Nikon Spinning Disk Inverted Confocal Microscope

User Guide

KING'S College LONDON

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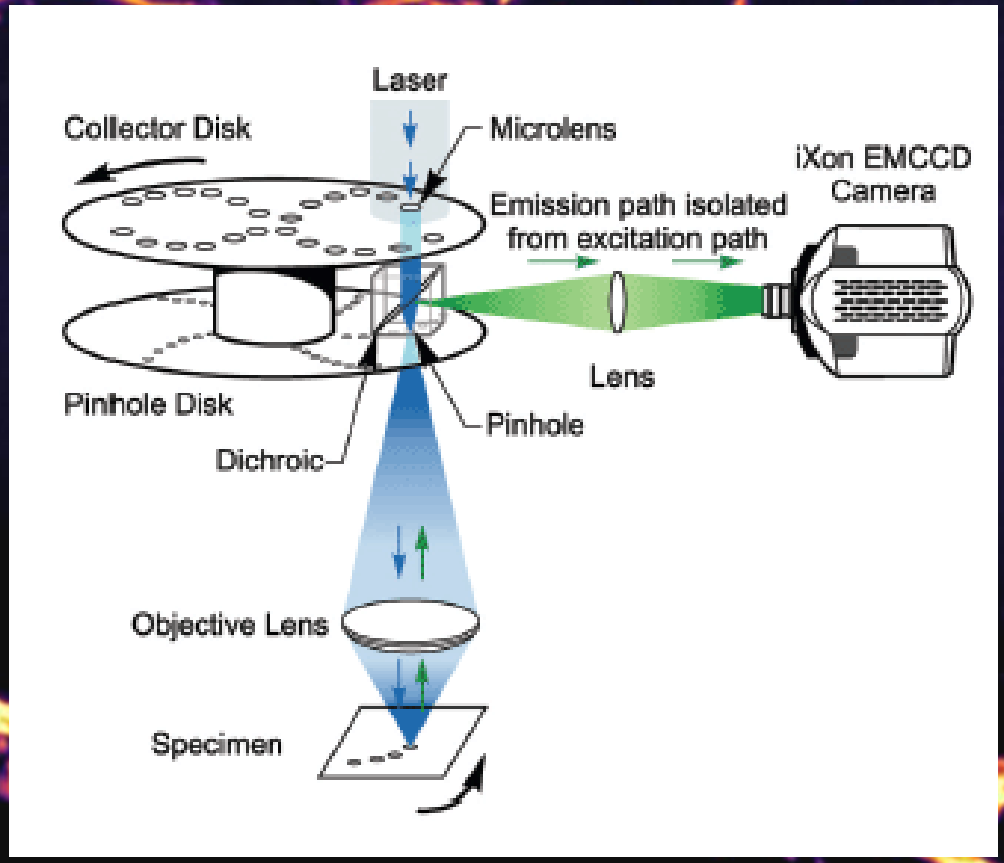
Introduction

Spinning disk microscopes use a Nipkow disk covered in pinholes arranged in a spiral pattern to simultaneously illuminate the entire field of view, making it much faster and gentler on live cells than a point-scanning confocal

Key advantages:

1. Fast acquisition – full sample illumination makes imaging much faster than point-scanning, ideal for imaging dynamic live-cell processes
2. Reduced photobleaching – faster imaging and reduced dwell time compared to point-scanning means reduced photobleaching of samples
3. iXon EMCCD camera is more sensitive than the PMTs used in other microscopes – this combined with reduced bleaching makes the spinning disk ideally suited to imaging dim, sensitive samples

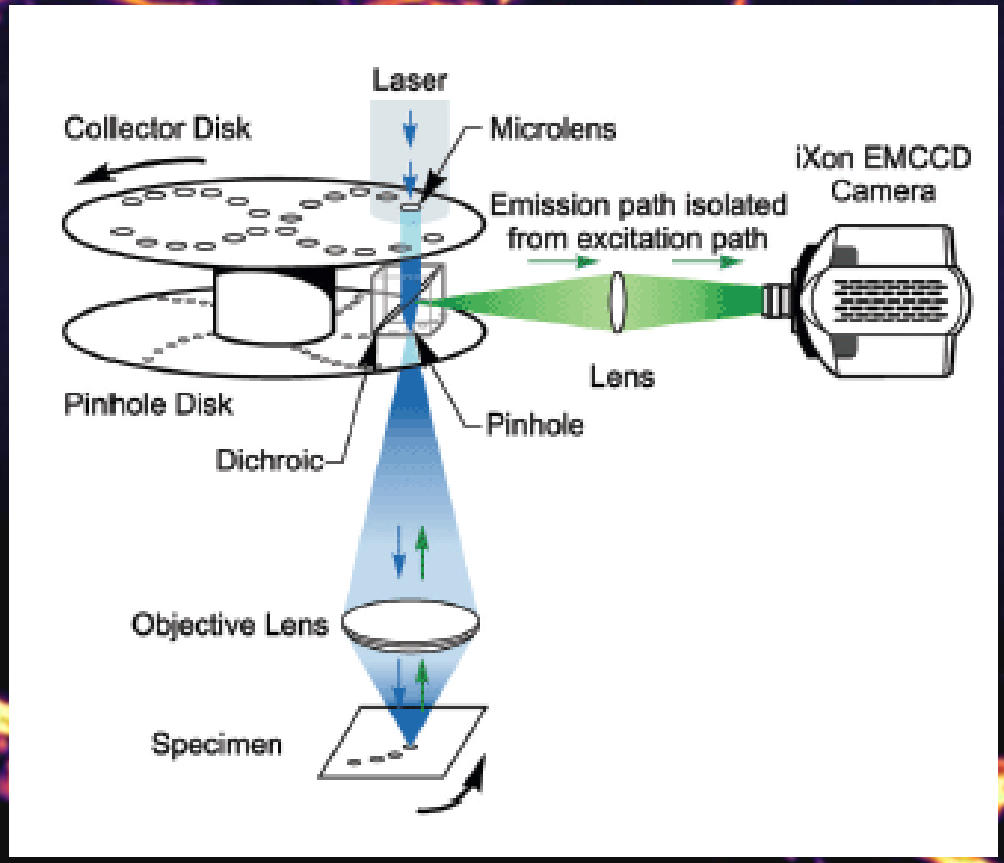


Key Limitations

Be careful:

With 10x and 20x lenses, the spinning disk is closer to a widefield microscope than a confocal.

Pinhole size is critical for confocal efficiency – the smaller the pinhole, the better the optical sectioning and therefore the higher the Z resolution. Because the pinholes in the spinning disk are a fixed size, the objective lenses determine confocal efficacy, with higher magnification lenses correlating to better Z resolution



Sample

Clean and load your sample, coverslip side always face towards the lens, the labelling area preferably towards the right - hand side, push it into a corner and clip it down.

Make sure to mount your sample 5mm away from the edge of the slide! You cannot image too close to the edge of the slide.

Only move the joystick while looking down the eyepiece and you can see your sample clearly in FOCUS!

STEP BY STEP INSTRUCTIONS

The remainder of this guide will provide step-by-step instructions for starting and operating the microscope. This guide may be a helpful reminder of what you were showed in training, but is not a substitute for completing your two training sessions

Step 1 – System On

To turn on the microscope, follow the numbered switches in order.

1. UPS back up power – quick press and release, wait 2 seconds to boot up. Turn on number 2 within 10 seconds

2. Microscope body – check no sample has been left on the stage and turn on the switch on the right side of the microscope, towards the back

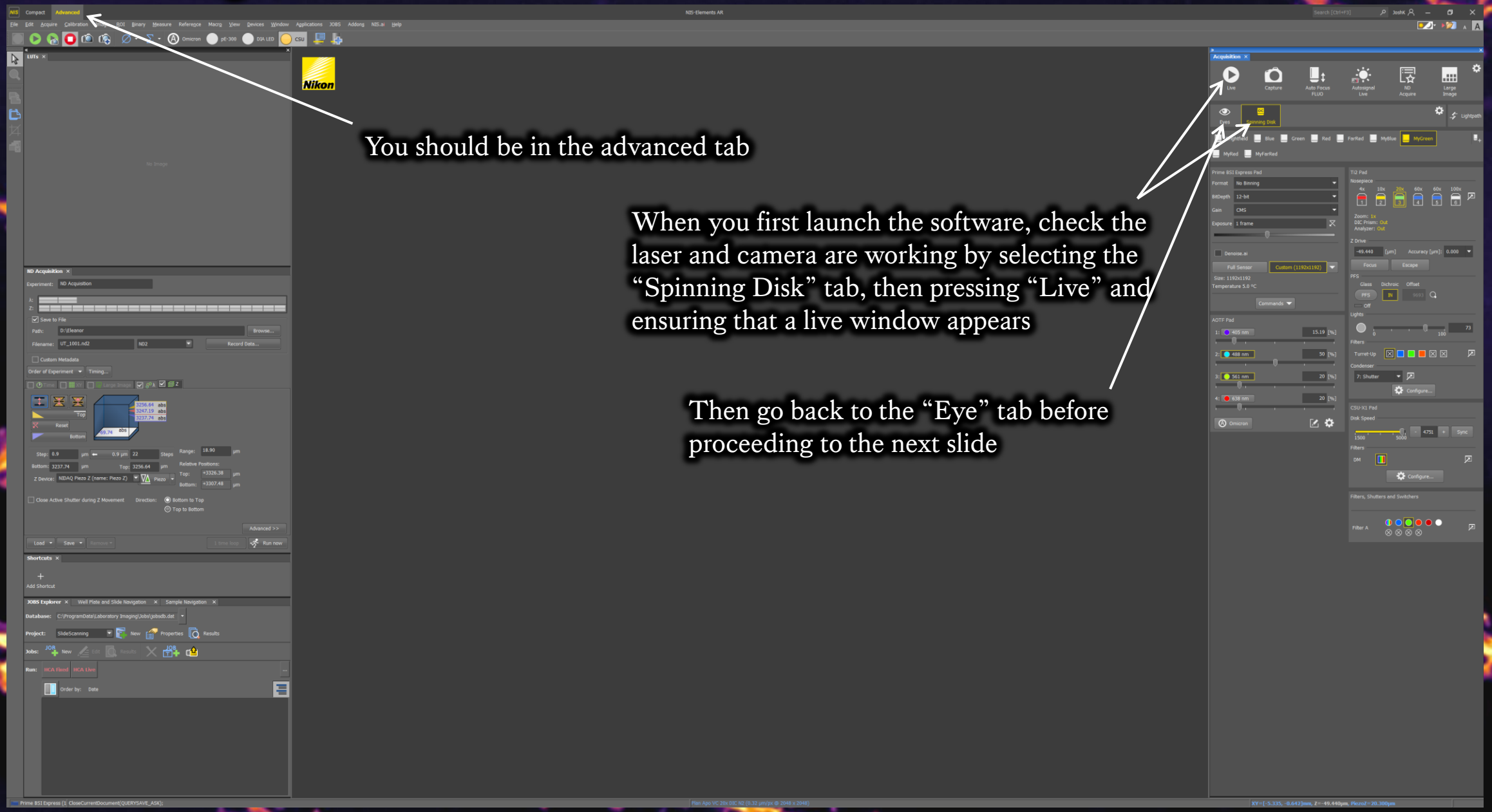
3. Button on the laser unit (to the right of the microscope table)

4. Key on the same unit as 3

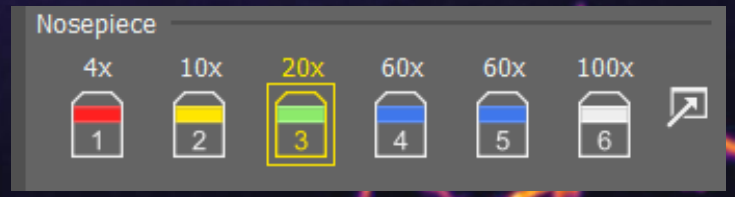
5. Key to the left of the microscope body

6. Camera – switch on the right side of the camera unit

Software Layout



Changing Lenses



ALWAYS lower the lenses as far as they can go before inserting the stage and/or clicking on another lens

If the display is not showing the current Z position, use the arrows on this button to navigate between different display modes

To lower the lens, turn this wheel clockwise (away from you) until the Z number on the display stops changing.

Once the lens is lowered to the bottom, you can select a different lens in the NIS-Elements software

Cleaning Oil Lenses

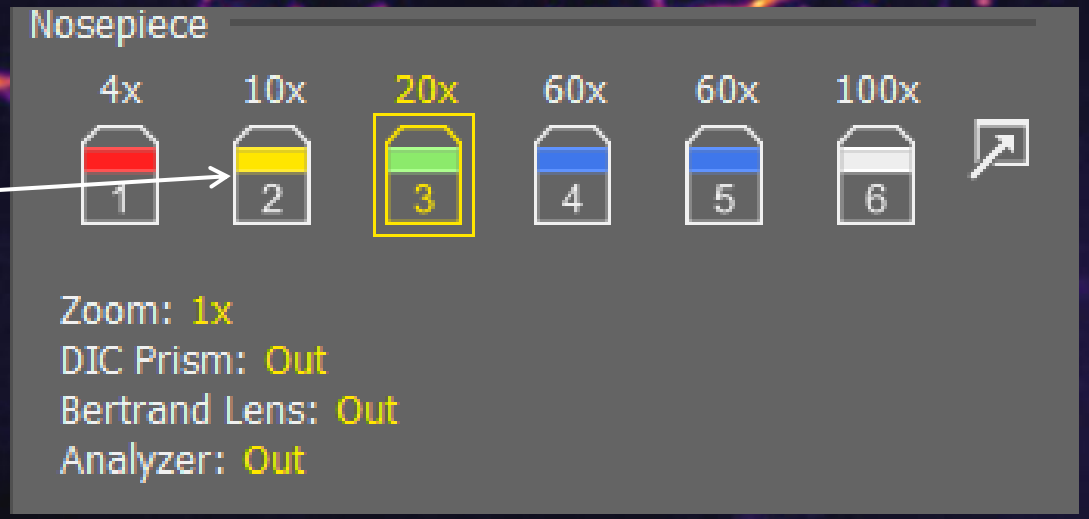
1. Wipe away any excess oil with dry lens tissue (normally after your session)
2. Wrap lens tissue around your finger and soak up some ethanol, then clean the lens from the centre outwards
3. Repeat previous step 3 times
4. Clean once more with dry lens tissue
5. During your session if you are switching between dry and oil lenses, just wipe away excess oil on the lens and slide and lower your objectives before switching. Clean the oil lens properly at the end of your session.

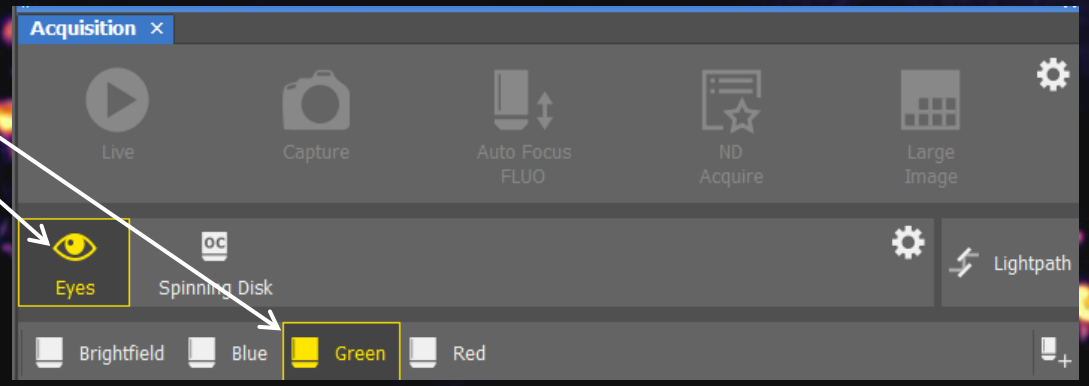
THIS is lens tissue:

THIS is not:

Focusing the Microscope

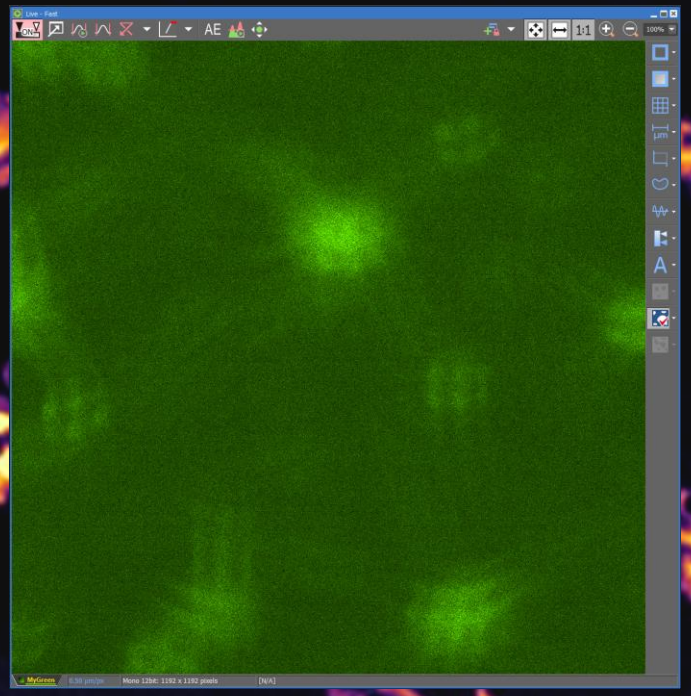
- Select one of the air lenses – 4x, 10x or 20x. We recommend using the 10x for first finding focus
- Select “eyes”
- Select an OC that matches a fluorescence that you know will be visible on your sample
- Whilst looking down the eyepiece, raise the lens until your sample is visible and in focus. You should see the background fluorescence get gradually brighter as you get closer to your focal plane

