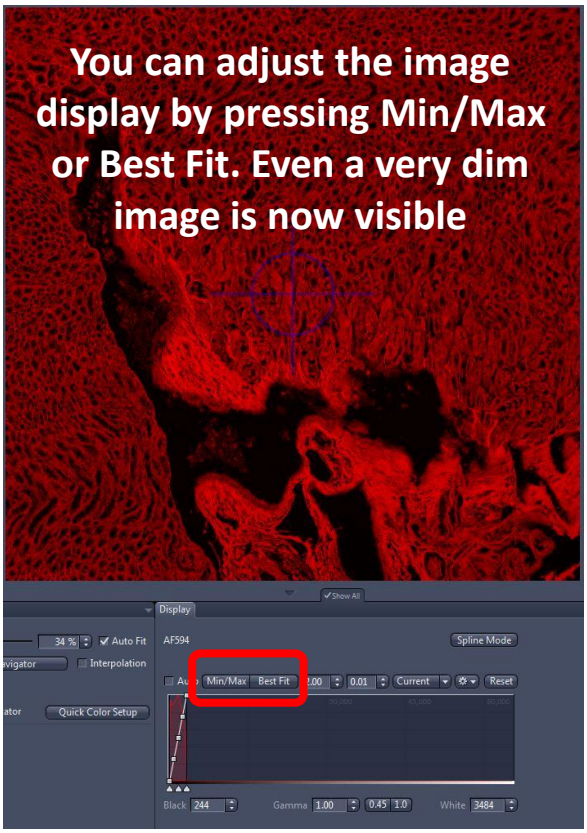
You can modify the channel name

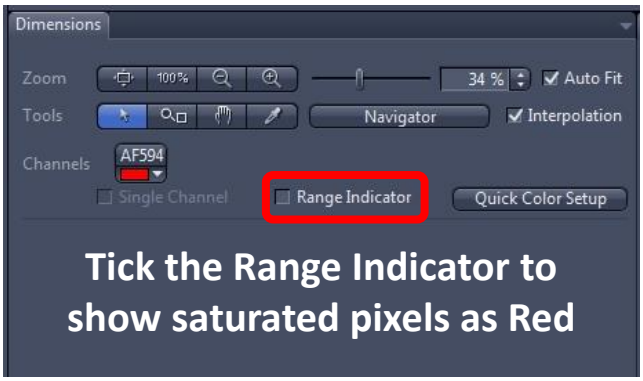
Exposure time. Longer = brighter

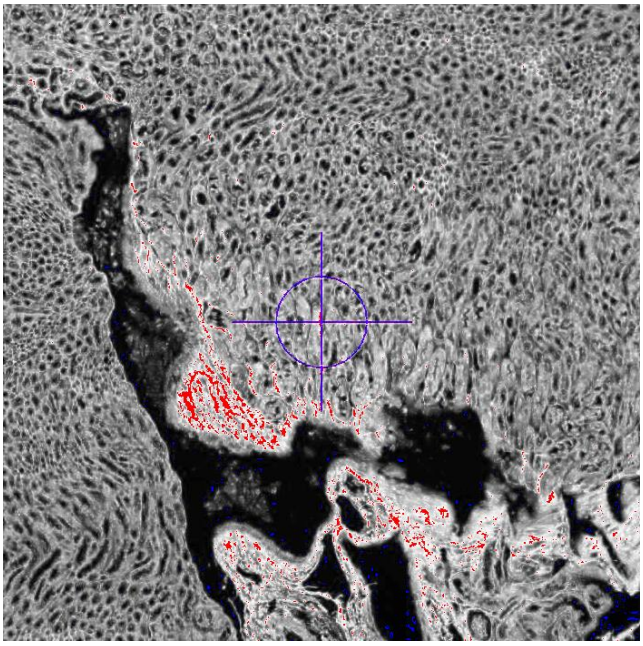
Typically the initial short (150 ms default) exposure time will be too dim. The image will look dark, and the histogram will be at the left edge (low values only). Increase bulb power and exposure time to make the image visible, without saturating any pixels…

