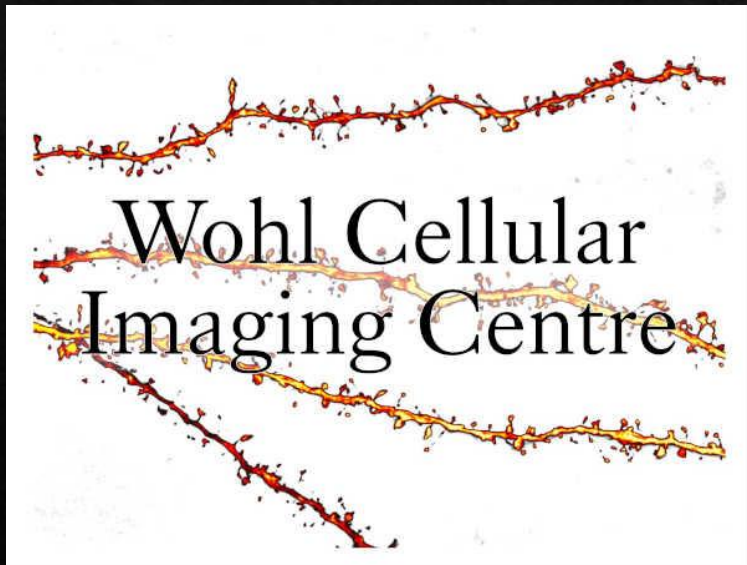


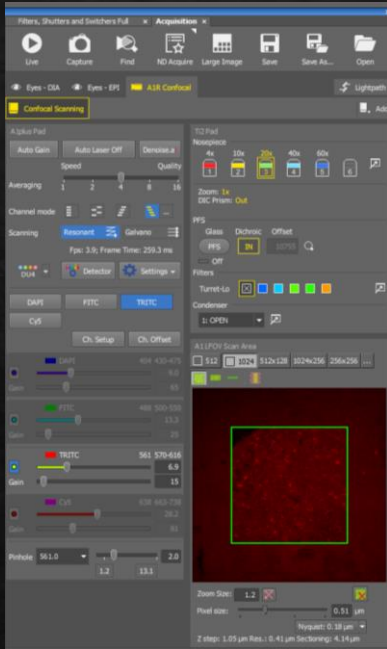


Scan Large Image

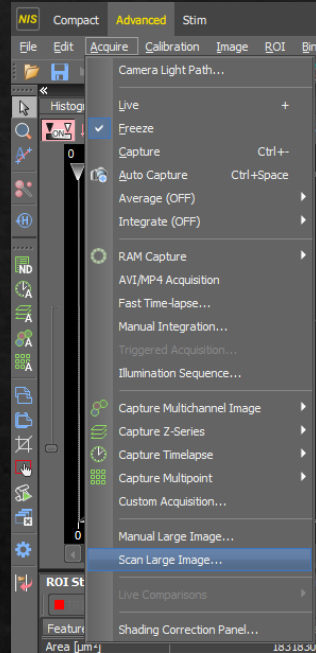


The Scan Large Image application allows users to define a region in the sample and set multiple focus points so the whole region remain in focus during imaging.