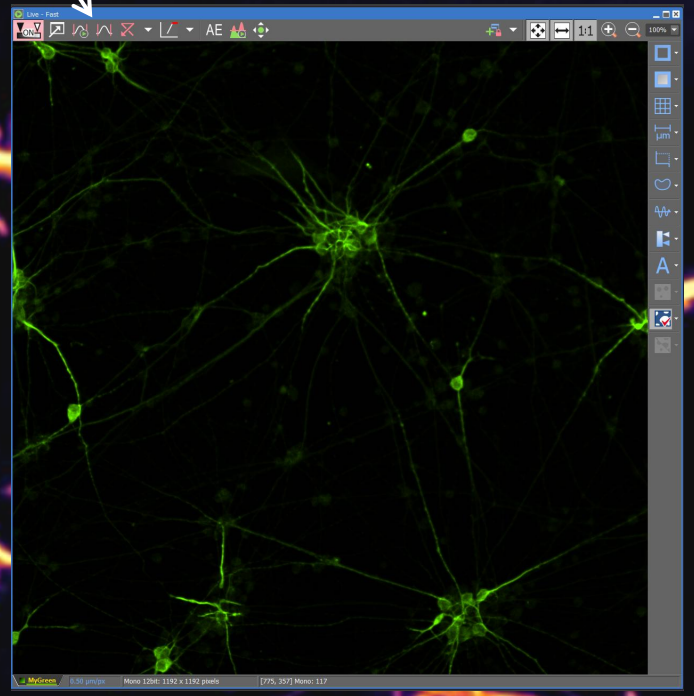


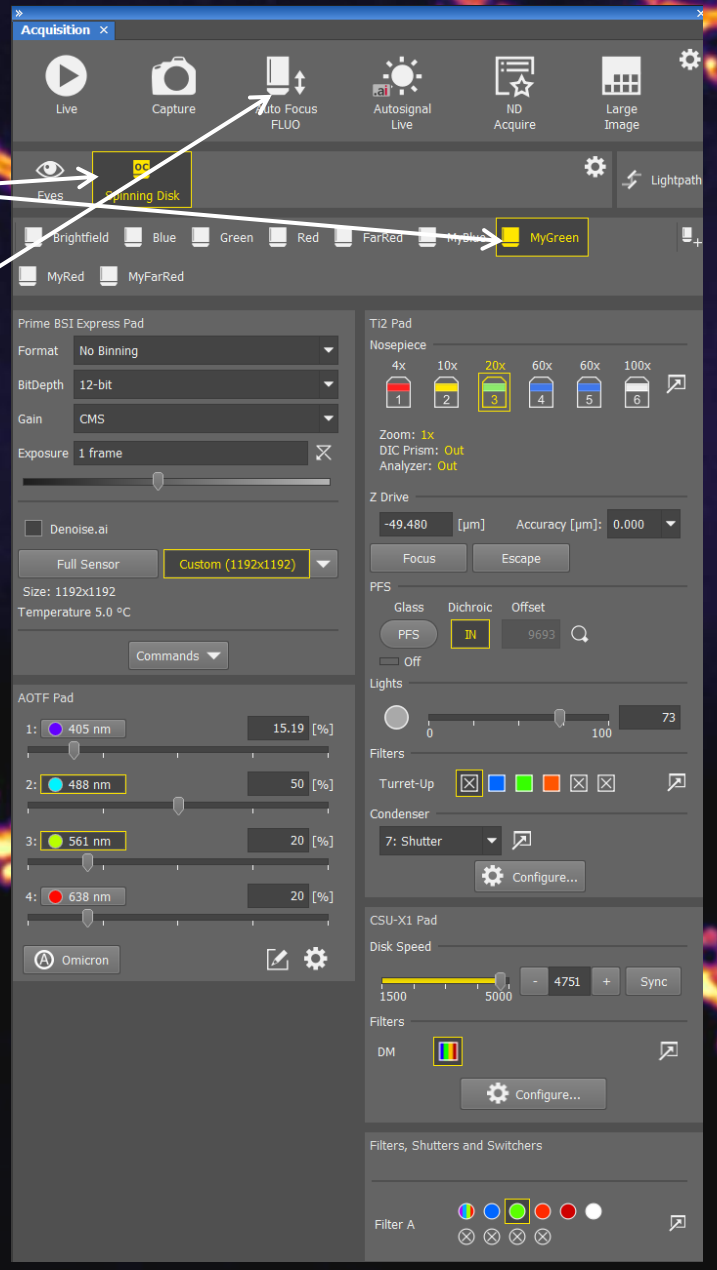


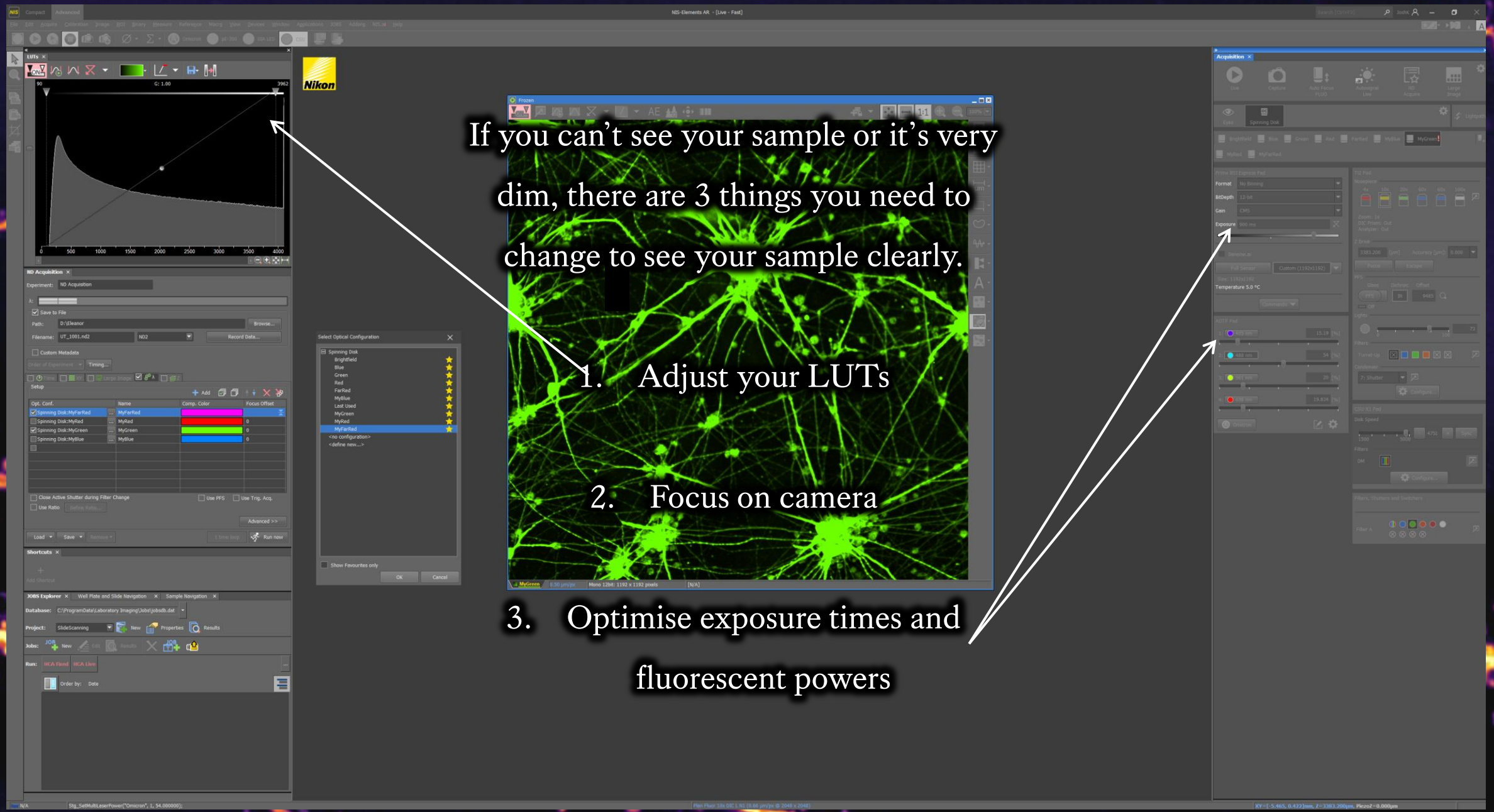
Switching to Live View

- Switch to “Spinning Disk” mode and select an OC corresponding to the same colour fluorescence that you used to find focus down the eyepiece
- On this microscope there is an offset of ~100µm between eyepiece focus and camera focus. You can adjust your focus by hovering over your live window and scrolling the mouse wheel. You can also use the “Auto Focus” button after switching to camera view to bring your sample back into focus
- Adjust contrast by using the auto-contrast button in your live window








If you can't see your sample or it's very dim, there are 3 things you need to change to see your sample clearly.
1. Adjust your LUTs
2. Focus on camera
3. Optimise exposure times and fluorescent powers

Each Button acts as a preset for Spinning Disk Camera

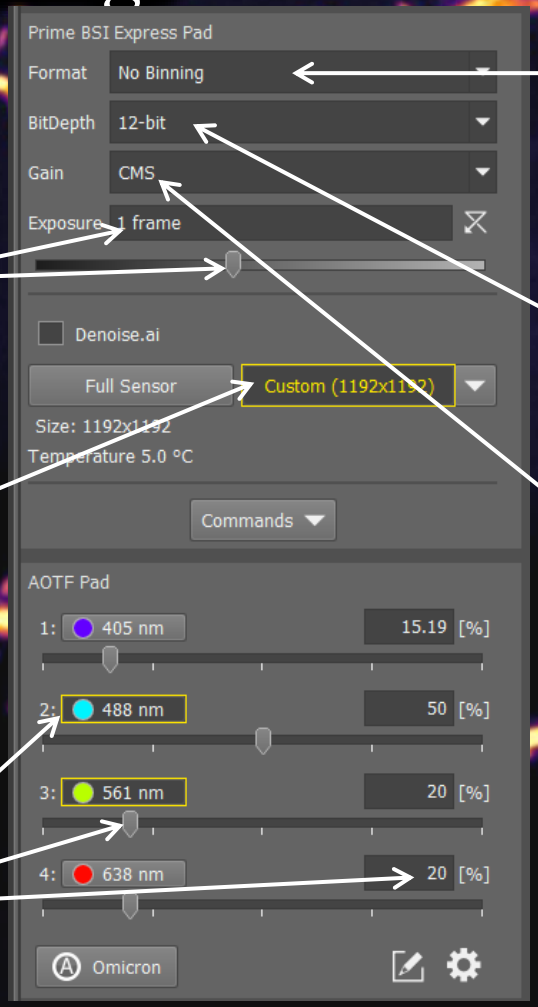
Each OC acts as a preset for camera and laser settings, allowing you to store and switch between multiple optical configurations

This slider controls the exposure time of the camera – increase to boost signal

This tab controls the area of the camera sensor being used – it is best not to use the full sensor, as the image quality is considerably reduced at the edges of the sensor

The laser panel allows you to turn individual lasers on and off by clicking the button, as well as using the slider or text box to control laser power

Settings



Binning controls how many adjacent pixels are averaged together – low for best resolution, high for boosting weak signal

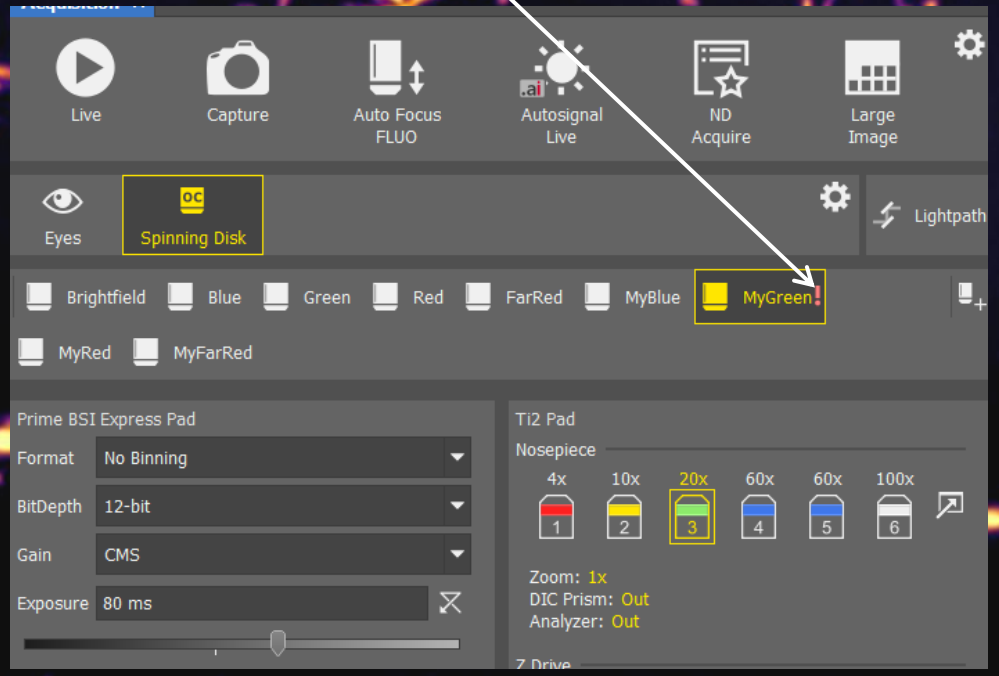
Bit-depth controls the way colour intensities are encoded by the camera. For most users, this can be left on 12-bit unless told otherwise by a technician

Gain settings can also be left on CMS unless told otherwise

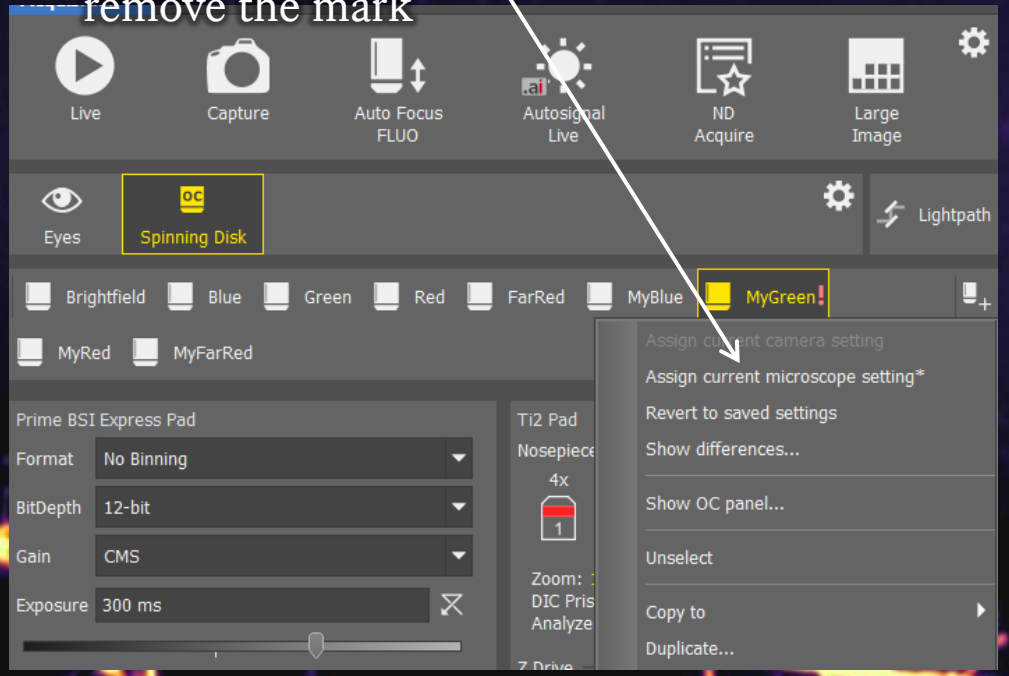
Filter settings have already been optimised for the colour in the OC name

Red exclamation mark

When you change the settings within an OC, this red exclamation mark will appear on the OC button



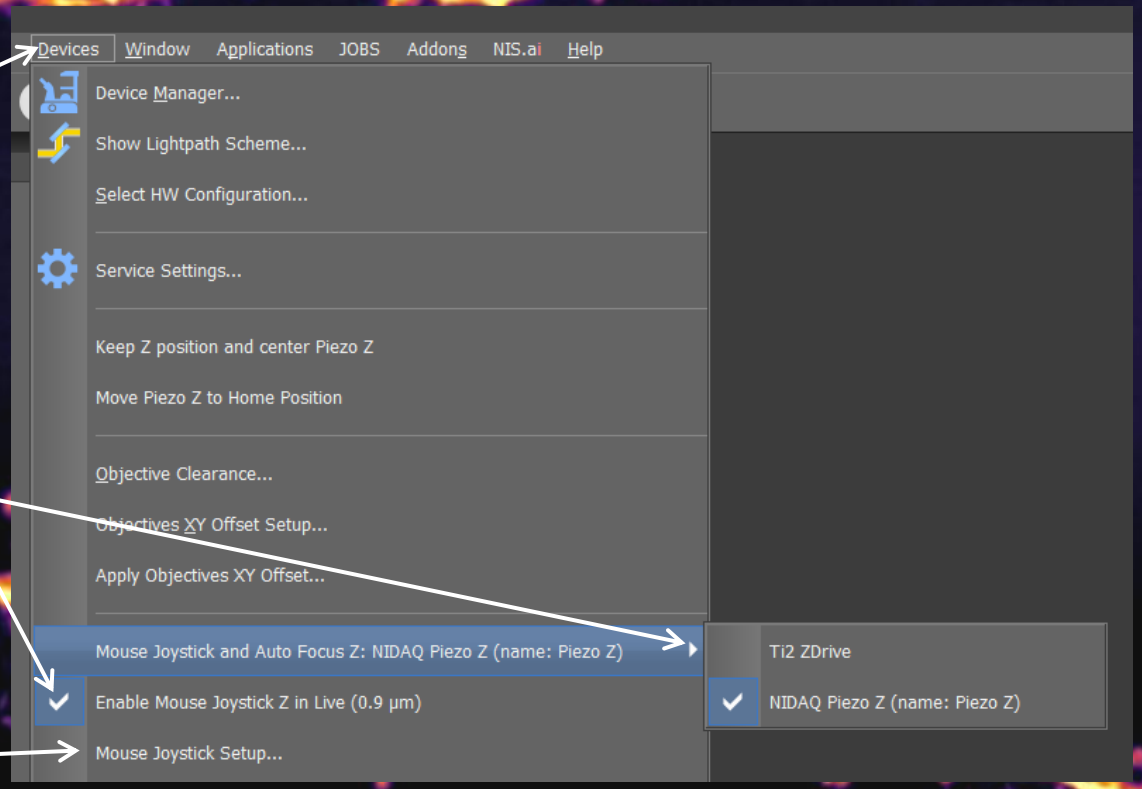
Right click on the button and select "Assign current camera/microscope setting" to save your edited OC and remove the mark



Using the Mouse Wheel in Live View

To ensure the mouse wheel is properly set up to control focus in live view:

- Go to the “Devices” menu in the menu bar at the top of the screen
- Ensure “Enable Mouse Joystick Z in Live” is ticked
- In the joystick menu highlighted in the image, select Ti2 ZDrive for control over a long range of Z values, or Piezo Z for finer control across a shorter distance
- In ”Mouse Joystick Setup…” you can change the distance by which the focus will shift for each partial rotation of the mouse wheel

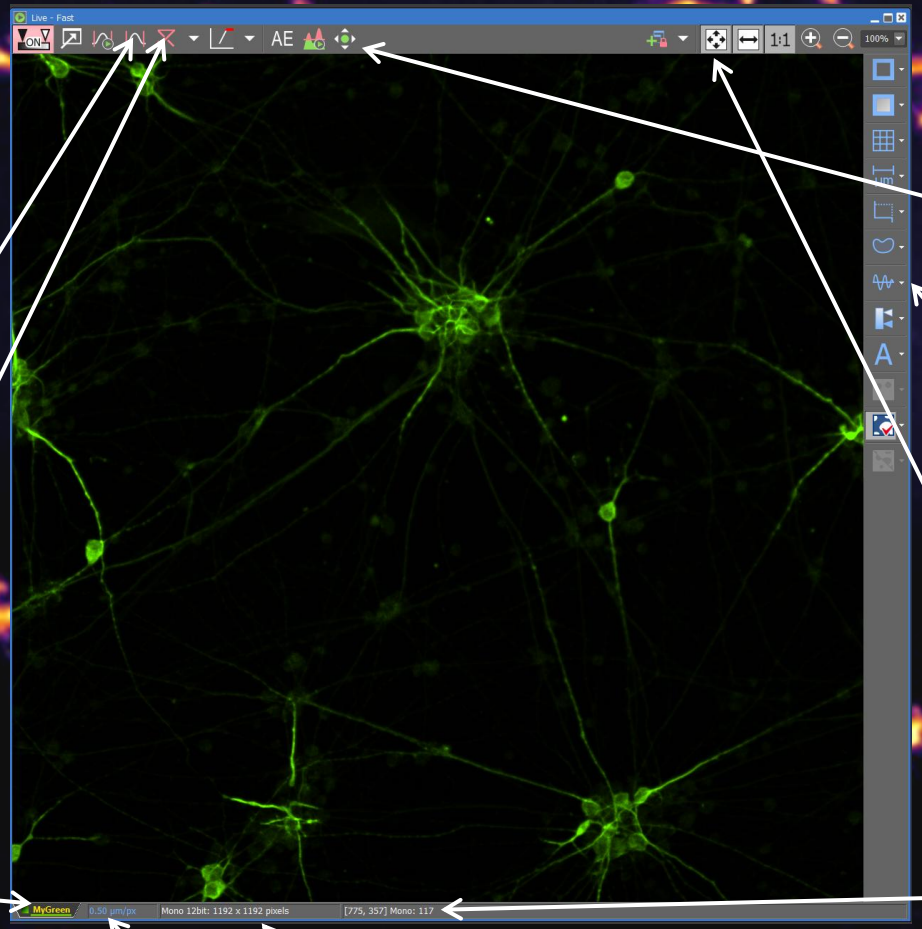


Using Live View

When you press “Live” this window will appear, giving you a live view of your slide using your current settings and channel selections

This button will adjust your contrast settings to allow you to see your sample better – this does not change your intensity data

Clears all contrast adjustments



You can toggle between available channels here – in this example I only had one channel enabled

This number shows the xy dimensions on your sample that each individual pixel in the image corresponds to. Adjacent number shows total image size in pixels

Whilst in Live mode, you can use your mouse wheel to adjust microscope focus

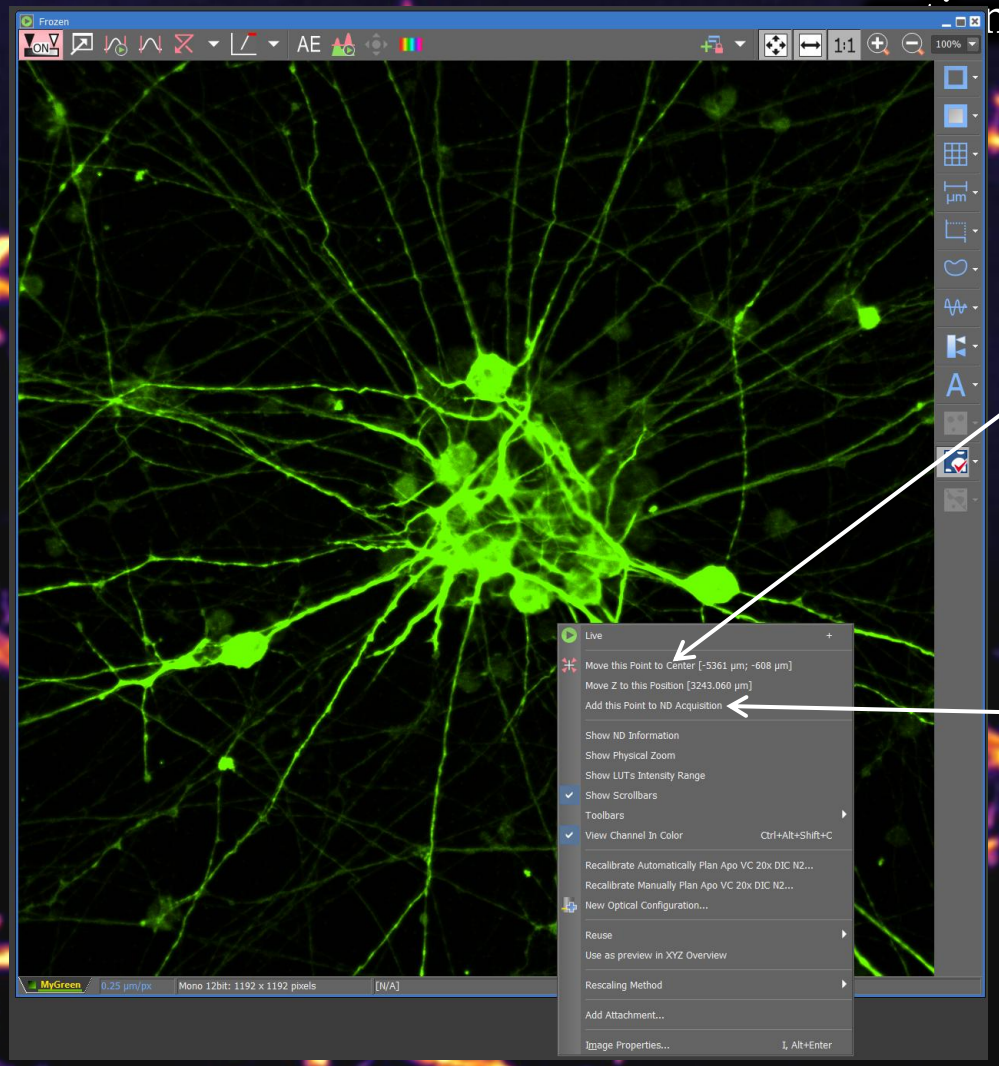
When selected, this allows you to click and drag your image to pan around your sample in live mode

There are various options in the right hand panel for adding shapes, scale bars and regions of interest to your image

Autofits your image to display the full field of view of the microscope within the window

Hovering your mouse over any part of the image will display the raw intensity value of that point at the bottom of the window

Right-clicking anywhere on your live image provides a variety of extra



Allows you to easily centre the microscope FOV on a chosen point. You can also do this by double-clicking anywhere on your image while in live view