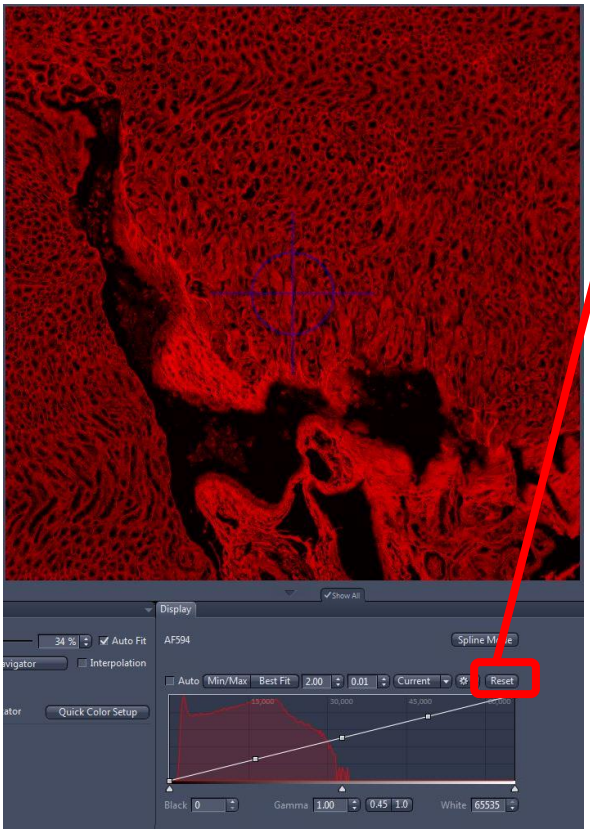


Channels set up – set exposures via Histogram and Range Indicator



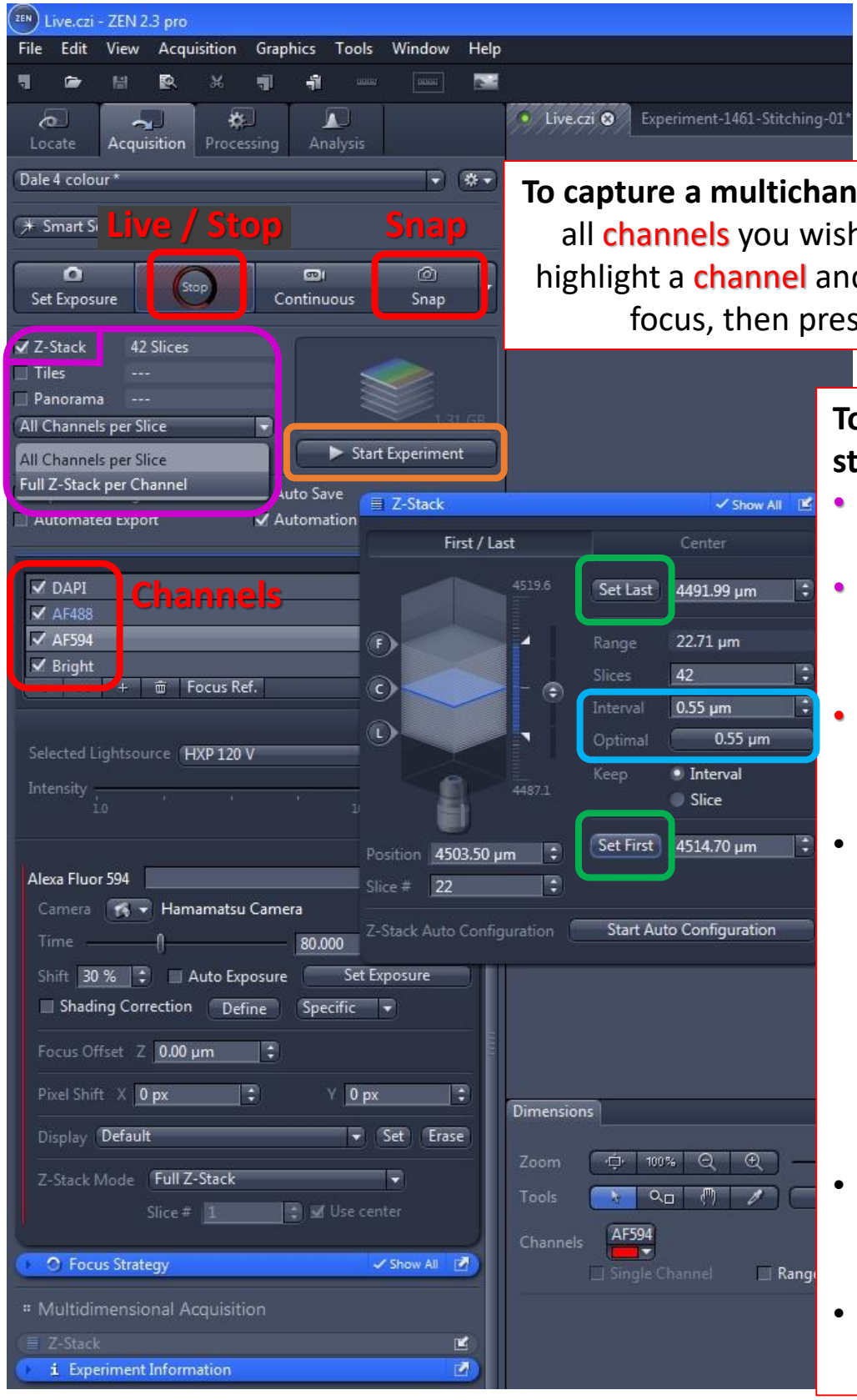






Reset the Display histogram, then adjust the bulb power and exposure time to approximately half fill the histogram, and avoid any saturated pixels.

Capture an image, or a Z-stack



To capture a multichannel image: tick all channels you wish to capture, highlight a channel and press Live to focus, then press Snap.

To capture a Z-stack:

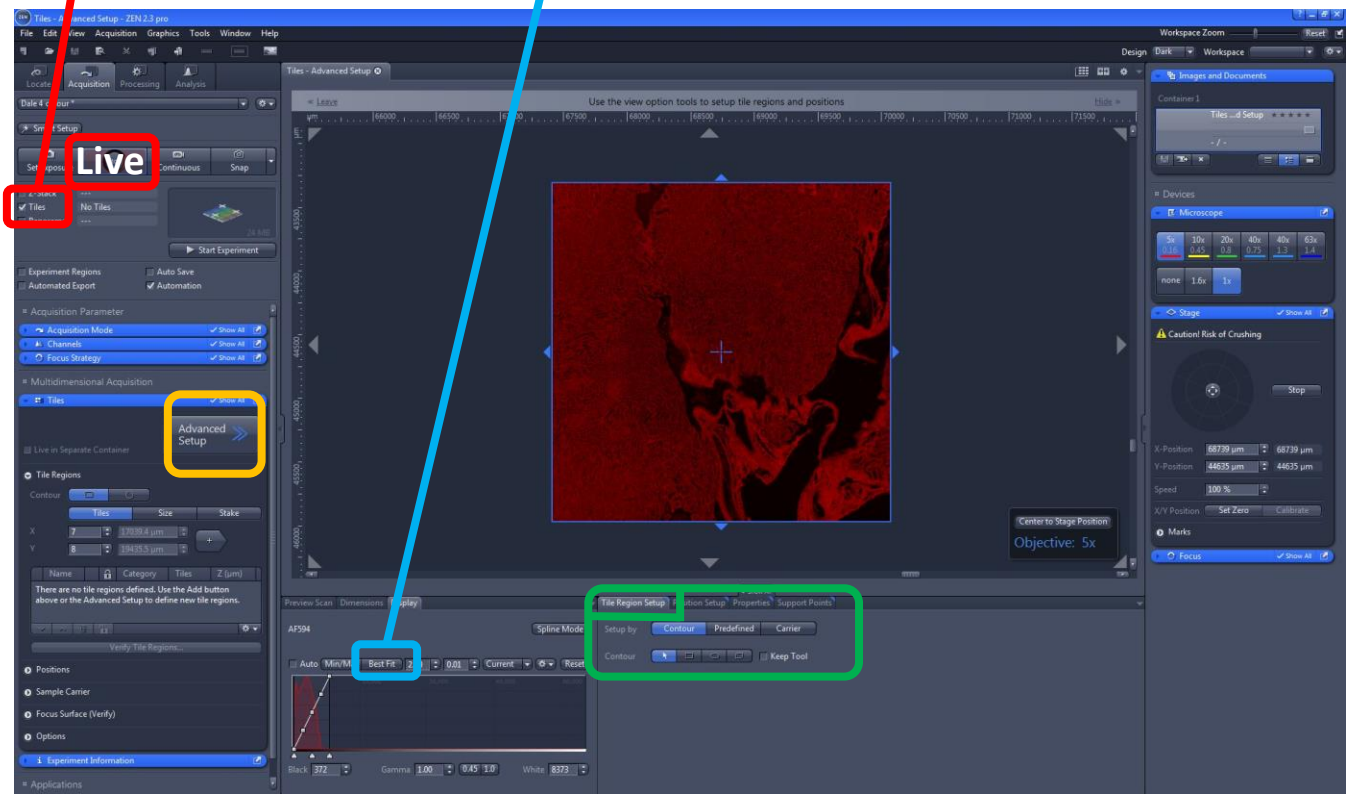
- Tick the Z-stack option
- Choose Full Z-stack per channel
- Highlight a channel and press LIVE
- Manually focus to the top and bottom of the stack to Set First and Set Last for the stack
- Make sure Interval = Optimal
- Press – Start Experiment