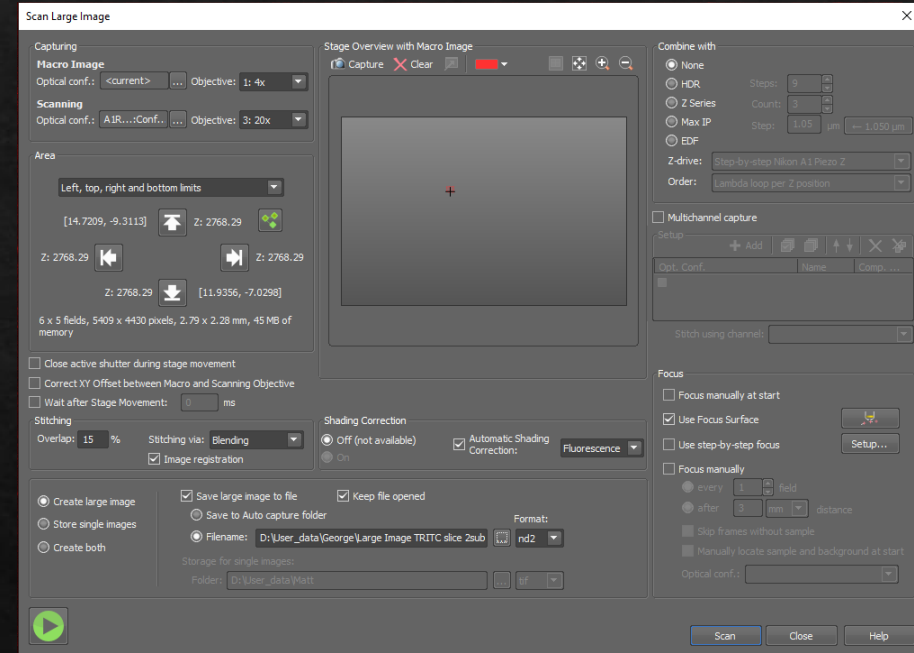
Step 1 – Bring up Scan Large Image window



Once you are happy with your confocal scanning settings...

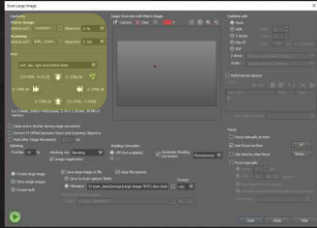


Click on scan large image here...

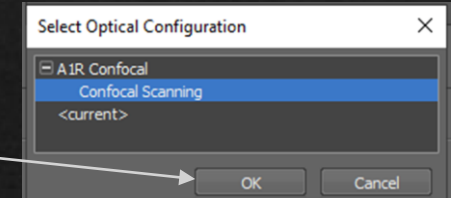


This window should appear.

Step 2 – Set the scanning area

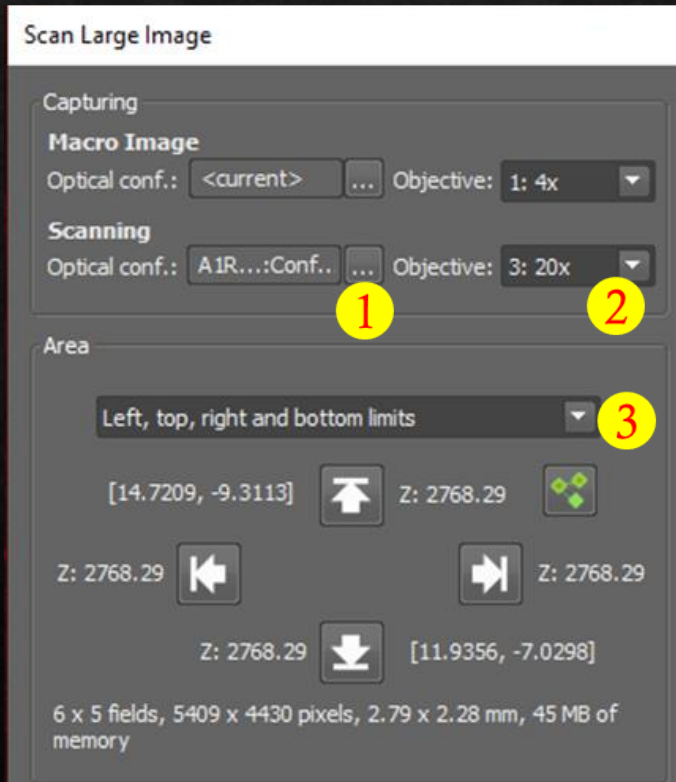
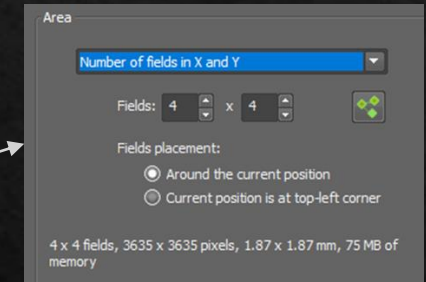


1 Click the ... which should open the 'Select Optical Configuration' box, click 'Ok'

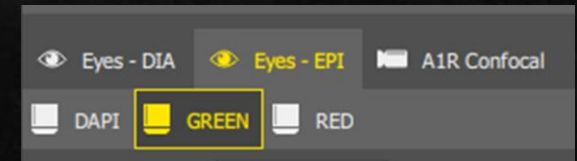



2 Select the lens you are currently using.

3 Most users set their scanning area by setting up 'Left, top, right and bottom limits'. The other option is 'number of fields in X and Y'.