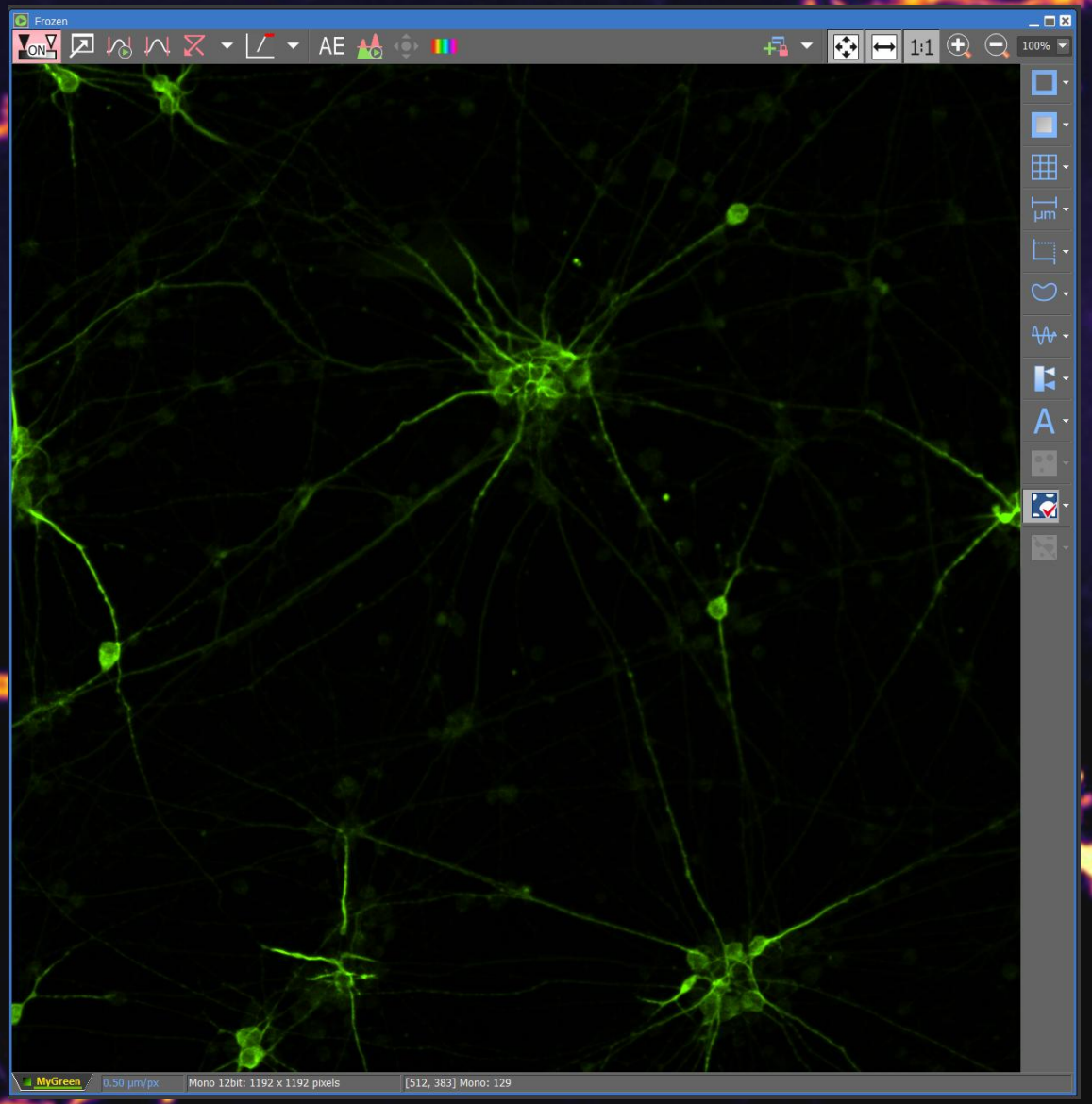
Adds this point to the ND acquisition XY tool, saving this location for imaging later. This tool is discussed in step 14

Using Live View When Frozen

Functionality changes slightly when the image is frozen

- You lose the ability to pan around your sample
- Mouse wheel now zooms in and out on your image rather than affecting focus
- Crucially, your sample is not being exposed to the laser

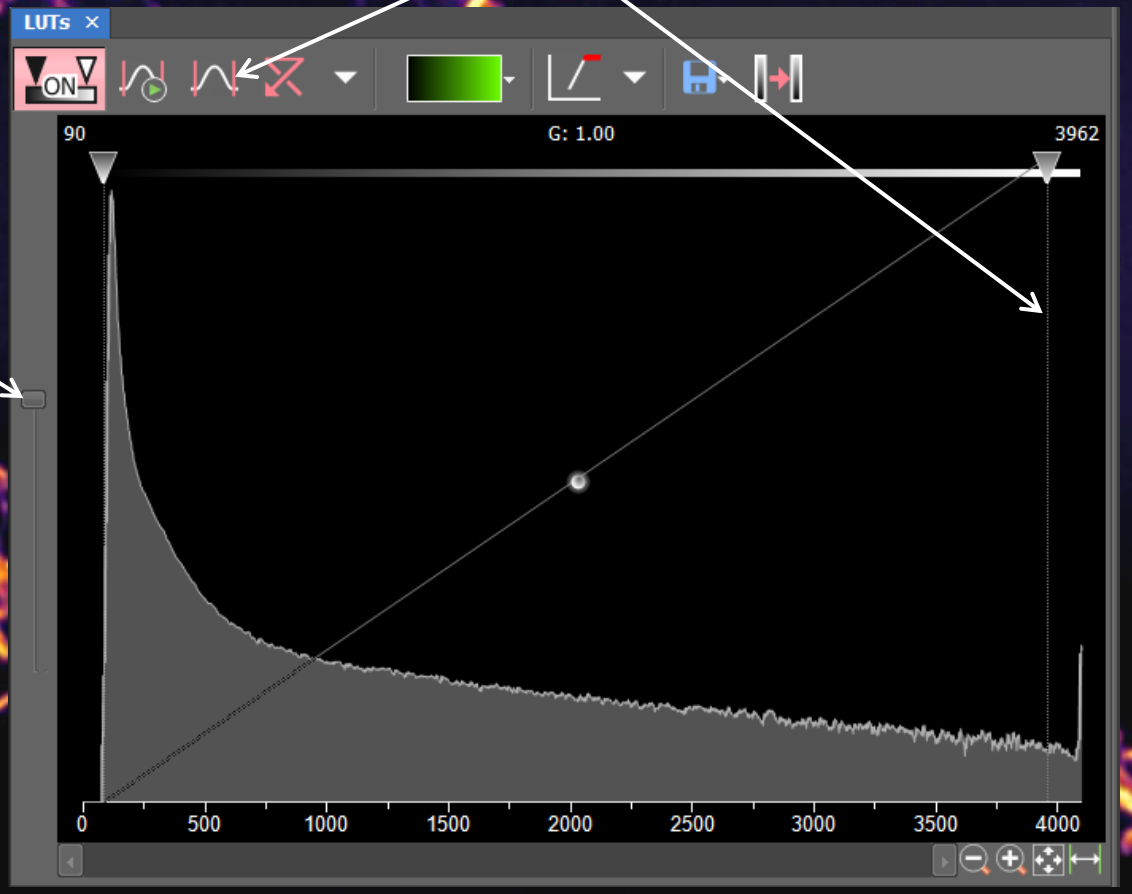
Always freeze your image whenever a live image is not needed – this minimises photobleaching



Adjusting Look Up Tables (LUTs)

LUTs do not change your signal intensity data, just how your image looks on screen. Make sure you can see your full signal intensity by dragging this bar to the top

Adjust your contrast to better view your sample. Click on auto contrast, or you can manually increase contrast by dragging this line to the left

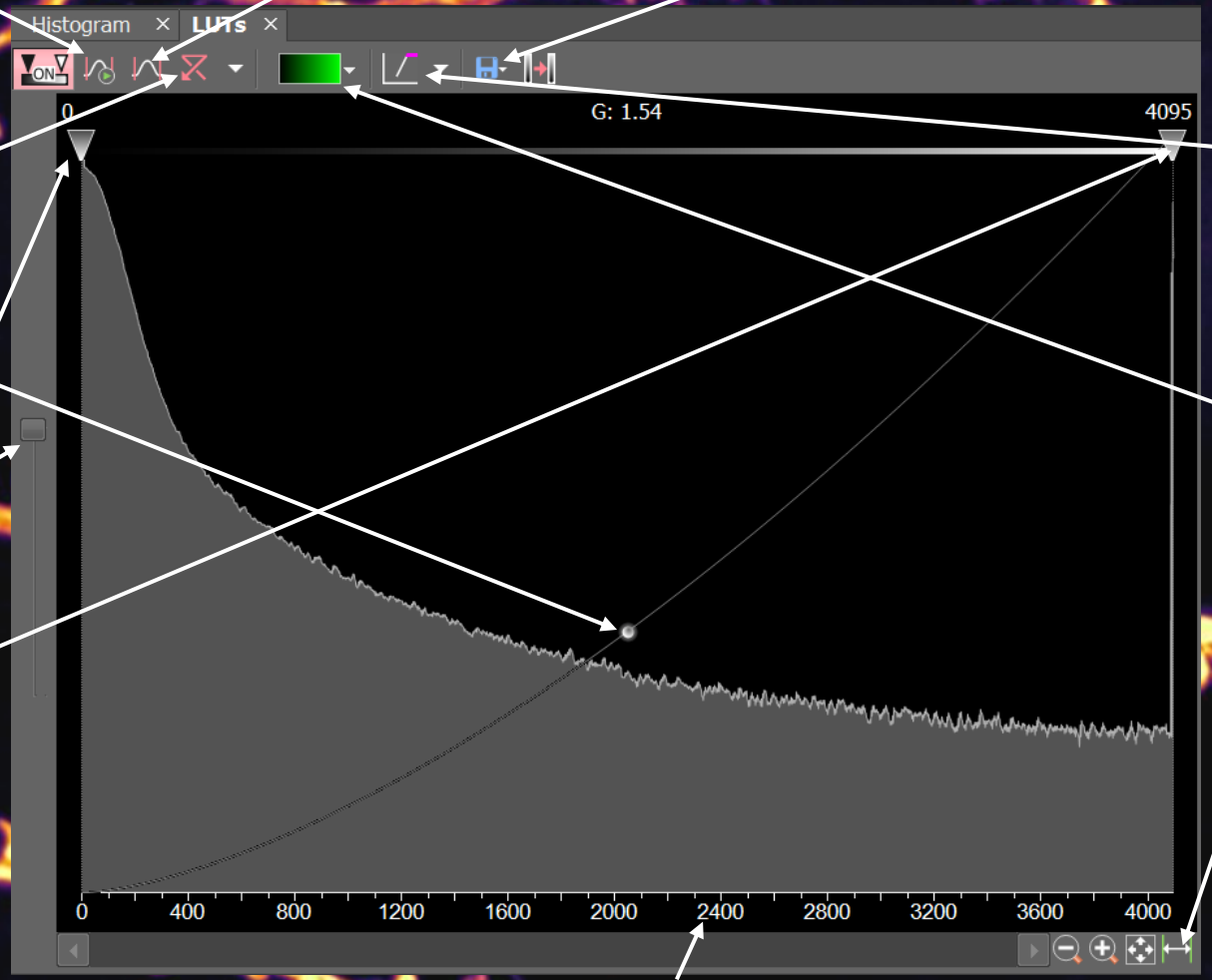


LUTs in More Detail

Continuous auto-contrast while in live view – can cause a flickering effect

Auto-contrast

To keep manual analysis consistent, you can save LUT settings to use across different images



Delete all contrast adjustments

Highlights saturated image regions. Saturated regions do not provide valid intensity information

Makes dim targets brighter, keeps bright targets the same

The camera is black and white – you can assign whatever colour you like to your image here

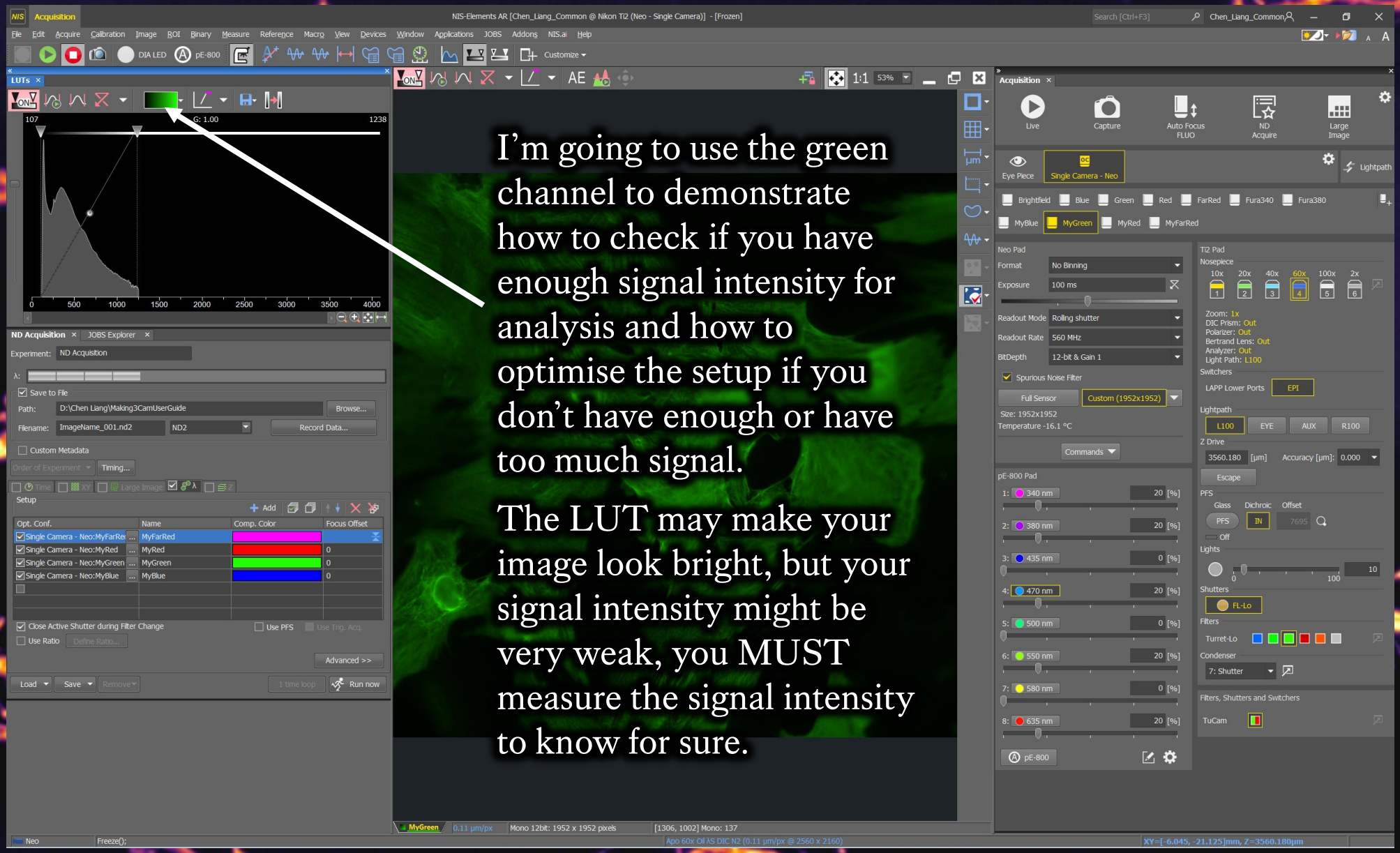
Controls the Y axis log graph. Always needs to be at the top like this

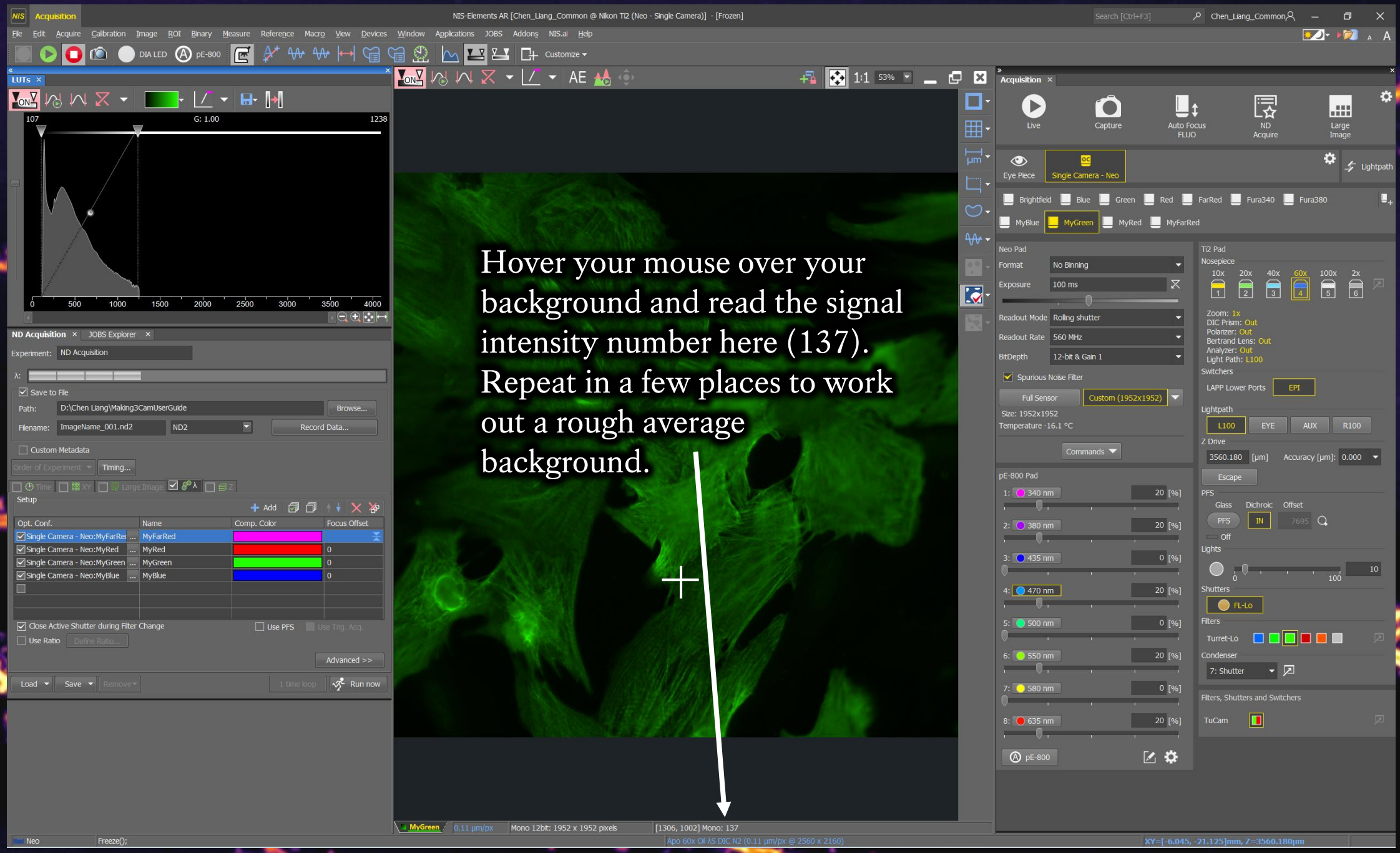
Fit the histogram to this space

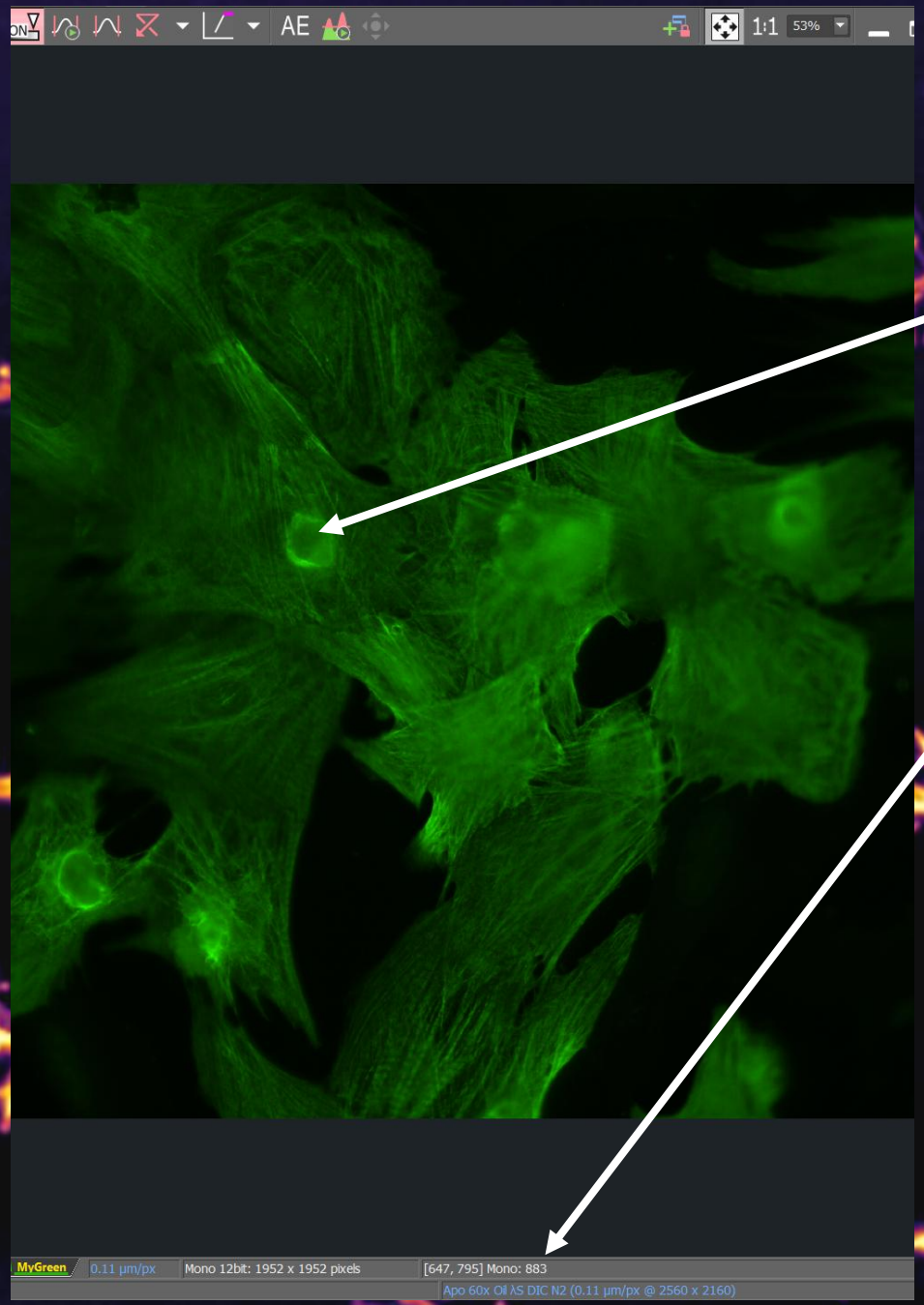
Alter contrast by changing min/max displayed values. Does not affect raw data

Drag and change the threshold for better visualisation. X-axis is relative to the 12-bit detector used by the A1R, so the limit is 4096 – try not to go above 4000 to avoid saturation