Tile scan

Using the Tiles option you can use a low mag objective (5x or 10x) to quickly scan the slide and draw a region that you would like to image with any objective. Once you've drawn your region (or regions) at low mag, switch to your imaging objective, and make a focus map using support points. Then select all the channels you'd like to image (and also do a z-stack if you wish), and start the experiment.

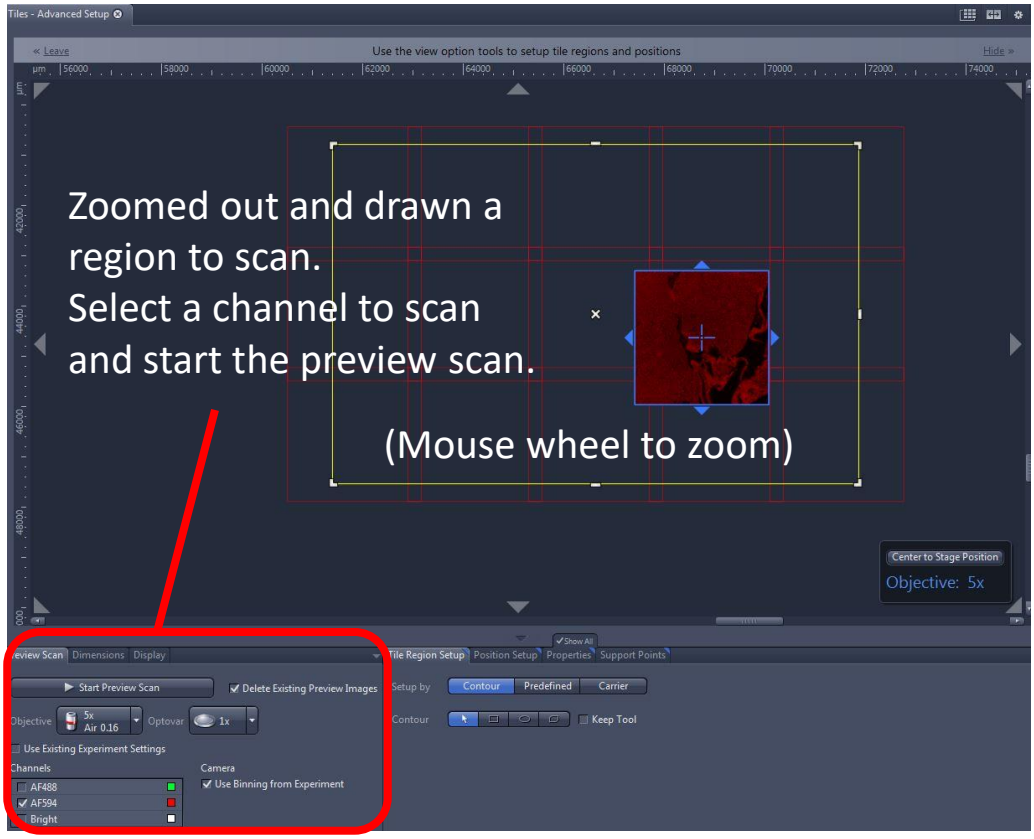
Select the Tiles option
Press Advanced Set up