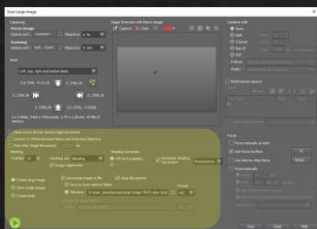
To set the scanning area, most users return to the EYES option, please make sure fluorescence or brightfield is working and you can see your sample in focus, before moving the joystick.



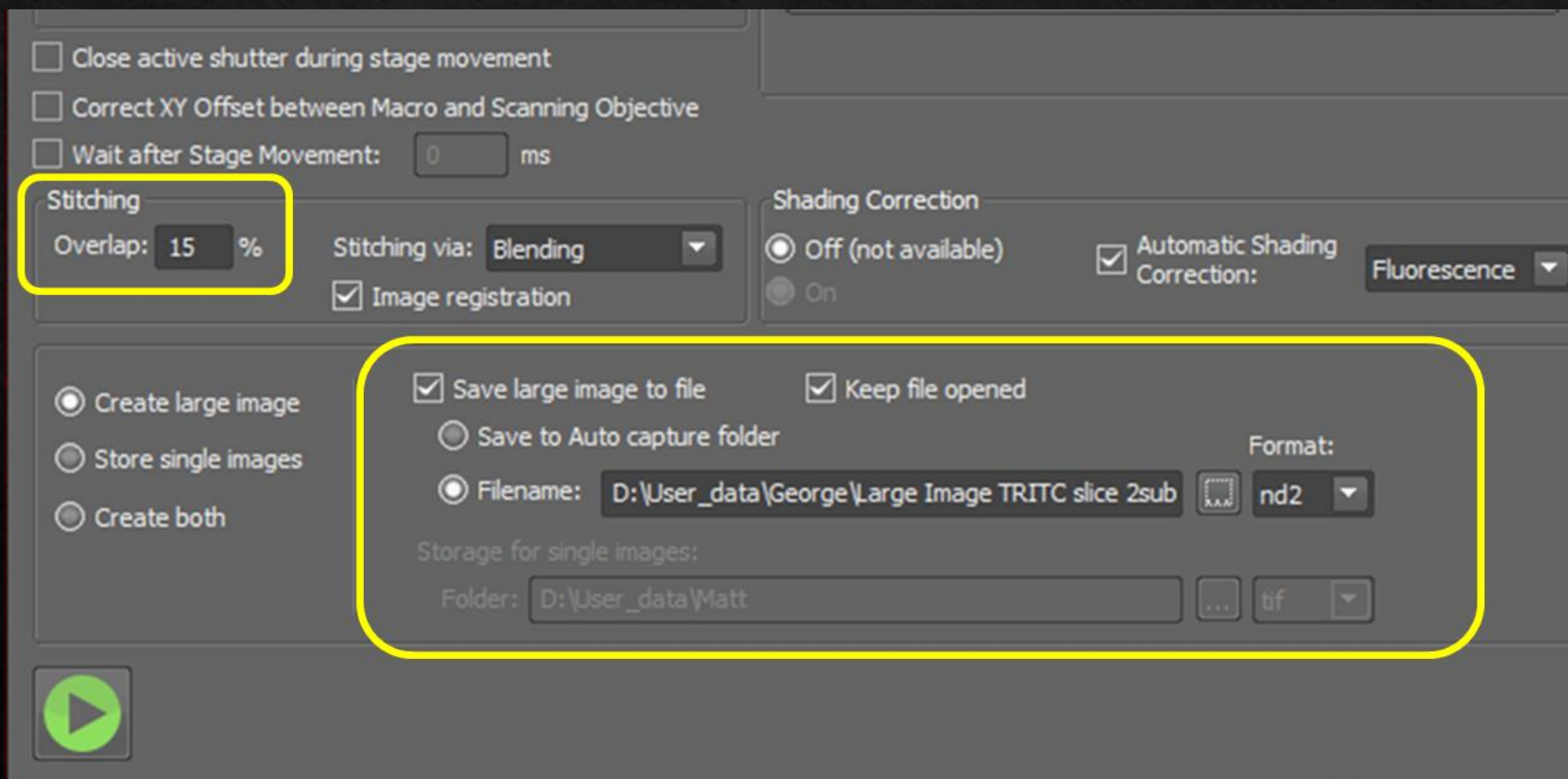
Go to the far left of the area you want to scan, click on  then go to the top, right and bottom respectively to set the scanning area.