How to tell if you have enough signal

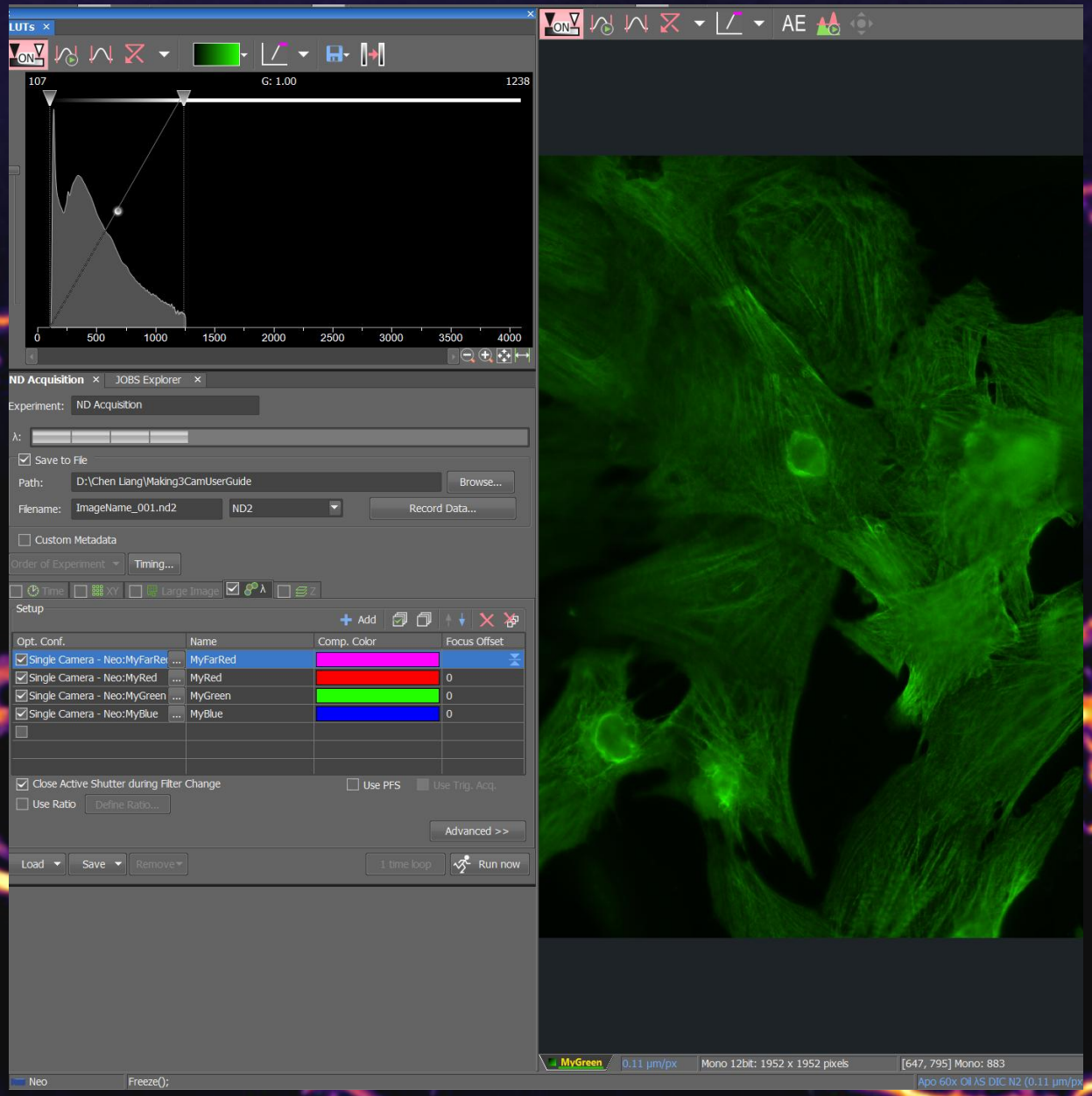



Hover your mouse over your background and read the signal intensity number here (137). Repeat in a few places to work out a rough average background.



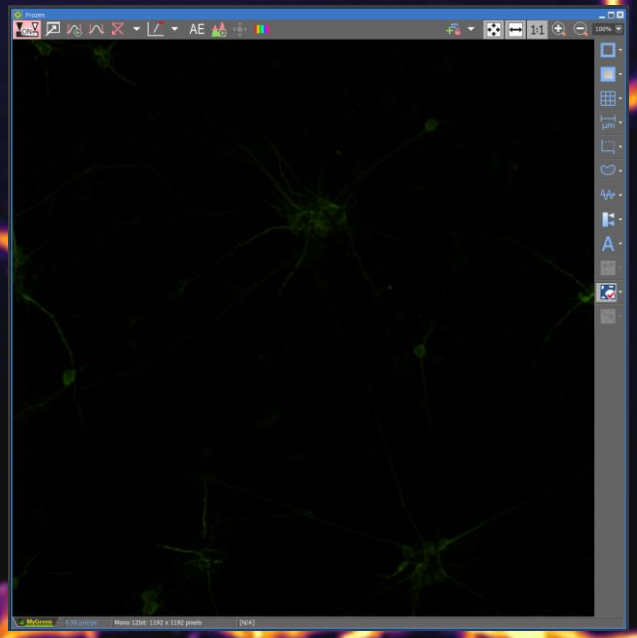
Now hover your mouse over a few focused targets you'd like to analyse, work out a rough average signal intensity (883) for your targets. It is important to measure the correct intensity, so if you need to zoom in to be more accurate, you can use ctrl + mouse wheel or if you are not on LIVE, then you can use your mouse wheel as a zoom instead of focus.

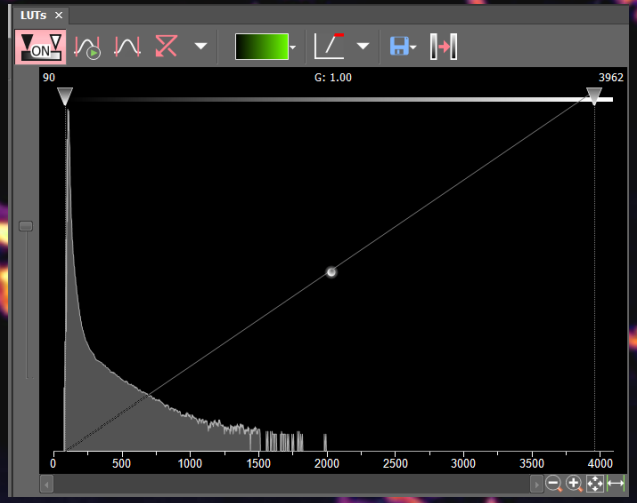
Now work out the rough difference between your target and background (900 – 150 = 750). Target signal intensity is 750 above background, if you want to do any automated analysis, this difference needs to be 1000 minimum.



If you don't have a minimum of 1000 intensity signal difference between your target and background, then you need to increase your signal intensity.

Optimising – Not Enough Signal





If your Image is hard to see and your LUT is not full, your signal intensity is too low

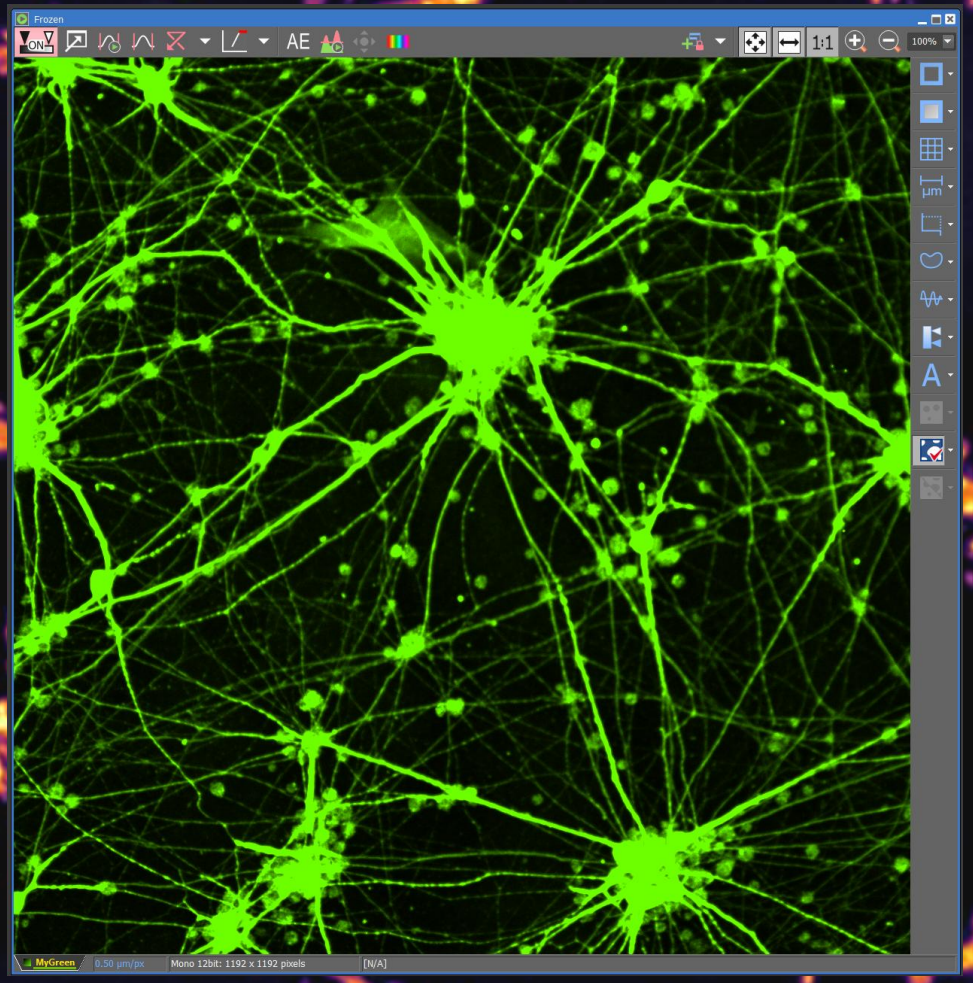
You can see intensity of individual points by hovering over them and reading off the intensity from the bottom. If the difference between your target and your background is <1000, your intensity is too low for robust automated analysis

To optimise your imaging settings when your signal is low:

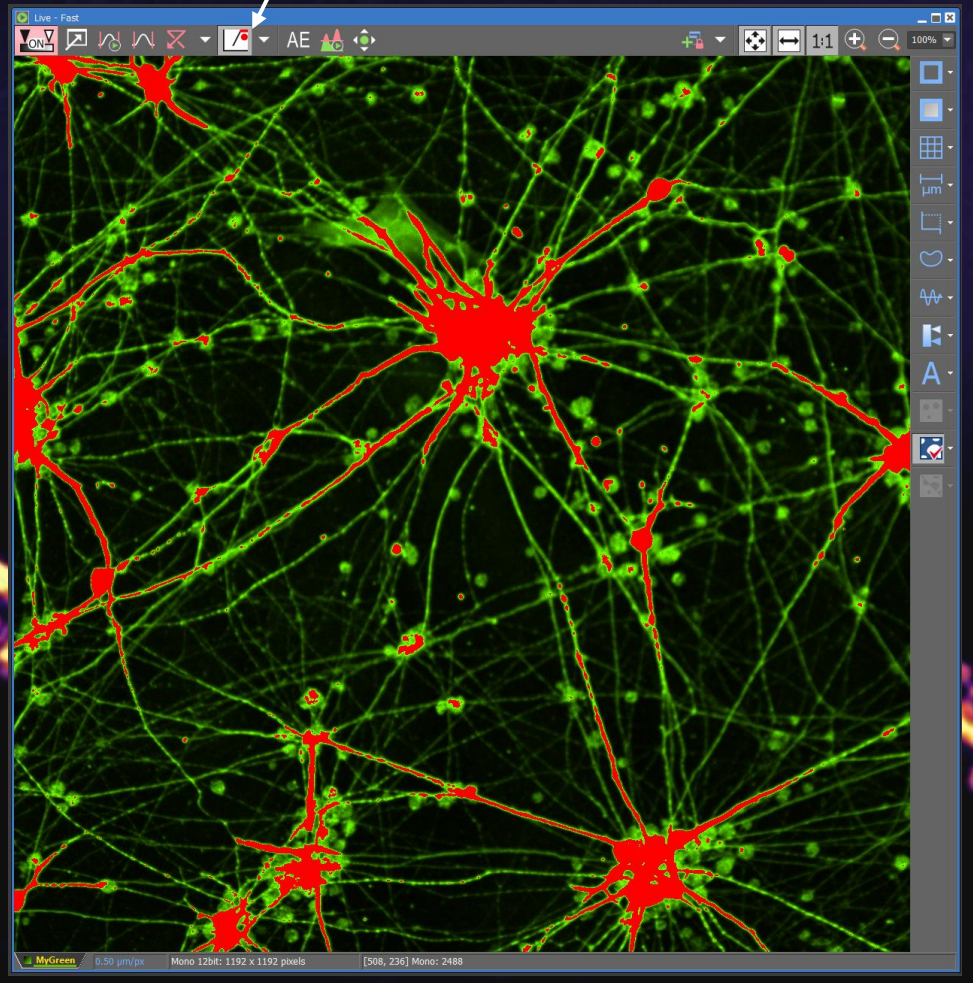
- Try using the "Auto Signal" button on the top right to automatically adjust your settings to suit your sample
  - Right-click on the button to choose whether your sample is fixed or live
- Increase exposure time
- Increase laser power
- Consider increasing gain if your signal is still too low and you can afford to sacrifice image resolution

Optimising – Too Much Signal

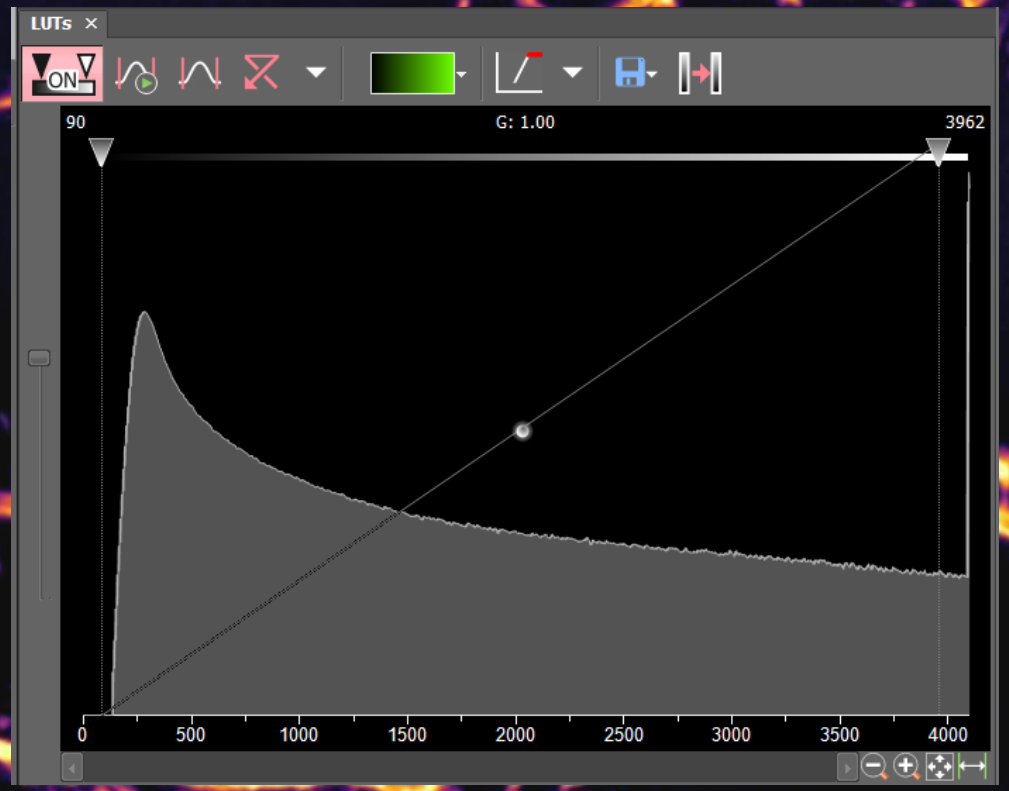
When your signal exceeds the maximum that the camera can detect, you get oversaturation. When this happens, you lose any useful intensity data in all oversaturated regions



To easily identify oversaturated regions, click this icon to highlight oversaturation in a complementary colour



Optimising – Too Much Signal

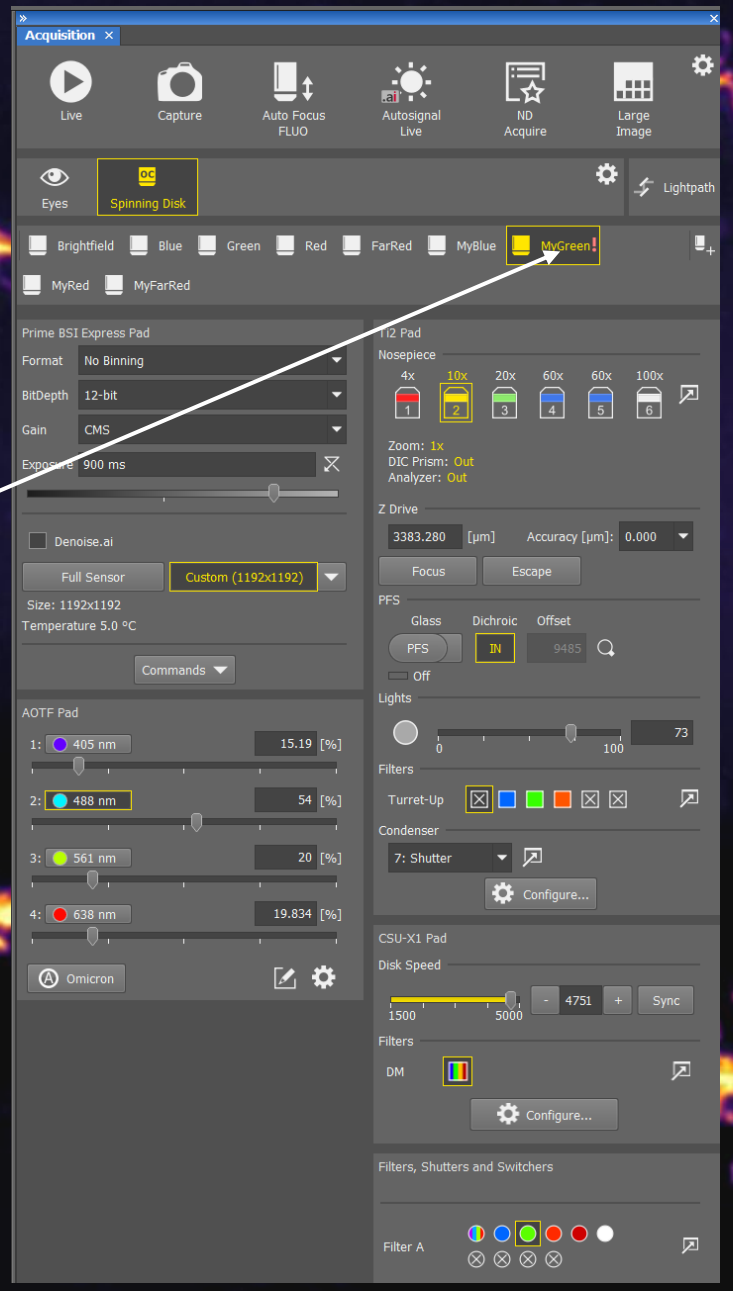


When your image is oversaturated, you will see a strong peak at the upper end of your LUT

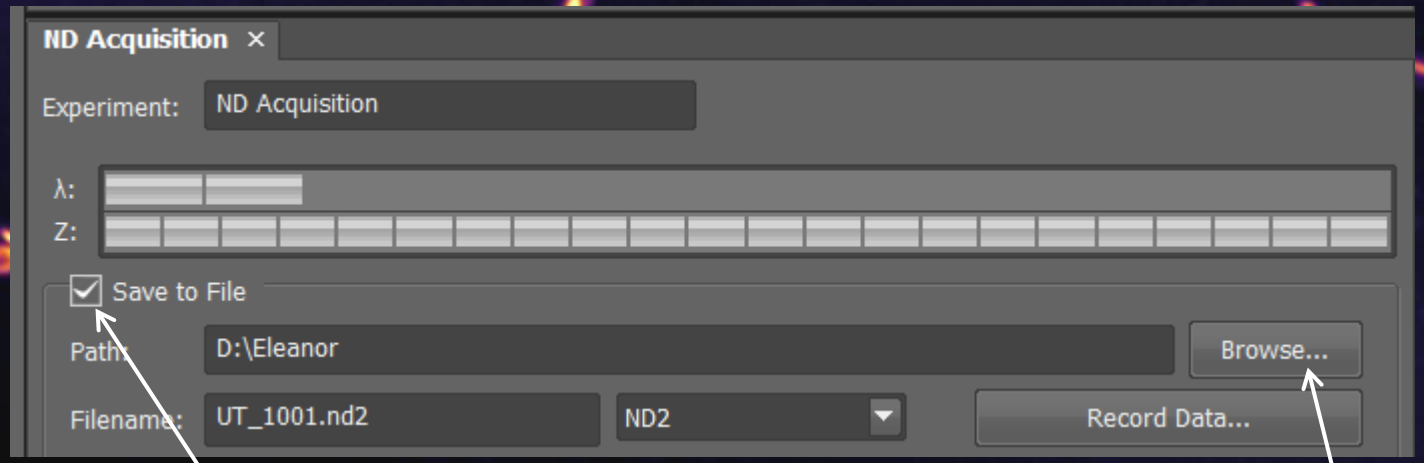
To optimise your settings to reduce signal:

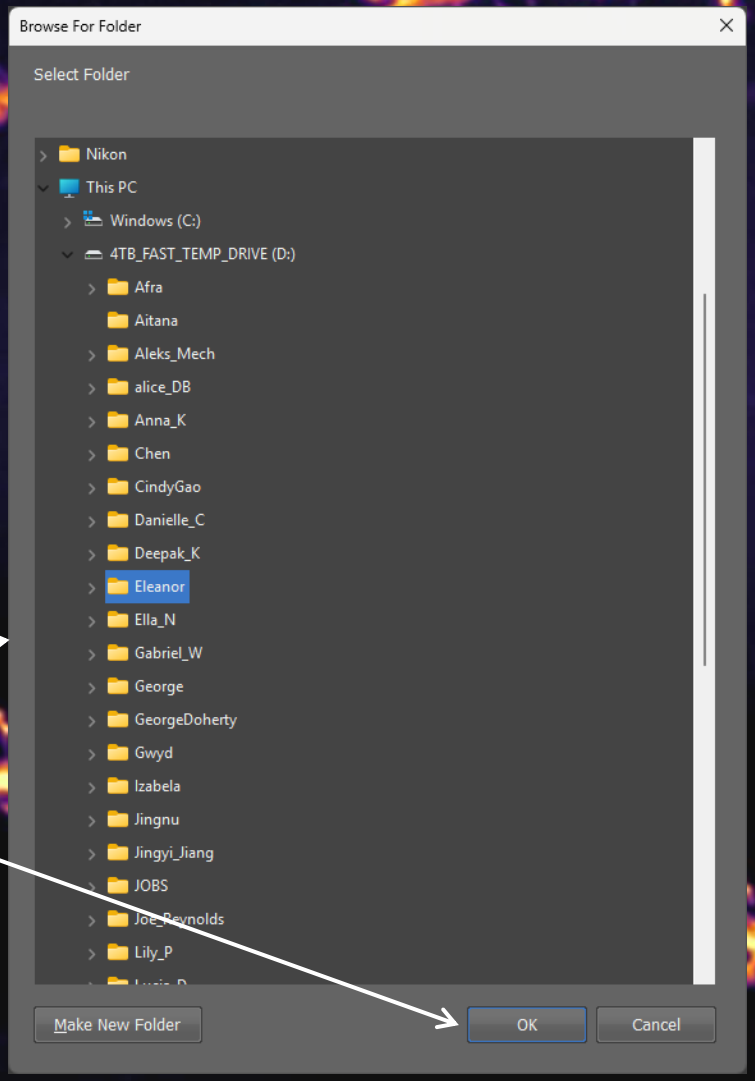
- Try using the Auto Signal button
- Decrease laser power
- Decrease exposure time
- If you cannot get your signal low enough, consider asking a technician about switching the camera to 16-bit acquisition

When you have successfully optimised your camera and laser settings for your channel, remember to right click → assign current settings



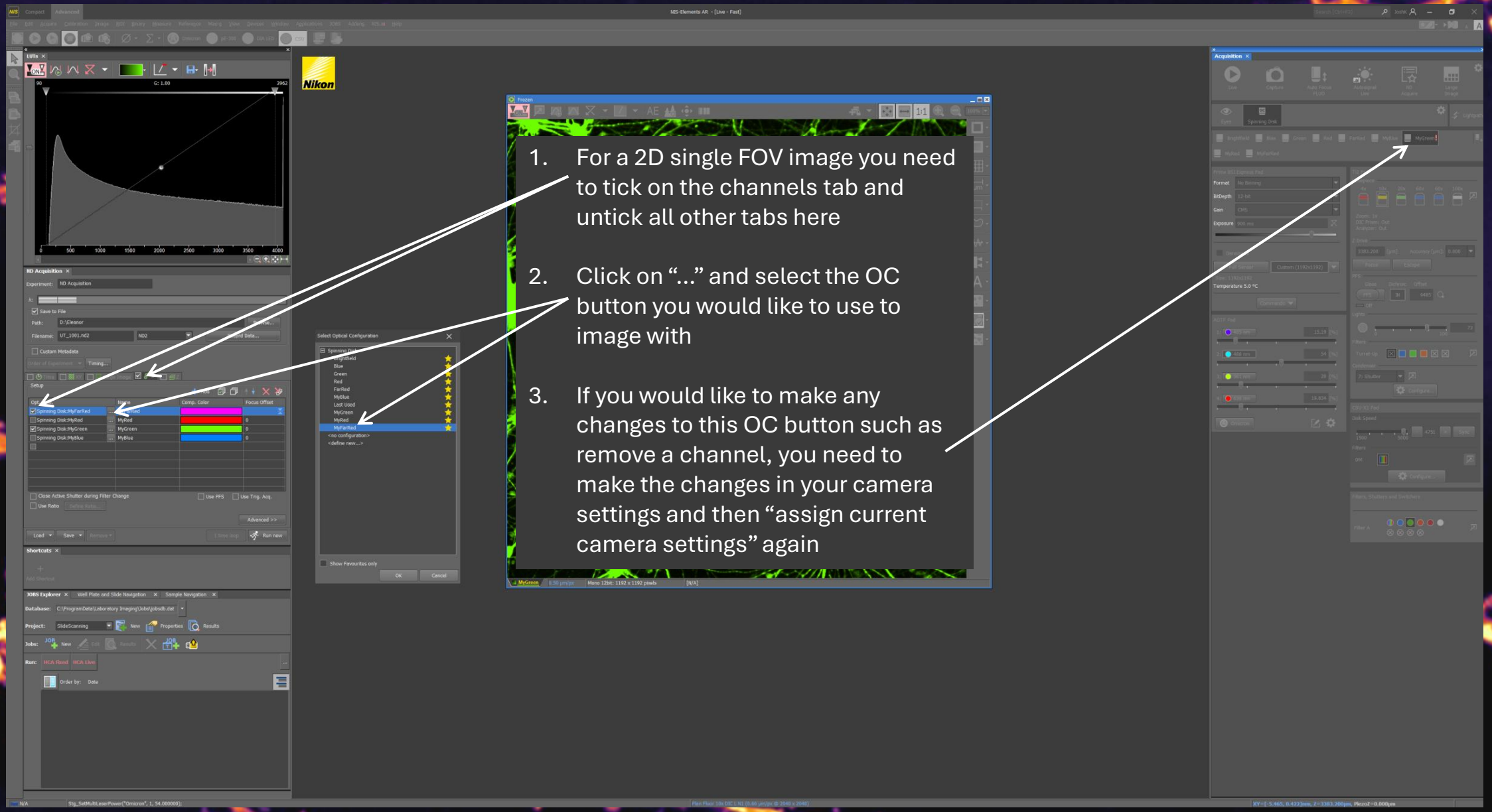
Set up Save to File





1. Under ND Acquisition in the left hand panel, ensure that "Save to File" is ticked
2. Click "Browse" to bring up this menu
3. Select the folder where you wish to save your images from this session and click "OK"
4. Enter a file name and end it with "_001" – this number will automatically increase by 1 each time you take an image

Image Acquisition – 2D Single FOV



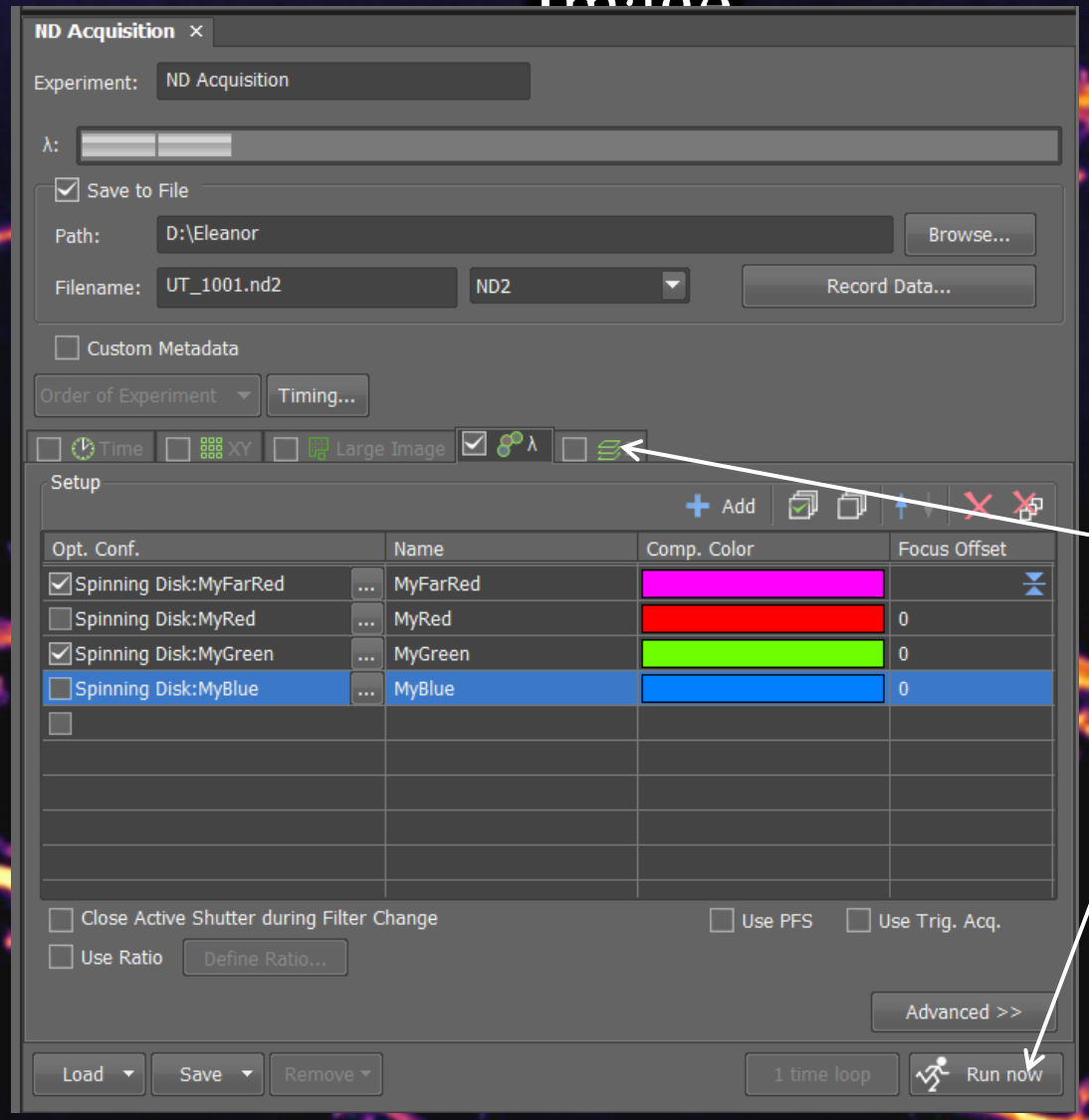
1. For a 2D single FOV image you need to tick on the channels tab and untick all other tabs here

2. Click on "…" and select the OC button you would like to use to image with

3. If you would like to make any changes to this OC button such as remove a channel, you need to make the changes in your camera settings and then "assign current camera settings" again

Image Acquisition – 2D Single FOV

Image



Once you have selected your desired OCs and the microscope is focused on your desired FOV, ensure the other ND acquisition tabs are not ticked and select “Run Now”

Image Acquisition – Z-stack Image

Set Middle: use mouse wheel to find the mid point of your Z-stack and set equal distance above and below the focal plane. Useful if your sample is symmetrical along the Z axis

Asymmetrical: find focal plane and set different distances above and below

Set top and bottom: use mouse wheel to find and define the top and bottom of your Z-stack

Step size

- You can set step size or number of steps
- Use recommended step size to capture all the information
- Fewer steps than the recommended is called under-sampling and you may lose information
- More steps than the recommended is called over-sampling, which is required for 3D deconvolution

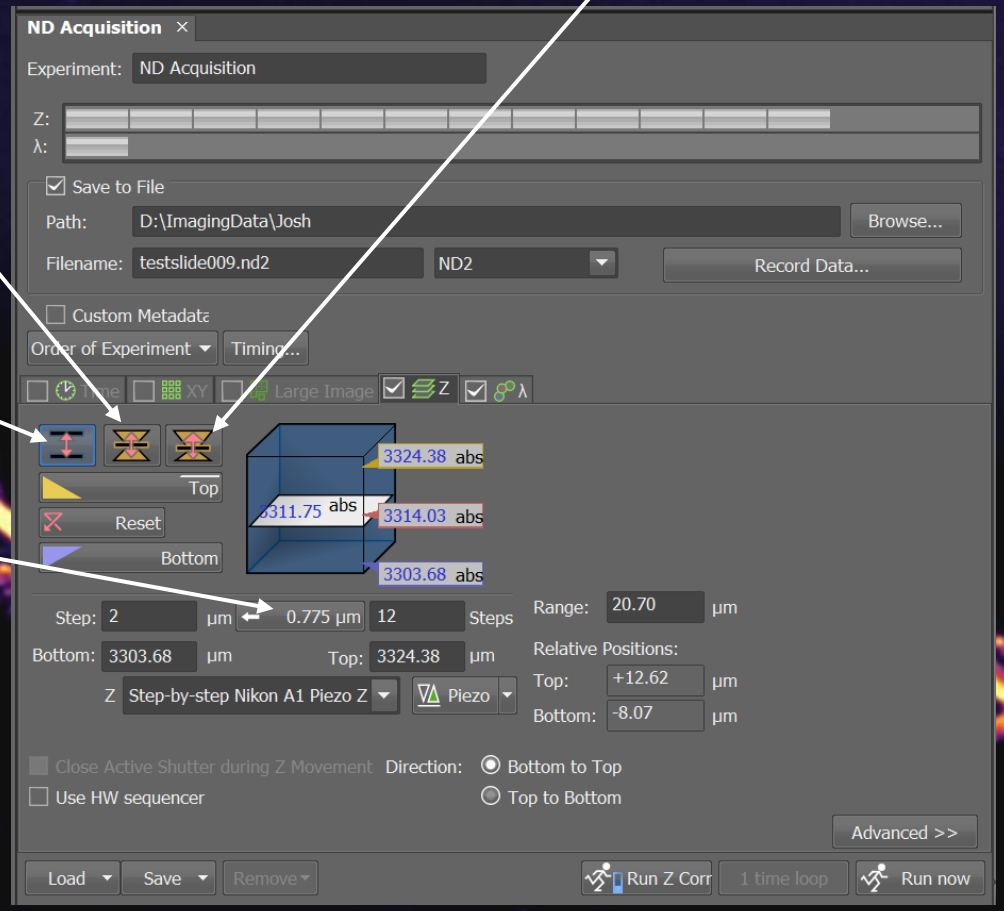


Image Acquisition – Z-stack Image

1. Tick the Z tab and untick others – can keep the channels tab ticked to continue using your selected optical configuration
2. Choose your method, I'll use the first one (top and bottom) for this guide
3. Press reset
4. Go on LIVE and click and hover your mouse on your live window. Scroll your mouse wheel towards you (lens going up) until the top of where you want your image, and click "Top"
5. Hover your mouse over your live window again and scroll away from you (lens going down) to the bottom of where you want your image and click "Bottom"

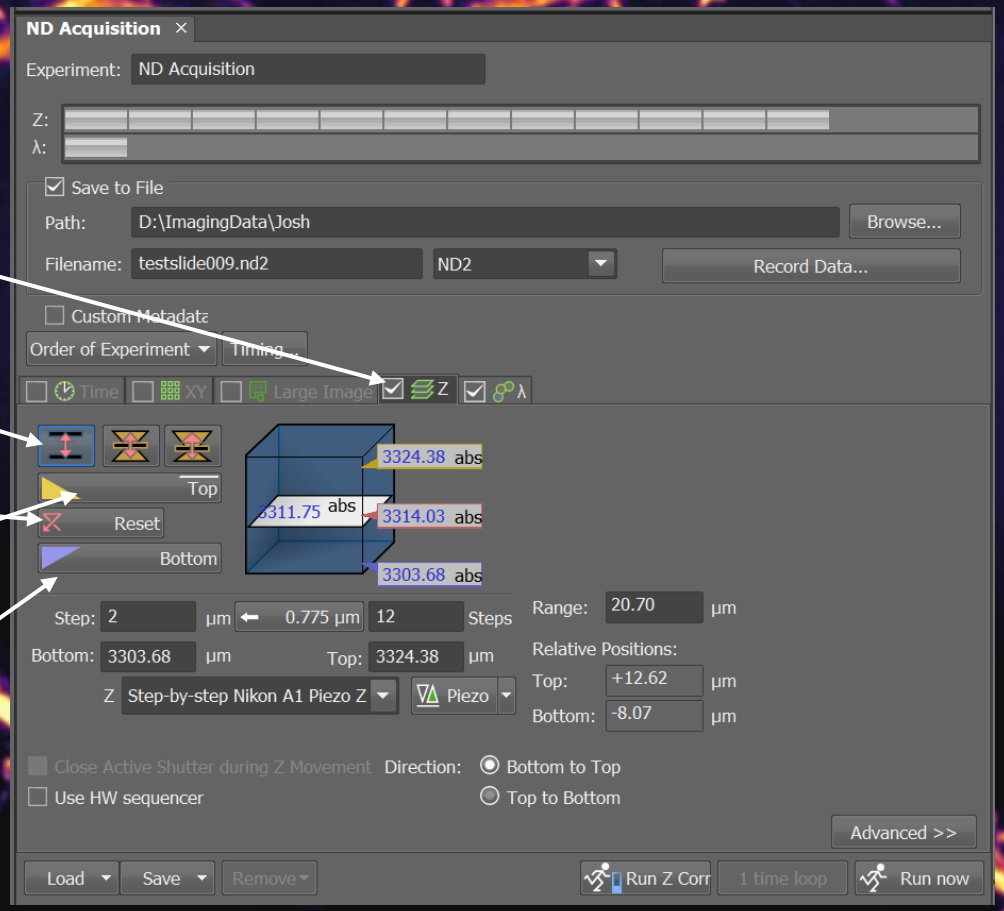


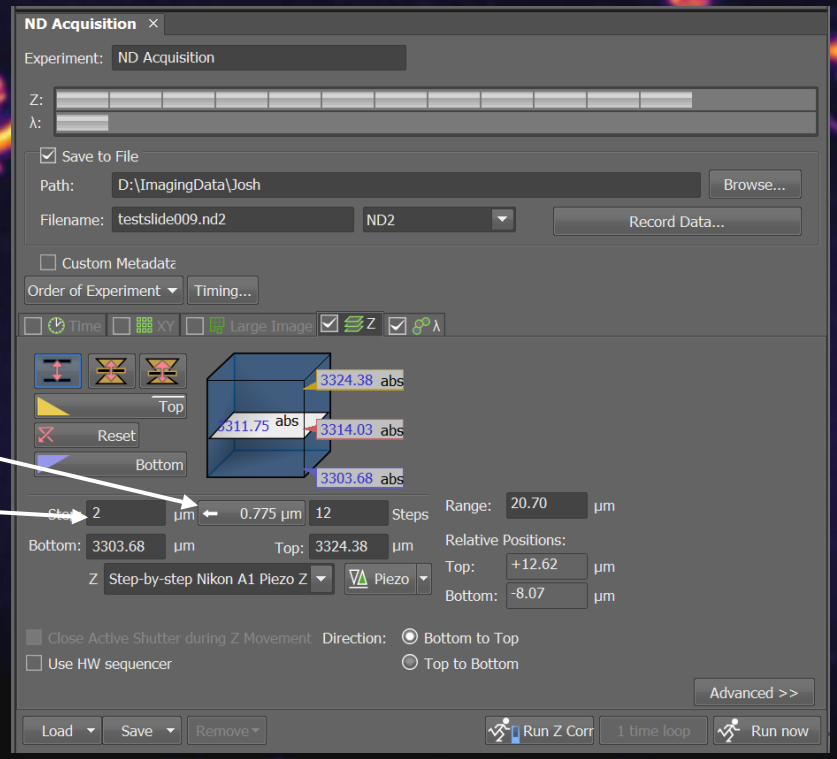
Image Acquisition – Z-stack Image

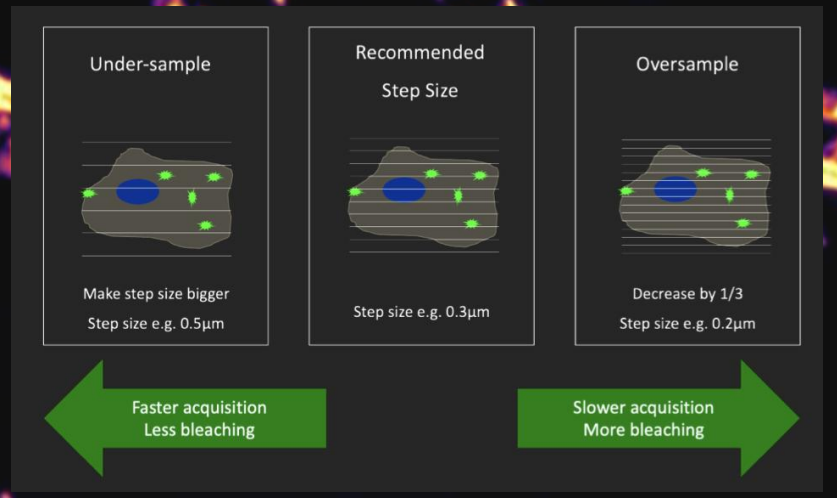
6. Click on FREEZE to stop imaging

7. Click on the recommended step size, this will give you the optimum Z-resolution

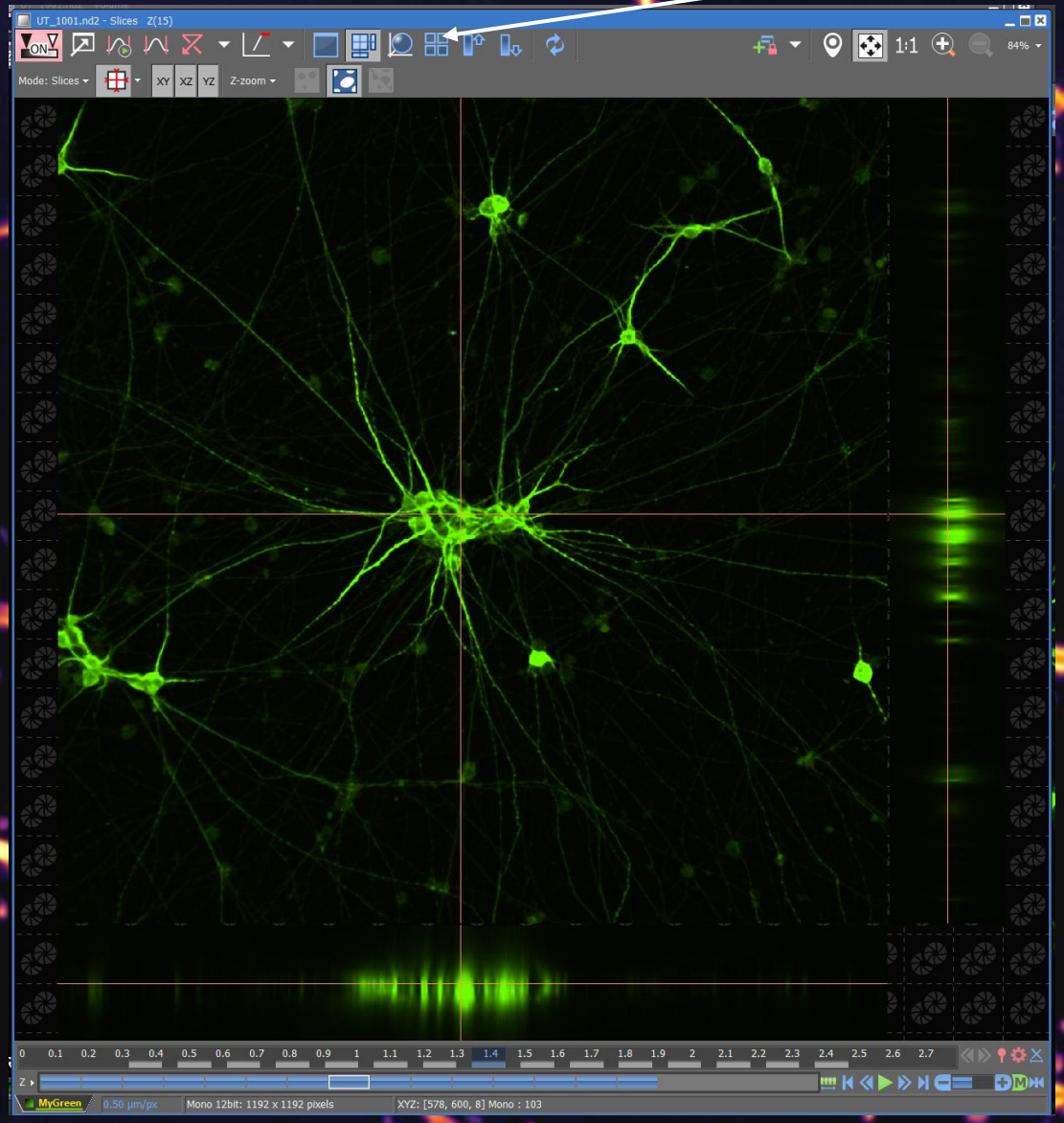
8. You have the option to manually change the step size here.
   - Under-sampling between each step will result in faster imaging but you may lose information.
   - Over-sampling will increase imaging time, but you can use the extra information in 3D deconvolution in the workstations to increase your image resolution post-imaging.
   - Please see our deconvolution guide here: https://www.kclwcic.co.uk/analysishelpguides