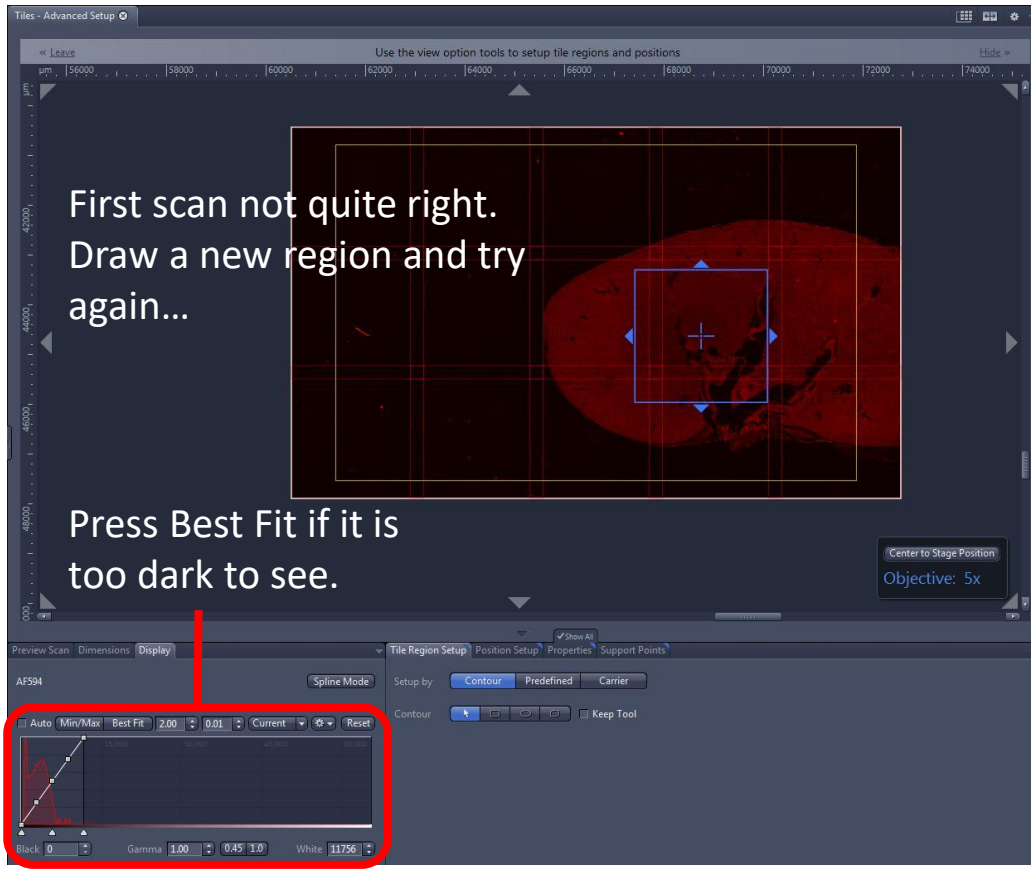
Scan at a low mag
(Best Fit on histogram so it is bright enough)



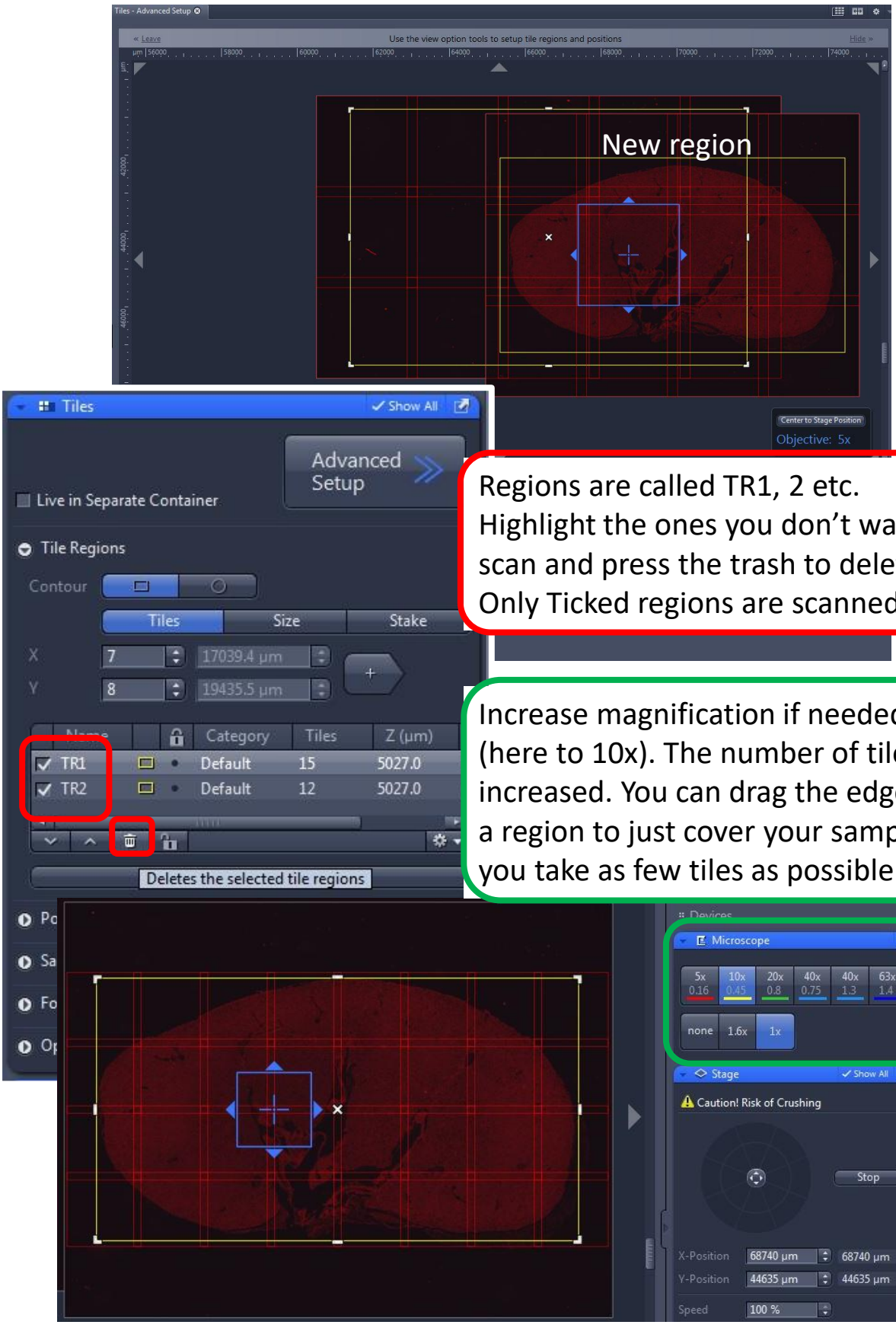
Press Live and zoom out the view (wheel on mouse).
Use the tools in Tile Region Set up to draw your first guess at the ROI...
Continued next page…