If this is greyed out, you need to repeat 1

Step 3 – Other initial settings...



These are good initial settings you can copy, please make sure overlap is 15% minimum.

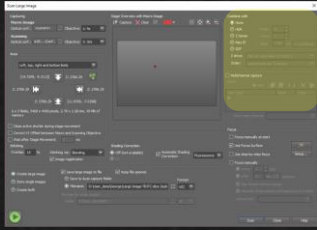


Save Large Image To File!!!

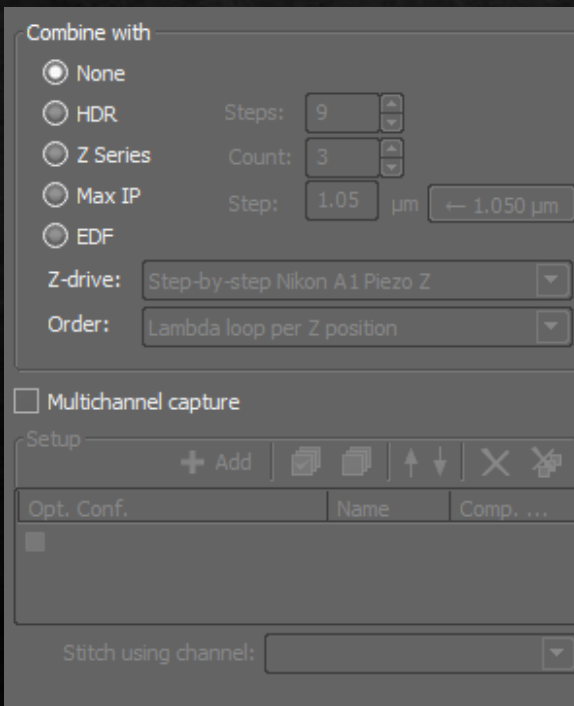
If you want to save your large images, please set this up.

Save it to your usual folder on the local PC, e.g. on the A1R inverted... This PC < Datadrive D < User Data < Your folder

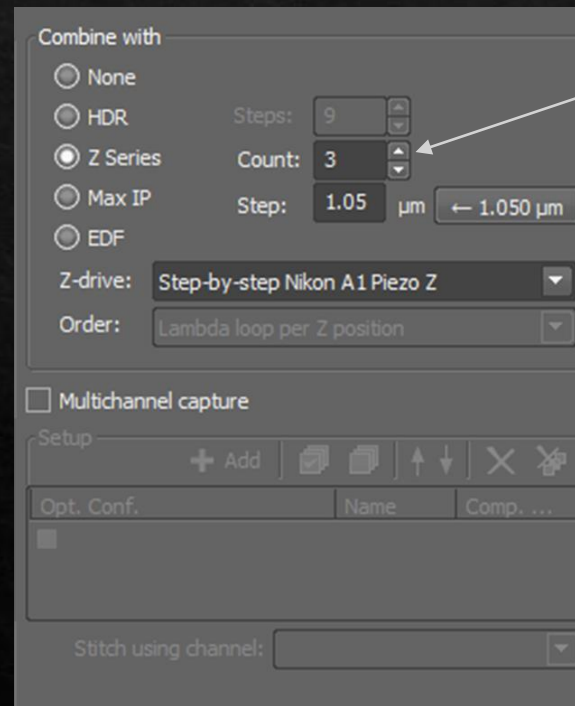
Step 4 – Do you need a 2D or 3D large image?