9. Click on “Run now” to take your Z-stack image





There are several viewing methods for 3D images, selected using these icons in the top row



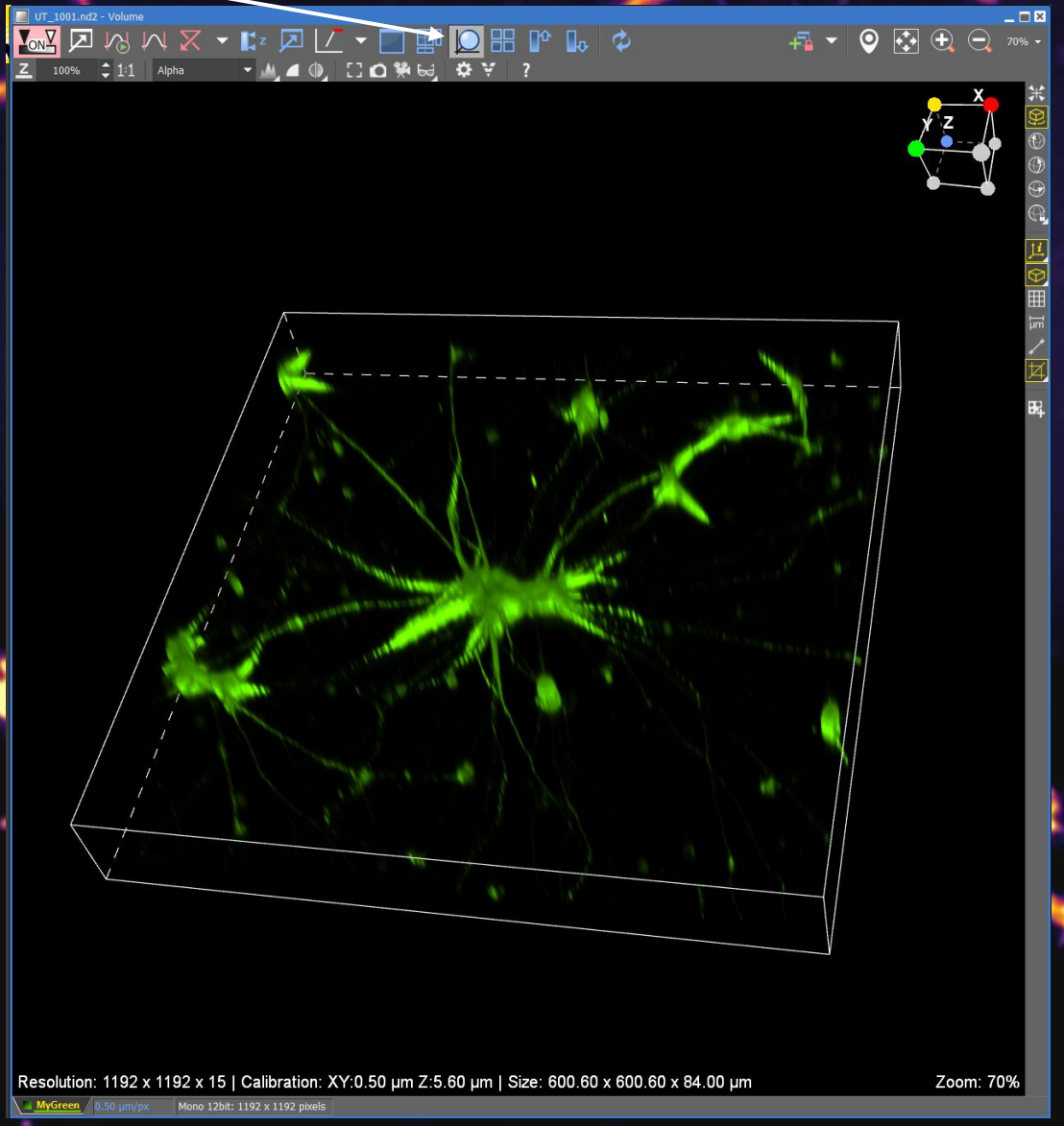
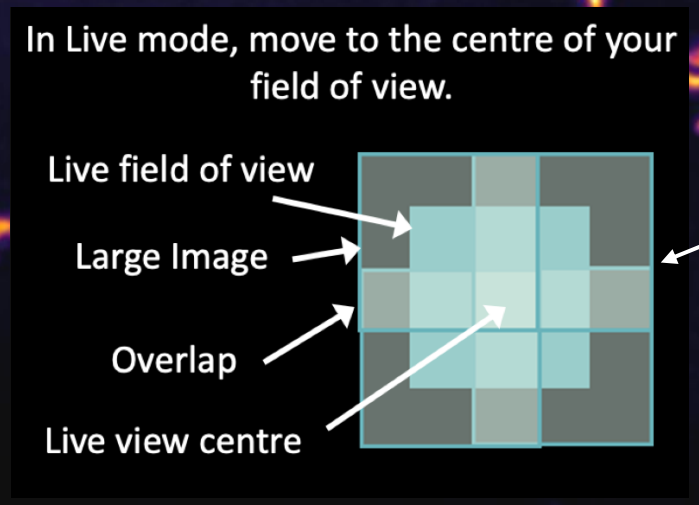
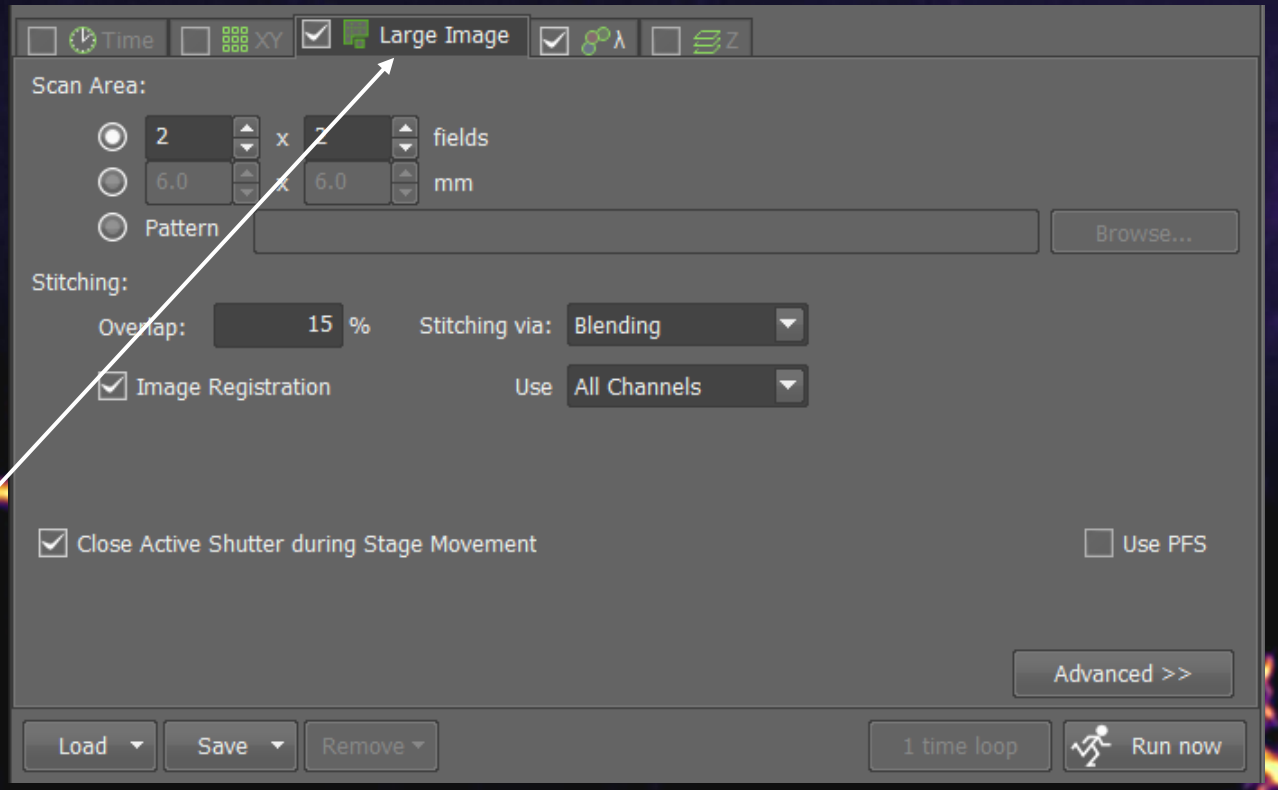


Image Acquisition – Large Image



1. Click on LIVE and focus your sample (especially after doing a Z-stack). Your live view will be the centre of your large image, this is a diagram of a 2x2 large image



2. Tick on the “Large Image” and “Channels” tabs, make sure the Large Image tab is on the left of the channels tabs (click and drag to rearrange)

Image Acquisition – Large Image

3. Enter in the Scan Area, how many fields of view you need in the X and Y axes respectively

4. Be mindful where you are on your coverslip, make sure you are not at the edge as your live image may go over the edge of your sample

5. If you want to stitch up your FOVs into one large image, overlap needs to be set to minimum 15%

6. Click on “Run Now” to take your Large Image

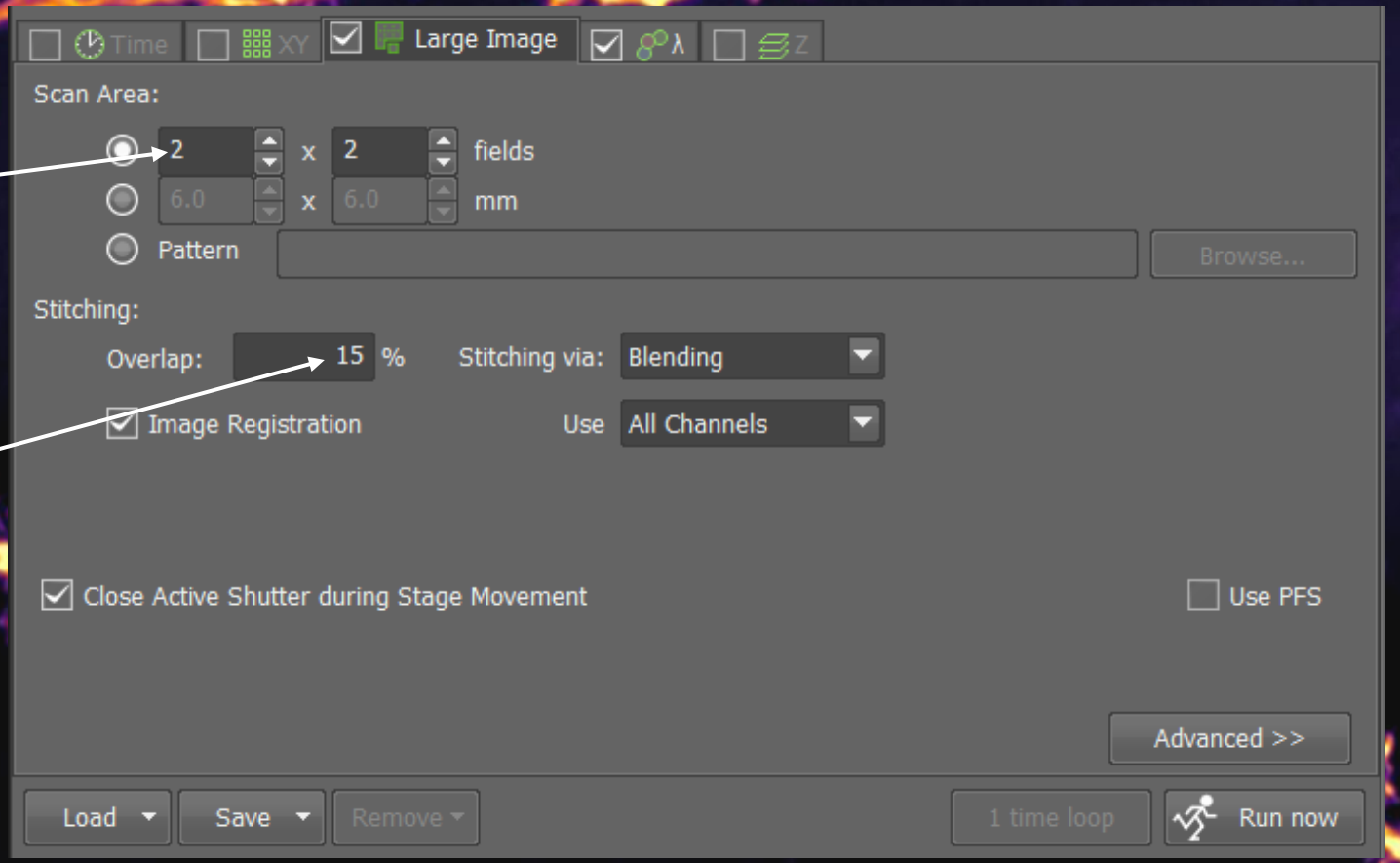
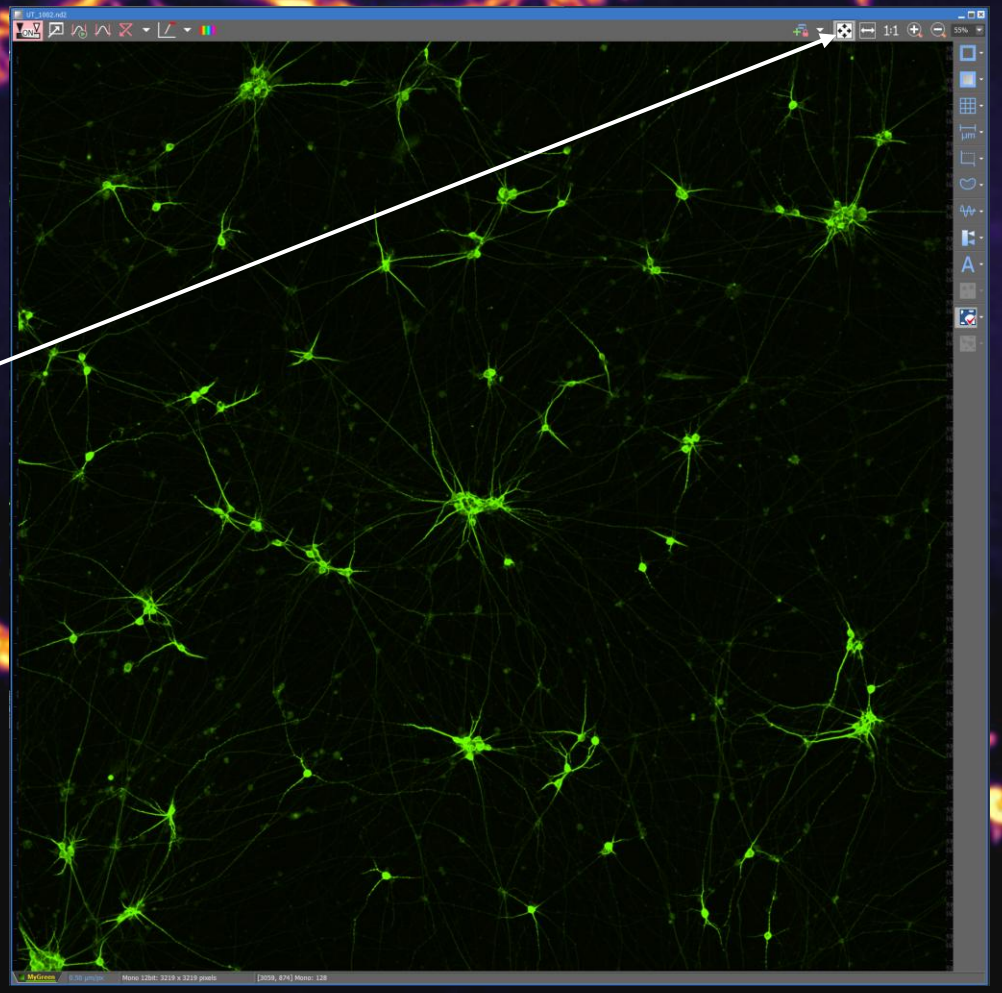


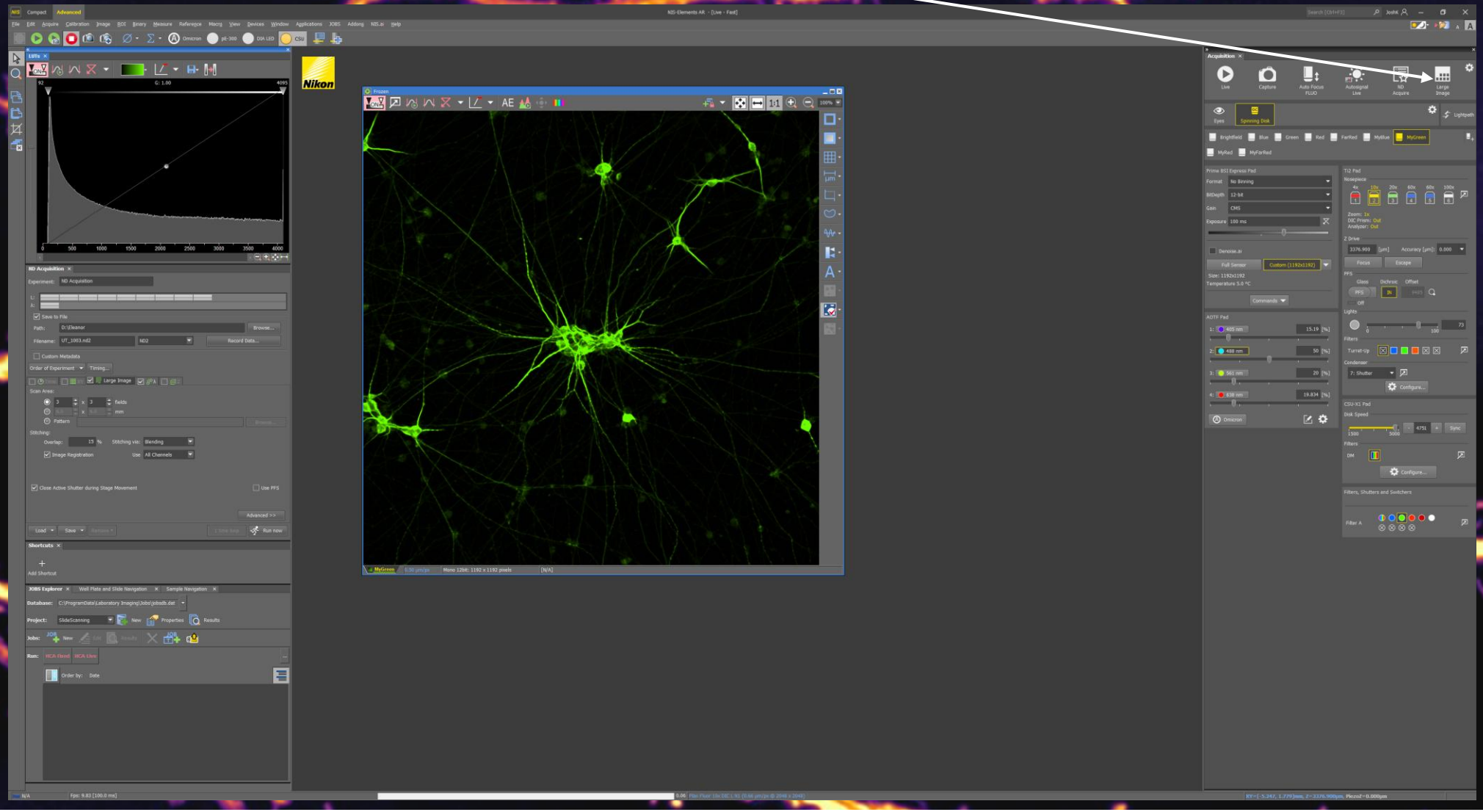
Image Acquisition – Large Image

Once your large image has been produced,
click here to view the full stitched up image



Alternative Large Image Options

Outside of ND acquisition, there ius a separate Large Image Scanning Wizard that gives you more control over the scan area, as well as some other features



Alternative Large Image Options

Tick “Use Z for focus surface” to ensure that your entire large image is in focus

Tick “Capture Multichannel” to use multiple channels in your large image capture

Give your image a name and select “Save file(s) to folder” before selecting your session folder

The scanning wizard gives you several options for defining your large image region – for this example we are using “Rectangle by sides”

1. In live view, pan to the FOV where you want the top of your large image to be and press this button

2. Repeat for each side of the image, ensuring to focus your image at each FOV to allow NIS-Elements to create a focus surface to keep your whole image in focus