Tile scan (2)


Tiles - Advanced Setup
Use the view option tools to setup tile regions and positions
Zoomed out and drawn a region to scan.
Select a channel to scan and start the preview scan.
(Mouse wheel to zoom)
Center to Stage Position
Objective: 5x
Start Preview Scan
Delete Existing Preview Images
Use Existing Experiment Settings
Use Binning from Experiment
Channels
AF488
AF594
Bright
Camera
Tile Region Setup
Position Setup
Properties
Support Points
Setup by
Contour
Predefined
Carrier
Keep Tool


Tiles - Advanced Setup
Use the view option tools to setup tile regions and positions
First scan not quite right.
Draw a new region and try again…
Press Best Fit if it is too dark to see.
Center to Stage Position
Objective: 5x
AF594
Spline Mode
Auto
Min/Max
Best Fit
Current
Reset
Black
Gamma
White
Tile Region Setup
Position Setup
Properties
Support Points
Setup by
Contour
Predefined
Carrier
Keep Tool

Tile scan (3)

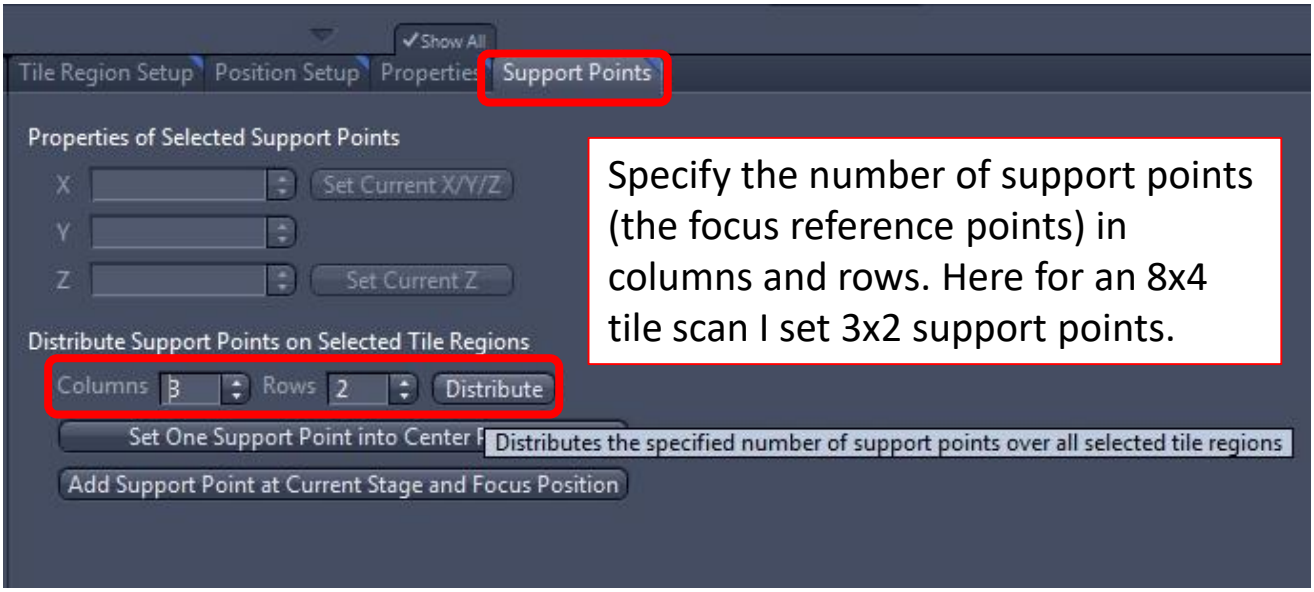


Regions are called TR1, 2 etc. Highlight the ones you don't want to scan and press the trash to delete them. Only Ticked regions are scanned.

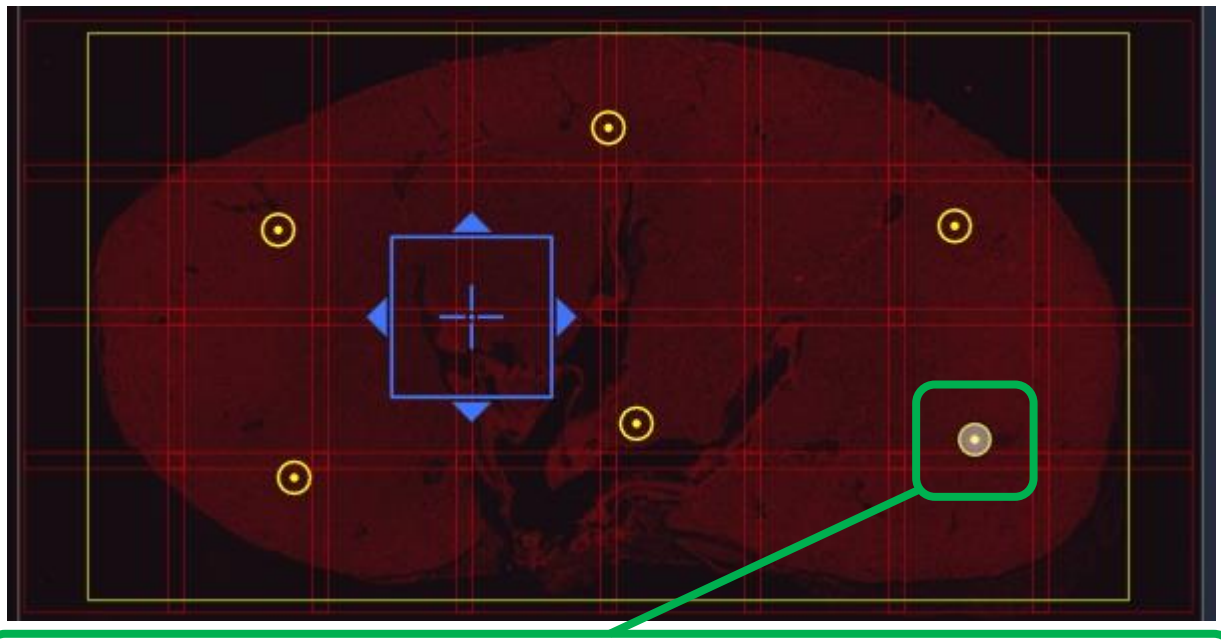
Increase magnification if needed (here to 10x). The number of tiles is increased. You can drag the edges of a region to just cover your sample. So you take as few tiles as possible.