3. Tick “Automated Shading Correction” if you wish to reduce the tiling effect created by large image stitching

4. Click “Run”

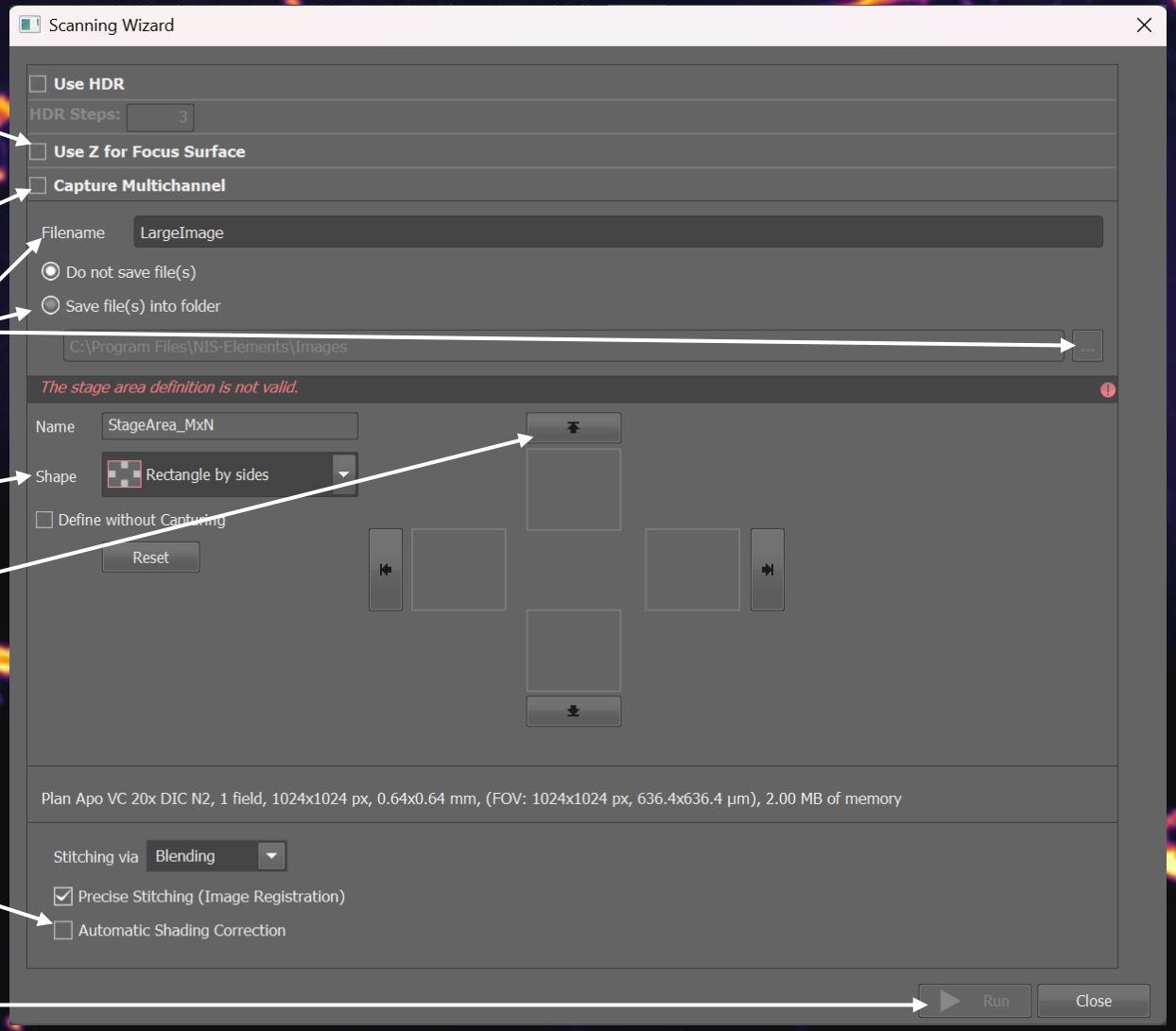
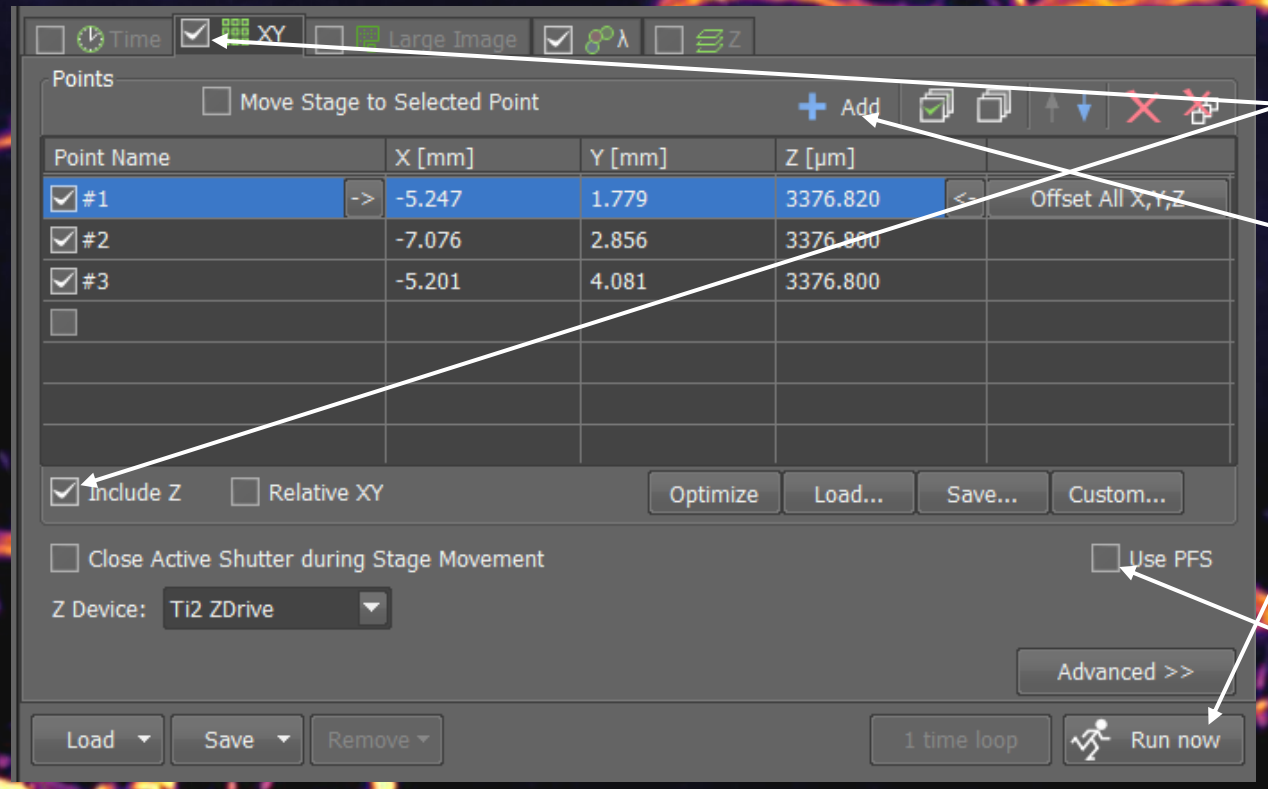
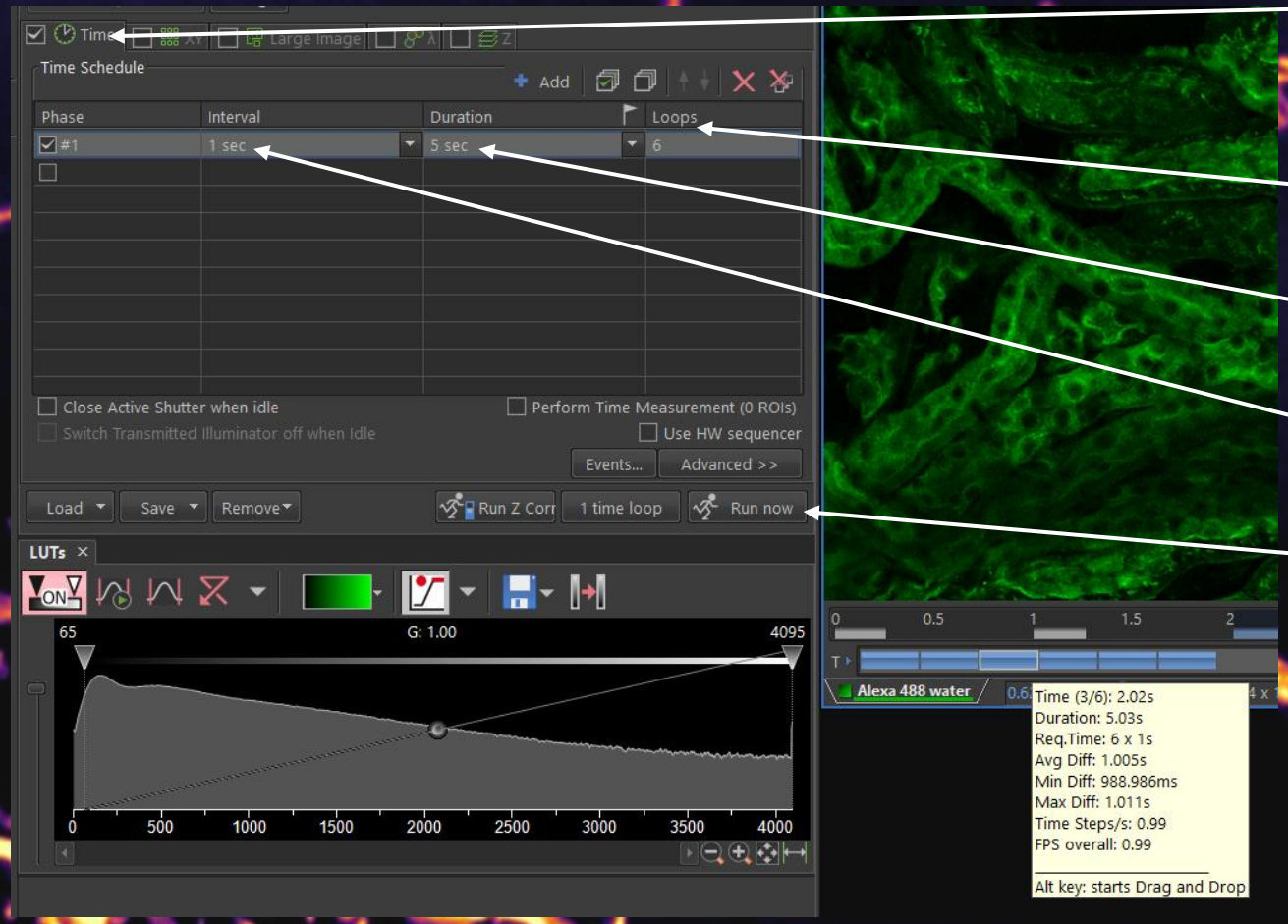


Image Acquisition – XY Positions



1. Tick the XY tab and click on “Include Z”, also tick on the channels tab
2. Click on LIVE and focus on your sample, then move to a field of view you want to image. Click “Add” to save this XY position
3. Move to another FOV, again be mindful where you are on your coverslip. Focus again if you need to, and add this position
4. Repeat for as many positions as you require and press “Run now” to take images at multiple XY positions
5. For live imaging we would encourage you to use PFS to ensure that the sample remains in focus throughout your image capture session

Image Acquisition – Timelapse Imaging

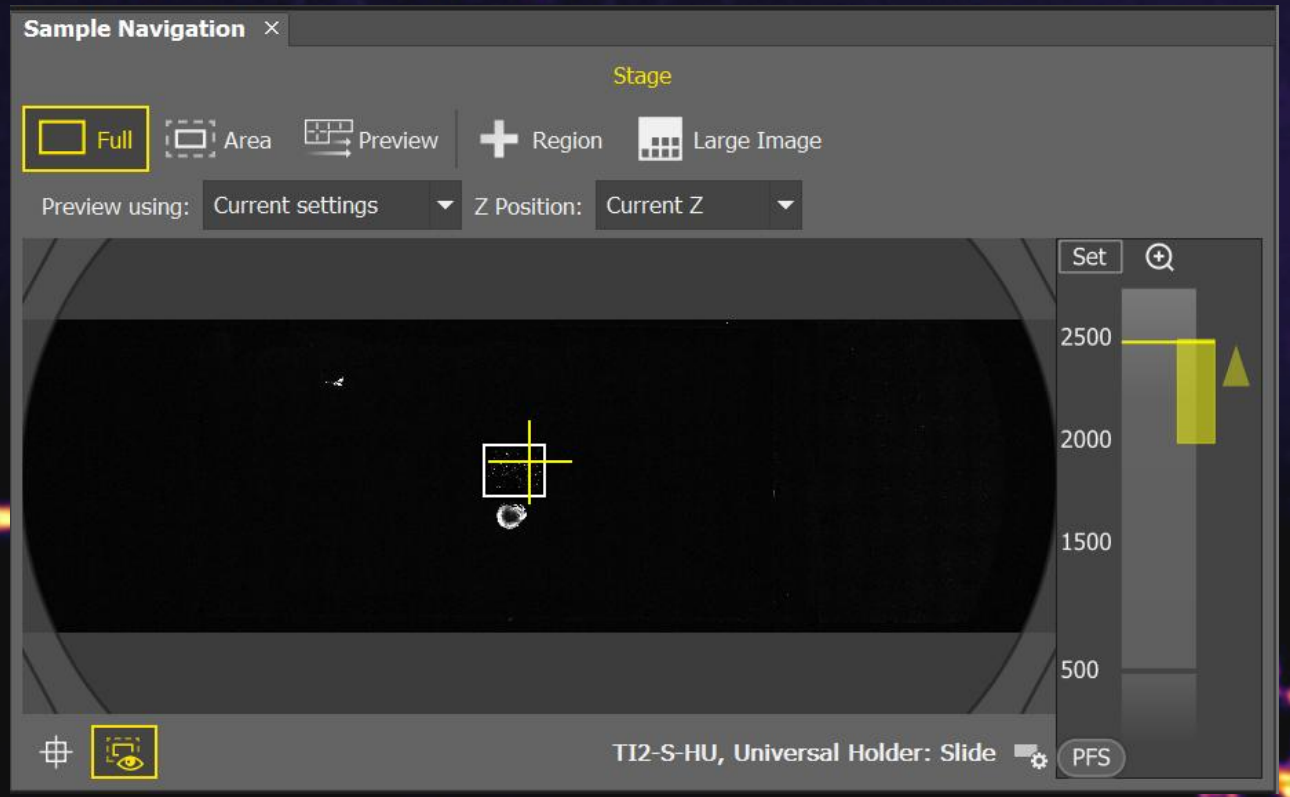


1. Tick on the Time tab and add a time-lapse, also tick on the channels tab.
2. Loops (how many images will be taken)
3. Duration (how long the overall time-lapse)
4. Interval (how long between each scan)
5. Click on LIVE and focus on your sample, move to a field of view you want to image and click on ‘Run Now’.
6. If you’ve not been trained to use Timelapse or set up live imaging, please let us know and we’ll be happy to help.

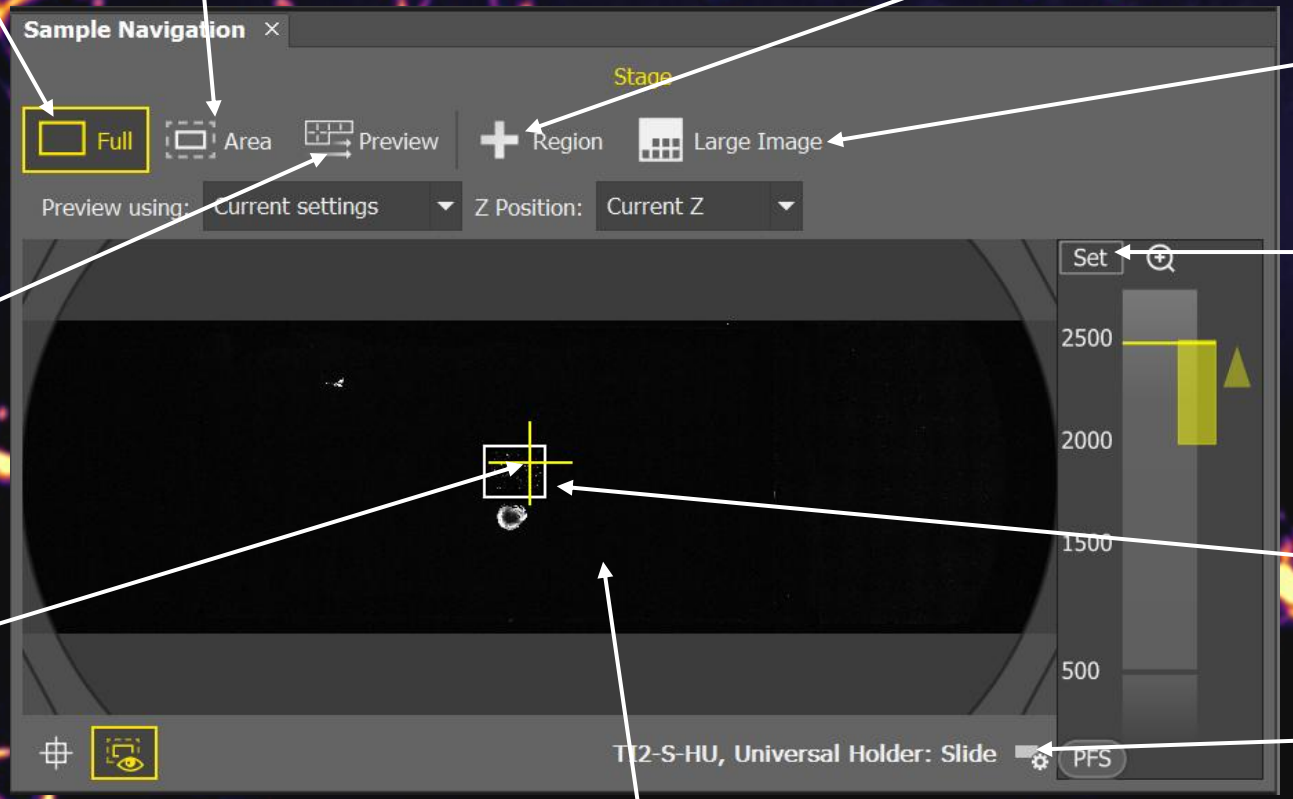
Optional - Sample Navigation

Sample Navigation provides a low magnification overview of the sample and stage position. It allows you to:

- Perform a low-magnification pre-scan of large areas
- Visualise where you are on the slide and sample
- Easily move around the sample and return to previously imaged fields of view
- Take more detailed pre-scans of manually selected smaller areas
- Use any desired lens and OC configuration to take large images of user-defined regions of interest



Sample Navigation – Interface Overview



Shows an overview of the full slide

Shows only the defined area in the white square – click and drag to move or resize, right click to confirm new area

Add a region of interest (ROI)

Perform a large image scan of selected ROIs

Scans either the full slide or area, whichever is currently selected, using current objective settings

Sets Z-position to current focal plane

Yellow cursor shows where the current FOV of the microscope is centred

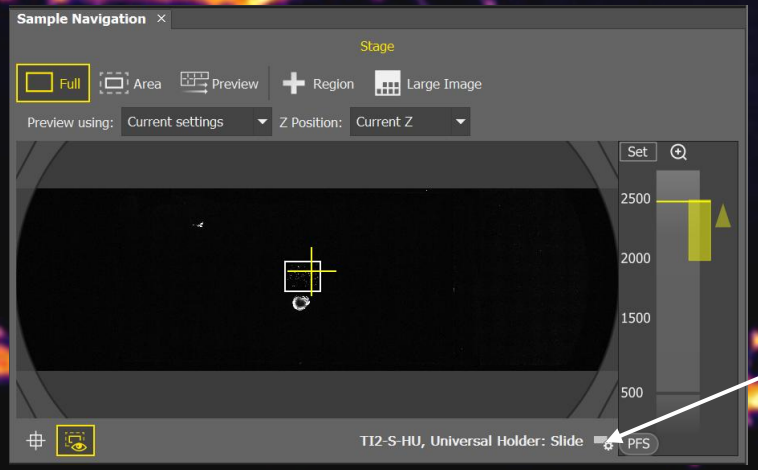
White box shows the currently selected area

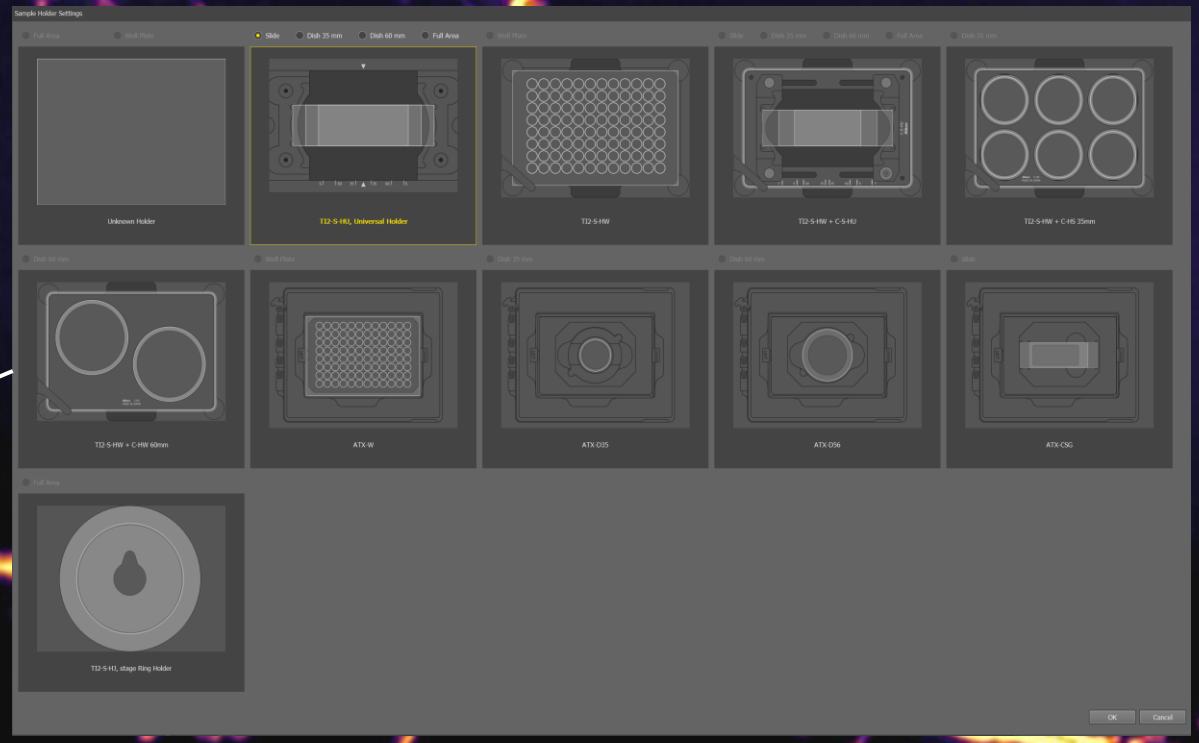
Change slide holder

Double clicking anywhere on the slide will centre the current FOV on that position