Tile scan (4)…make a focus map – set support points

First add support points, choose the Support Points tab below you image



Specify the number of support points (the focus reference points) in columns and rows. Here for an 8x4 tile scan I set 3x2 support points.



The points are initially aligned as a grid, you can select each point with the mouse and drag them anywhere you like

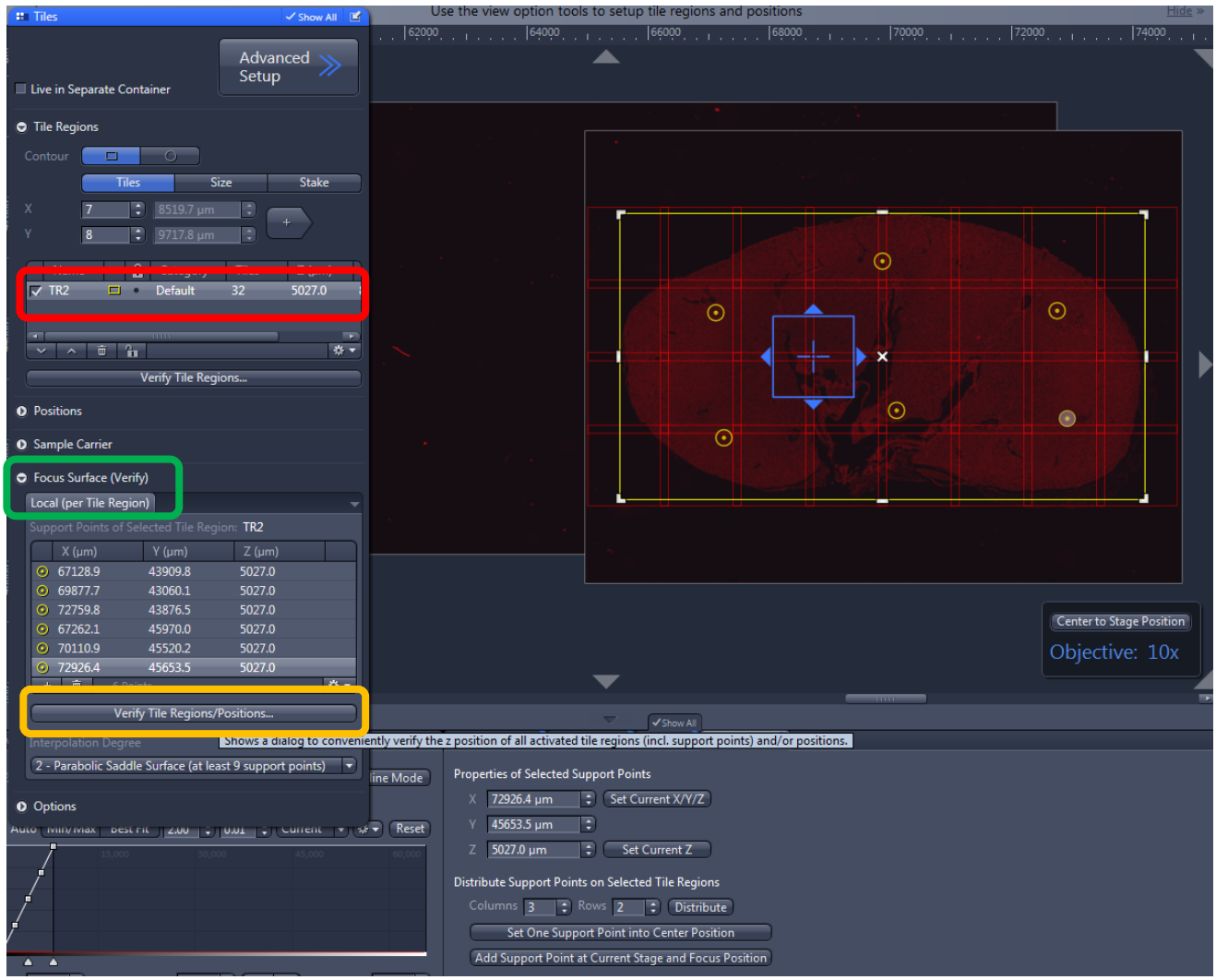
Tile scan (5)…make a focus map – verify support points

In the Tiles Advanced Setup window:

Make sure your Tile region (TR) is ticked.

Select: Focus Surface (Verify)