Sample Navigation – Slide Holder Selection

Ensure you have selected the slide holder that you are using – for the slide holder mounted on the A1R by default, the Ti2-S-HU Universal Holder is the correct option





Using Sample Navigation

1. Ensure the correct objective is selected – use a low magnification objective on zoom 1 for previewing

2. Focus on the sample

3. In sample navigation, select:
   - Full
   - Preview using: current settings
   - Z position: current Z

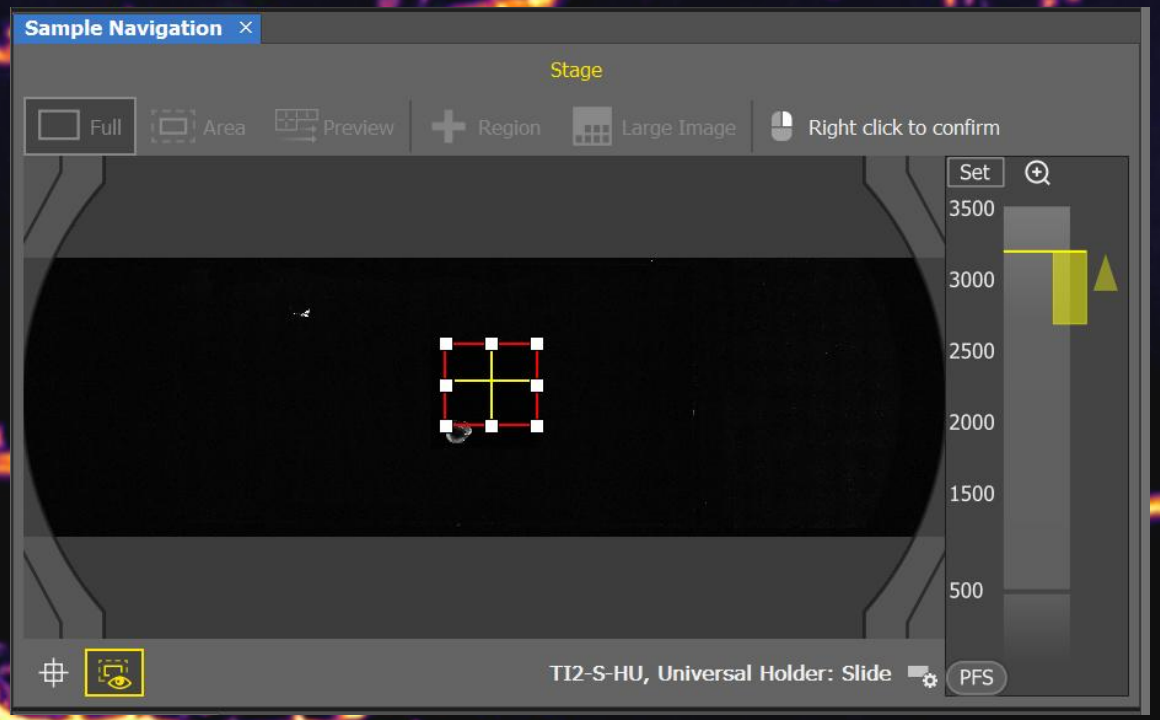
4. Select “Preview”

5. Sample navigation will now show a full preview of the slide as shown here

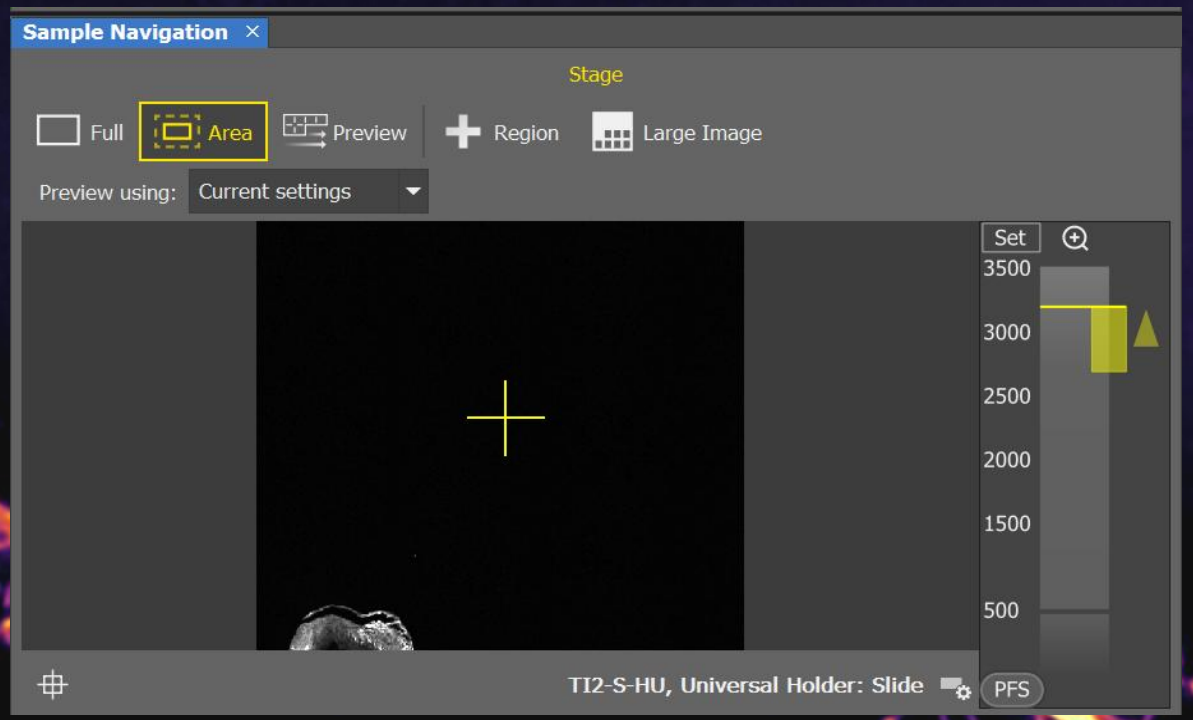
6. Double-clicking anywhere on the sample will move the microscope to centre on the location you have clicked

Sample Navigation - Full vs Area View

Full view, with selected area highlighted in red



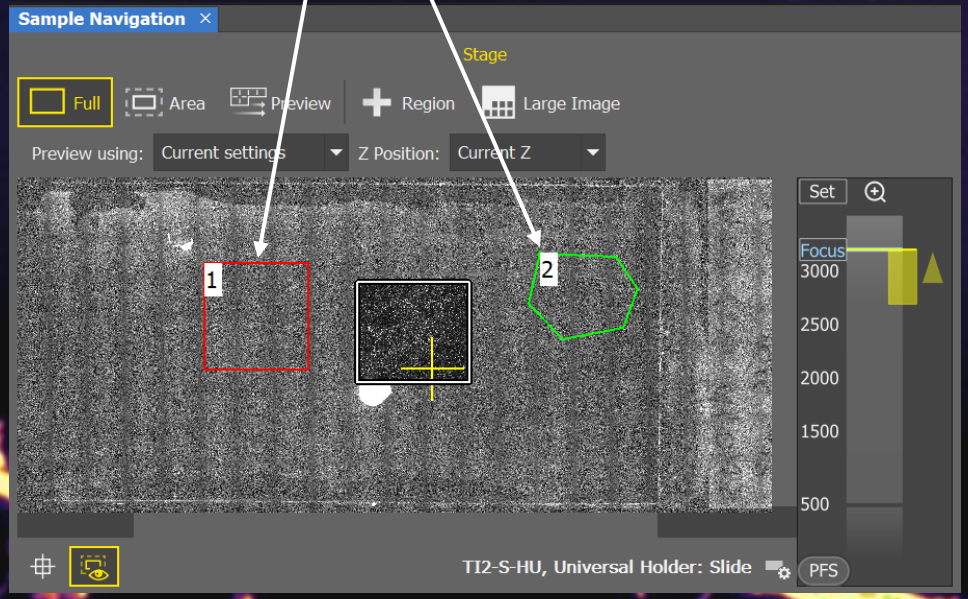
Area view of the selected area



Preview scans can be performed on both the full slide and the selected area - area scans will be faster, useful if you want a pre-scan of the area under a coverslip rather than the entire slide

Sample Navigation – Regions of Interest

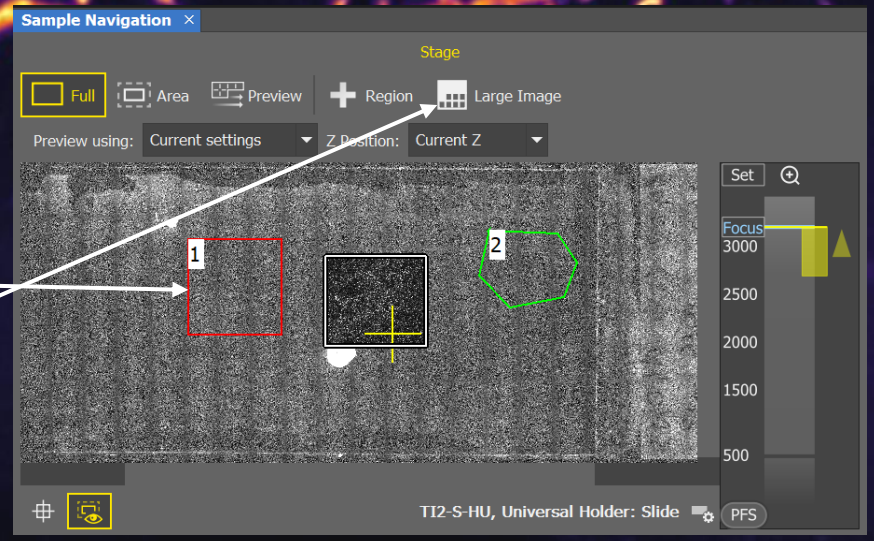
Clicking the ”Region” button will give you 4 options for drawing an ROI. Example ROIs are displayed here

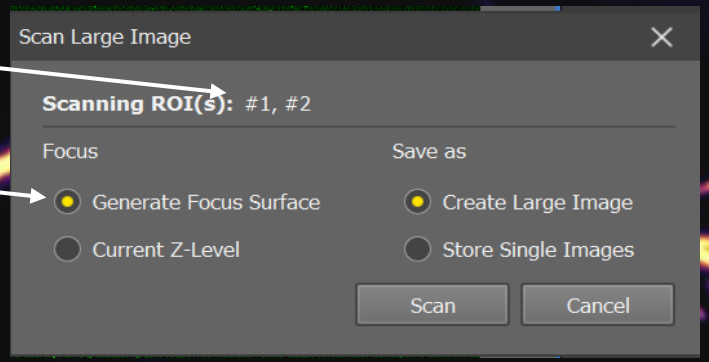


Sample Navigation – Large Image

Highlighting regions of interest allows us to take a large image of these regions

1. Select your region(s) of interest in sample navigation
2. Ensure you have the optical configuration that you want to use for imaging selected
3. In the sample navigation panel, click “Large Image”
4. Ensure all of the ROIs you wish to image are listed
5. Choose to either have NIS-Elements automatically generate a focus surface (recommended to keep entire image in focus), or image at your current Z-level across the image giving a true cross-sectional image of your sample
6. Click “Scan”



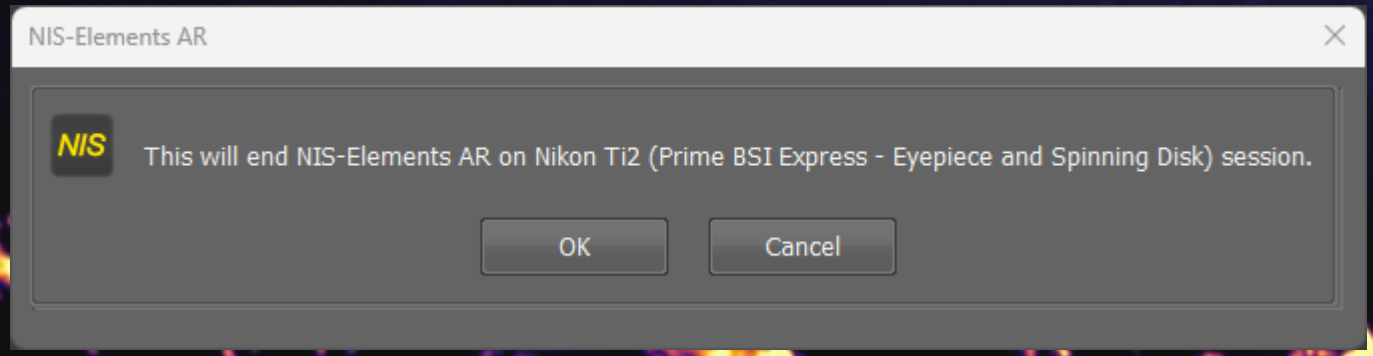


Ending Your Session

1. At the end of your session, ensure the image is frozen and click on “Eyes”
2. Lower the lens to the very bottom
3. Remove your sample and sample holder
4. Clean any immersion lenses you have used as described at the beginning of this guide
5. Always leave the microscope on the 20x lens, lowered to the bottom
6. Please clean up after yourself. The bins in the WCIC are for paper only, if you have any gloves / media / cells / slides you would like to throw away, please do so in the wet lab

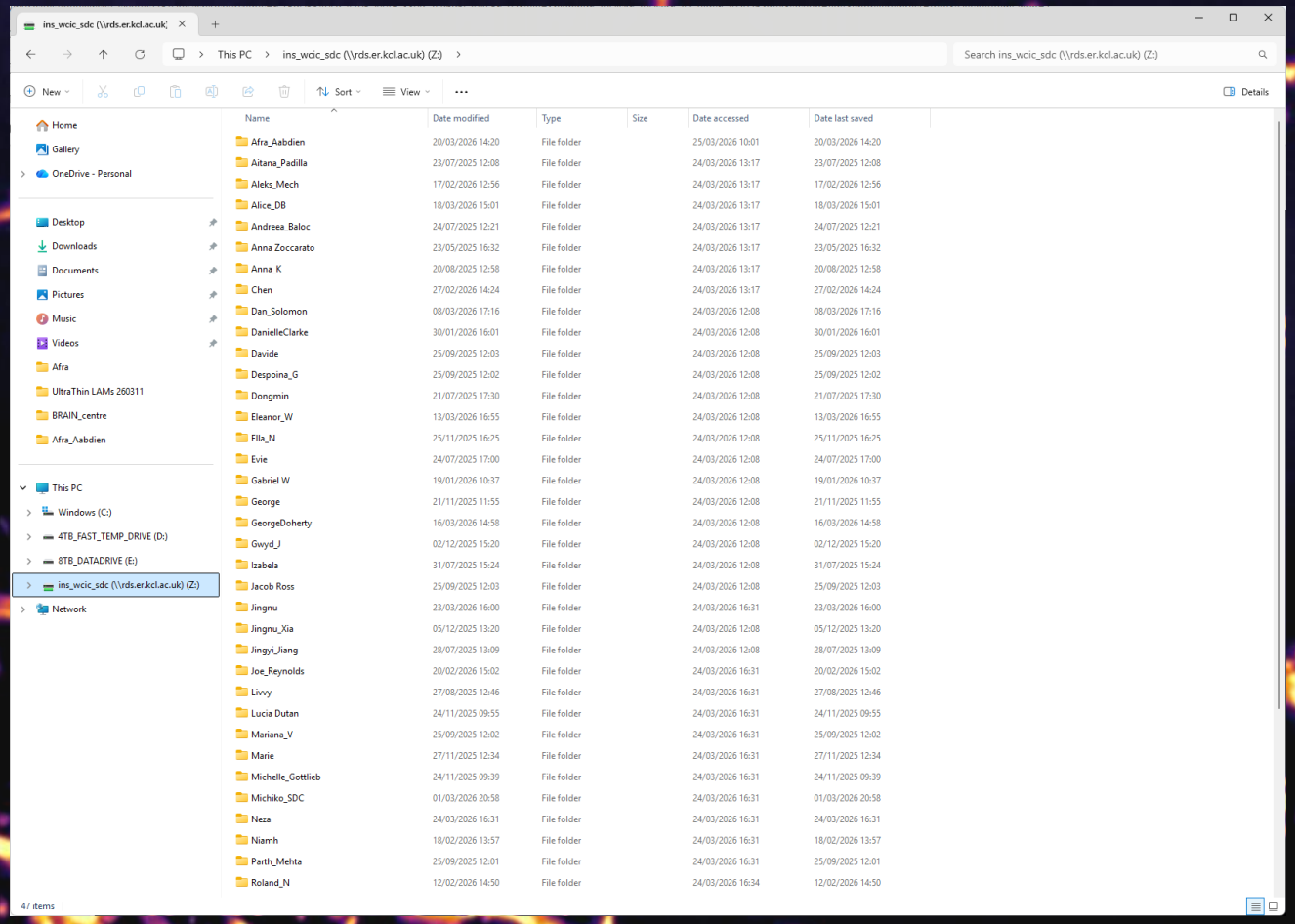
Closing the NIS-Elements Software

When you close NIS-Elements this warning will appear – click “OK”



Assuming you had Save to File set up correctly, all your images should already be saved

Data transfer



1. Go to file explorer
2. Find the folder where you have been saving your images from todays session
3. Copy
4. Find your folder in the shared network drive – if this is your first time using the system, create one in your name
5. Paste today's folder into your network drive folder – this can be accessed from anywhere on the KCL network. Access instructions are on the PPMS website under "Documents"

This shared network is for data transfer only, it is not a backup storage, please connect your own computer to this shared drive (instructions can be found in your PPMS booking system > Documents > Accessing The Network Drive) or book and go to the workstations with an external storage device, and copy and paste your data into your personal backup storage.

DO NOT plug in USB or external storage into the Microscope PCs!!!!

Check PPMS Booking System

1. Always check PPMS at the end of your session to see if anyone else is using the microscope after you

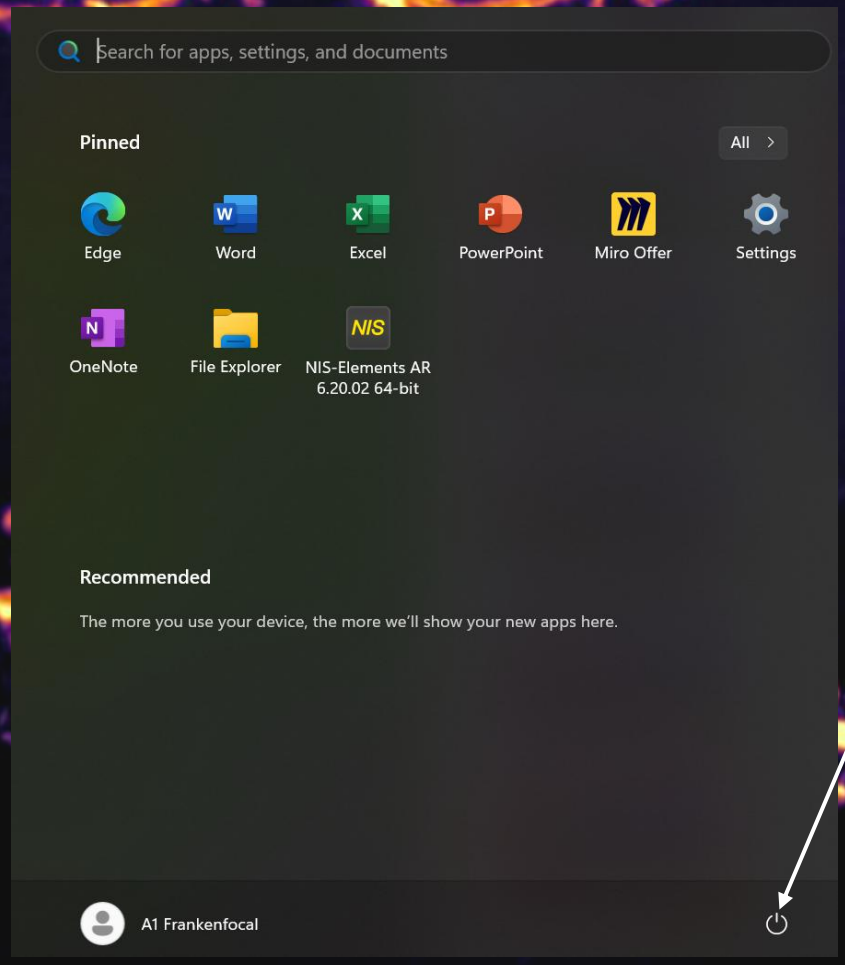
2. If another user is booked on within 2 hours, make sure NIS-Elements is closed and you have transferred your data, then leave the computer and microscope running and log out of PPMS on the bottom right

3. If no one is booked within 2 hours of the end of your session, please close the NIS-Elements software, then shut down the computer and microscope by following the numbered switches in reverse order, allowing a few seconds between each

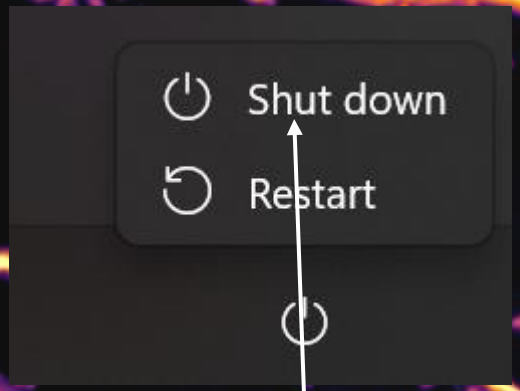
4. Please do not switch off the computer using the button on the computer – instructions for shutting down the computer are on the next slide

Powering Off the Computer

1. Open windows menu



2. Select power options



3. Shut down

Please don’t email us if you need help during your session.
If you need urgent (in session) help, please contact us on WhatsApp or Using Teams:

George Chennell 07435 737 423
Chen Liang 07469 356 576
Josh Kellet: 07553 416 467
Or use Teams Instant Message

Happy Imaging