Press Verify Tile Regions/Positions…



Tile scan (6)…make a focus map – adjust focus

A new window opens: Verify Tile Regions / positions…

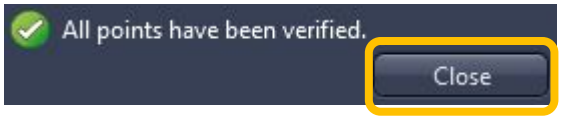
Click Move to Current Point

Press Live to see the desired channel, adjust the focus, then

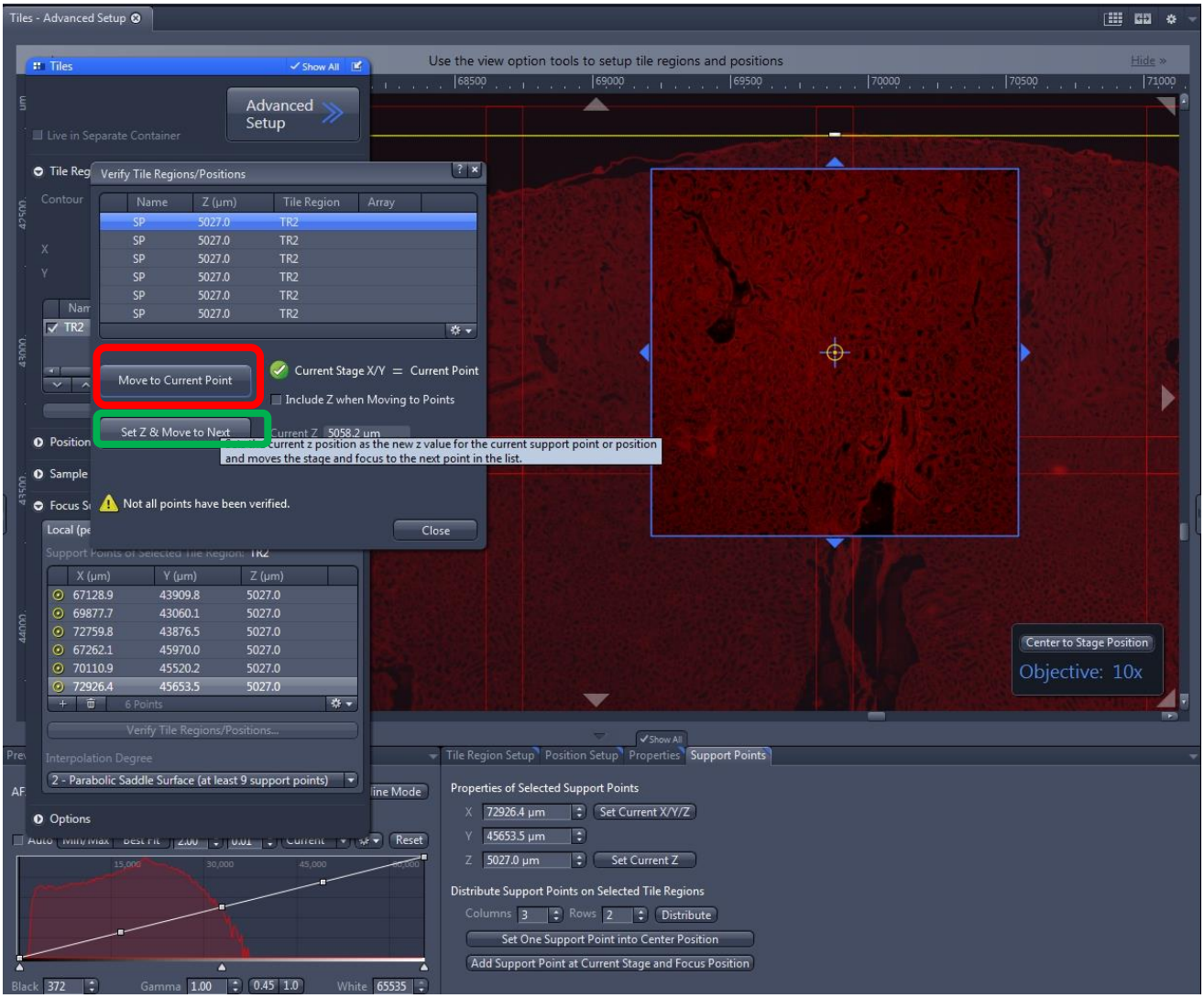
Press: Set Z & Move to Next It moves to the next support point.

Keep doing this and setting the focus for each point,

until the window changes to:



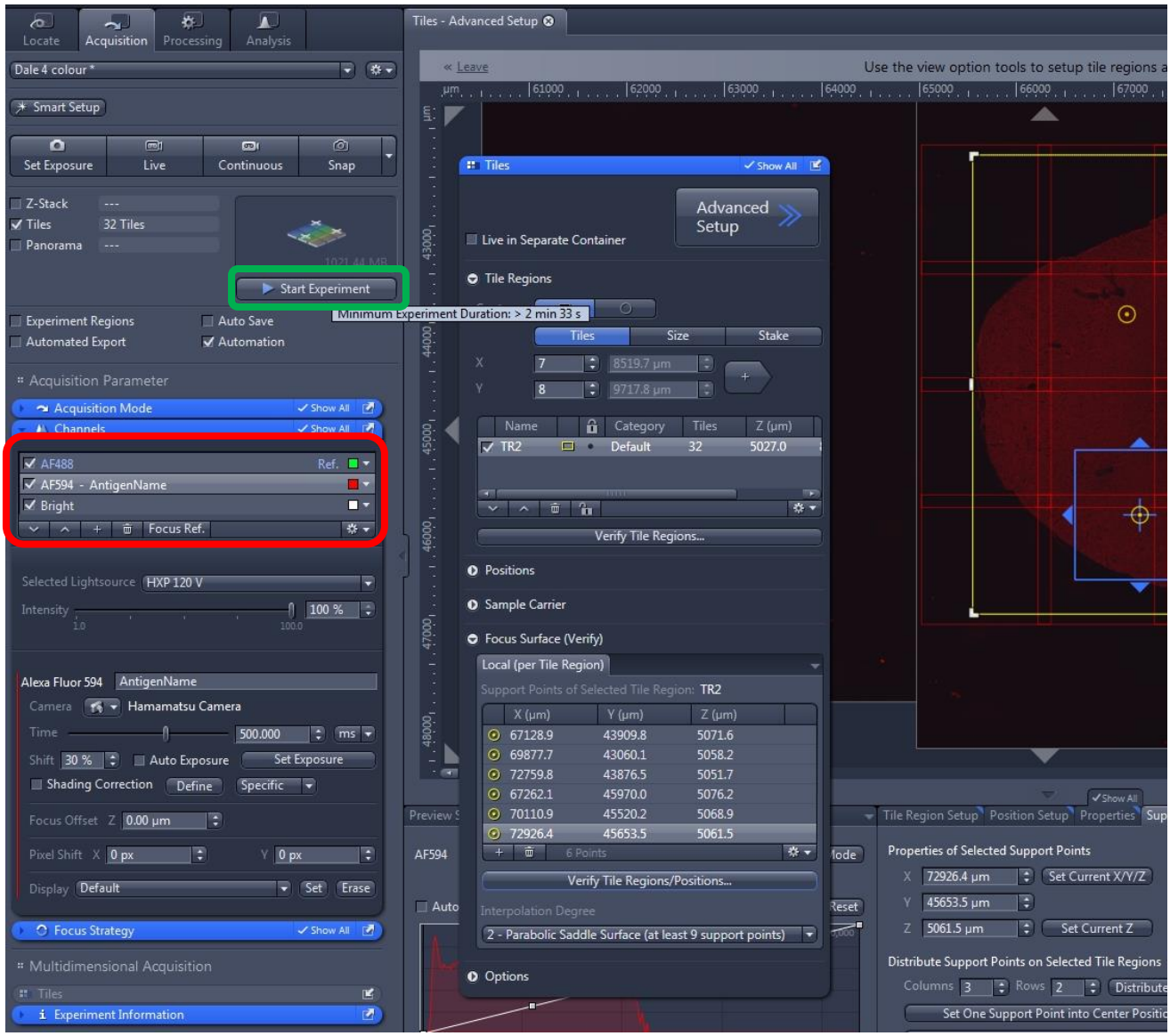
Finally close the window.



Tile scan (7)…Capture the image

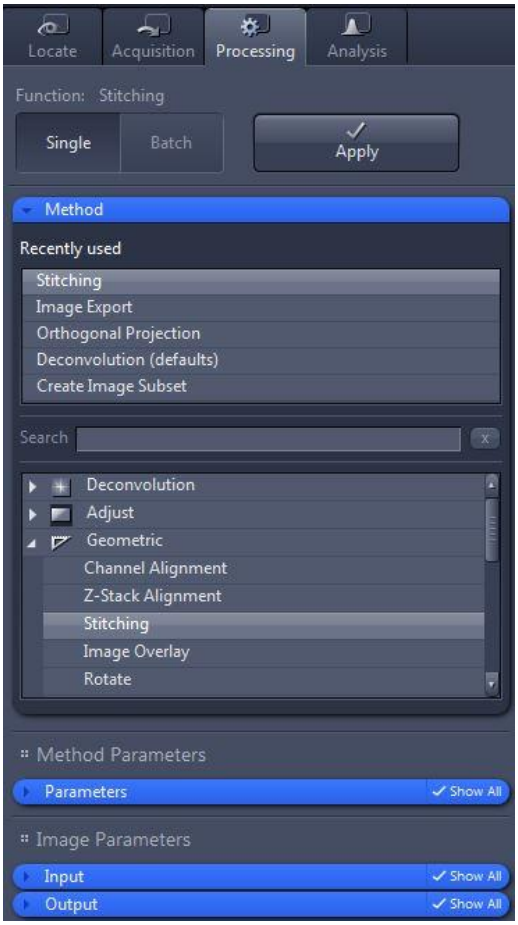
Make sure your Channels are ticked.

Press: Start Experiment

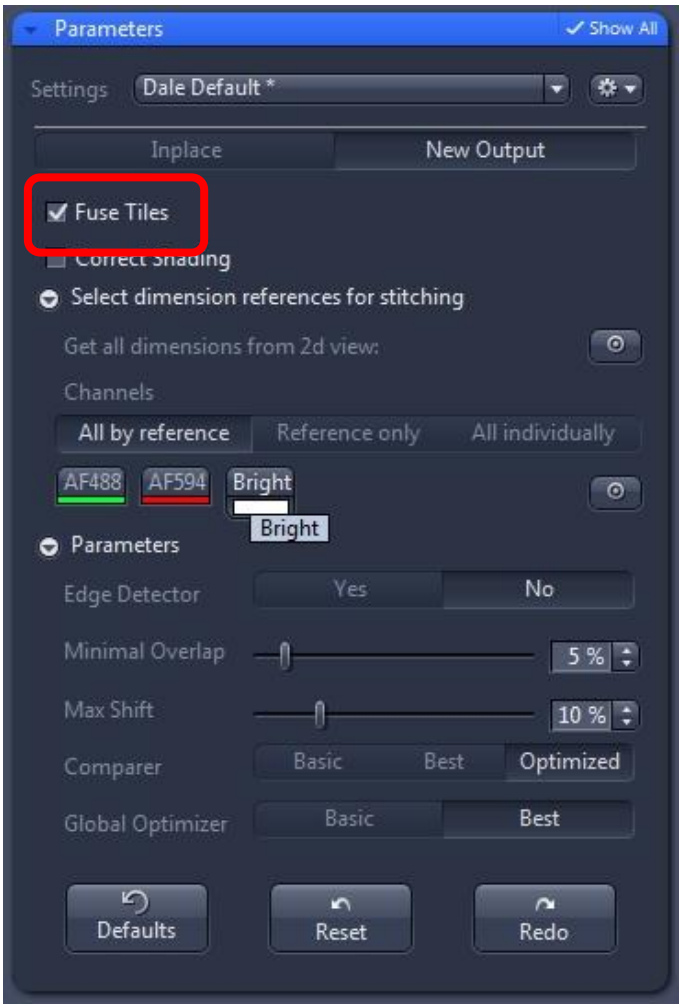


Tile scan (8)…Stitching the image

Go to the processing tab, choose the method: Stitching



Open up the Parameters window, select the Settings “Dale Default”. This sets the output to have a single fused image, in a New Output window.



By default it will use the first channel captured to align the image, (normally Dapi) but you can choose any channel that has sufficient content to perform the stitching.

Tile scan (9)…Stitched Image