2D Settings



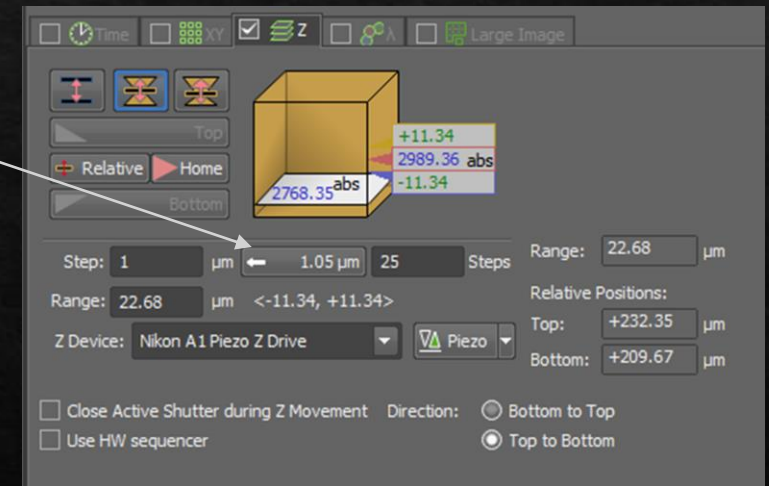
3D (Z-stack) Settings



Choose how many steps here

Recommended step size

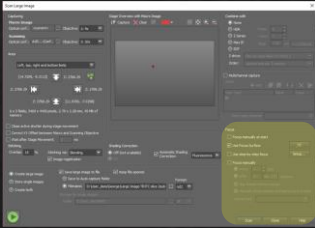
Setting up Z-stack in this application is similar to this one...



... your focus plane will automatically be used as the middle of your stack.

Step 5 – Focus

There are multiple ways to set up focus, depending on what your sample needs...



Manual focus

If your sample/mounting is very flat and monolayered, you can **'Focus manually at start'** and that might be enough for you to remain in focus.

If you want to be a little more precise, you can choose **'Focus manually'** which means the machine will pause throughout imaging, so you can adjust your focus, using your mouse wheel if/when necessary.

A screenshot of the 'Focus' settings dialog box. The dialog has a title bar 'Focus' and several options:

- Focus manually at start
- Use Focus Surface (with a button showing a glass icon)
- Use step-by-step focus (with a 'Setup...' button)
- Focus manually
 - every 1 field
 - after 3 mm distance
- Skip frames without sample
- Manually locate sample and background at start
- Optical conf.: [dropdown menu]

At the bottom of the dialog are three buttons: 'Scan', 'Close', and 'Help'.

More automated focus

If your sample/mounting is tilted, you can **'Use Focus Surface'**.

If your sample is folded or not very flat, then **'Use step-by-step focus'** might be the better option.

The rest of this guide will help you set up the above options.

Step 6 – Use Focus Surface

This allows you to define focus at different points within the scanning area you've already set up.

The screenshot displays the software interface for microscope scanning. Key components include:

- Capturing Panel:** Shows 'Macro Image' and 'Scanning' settings. The 'Scanning' objective is set to 3: 20x.
- Stage Overview with Macro Image:** A central window showing a grid of scanning fields with a red square indicating the current focus area.
- Combine with Panel:** Includes options for 'None', 'HDR', 'Z Series', 'Max IP', and 'EDF'. The 'Z Series' is selected with a count of 3 and a step of 1.05 µm.
- XYZ Overview Panel:** A panel with tabs for 'ND Acquisition Overview', 'Focus Surface', 'PFS Surface', and 'Document Overview'. It contains navigation icons and a coordinate system.
- Graph Panel:** A plot showing Z-position (from 2668.3 to 2868.3) versus field numbers (#1 to #4). A red dashed line indicates the focus surface at Z = 2768.3.


Callout Box: A yellow box with the text: "First click on this button to bring up XYZ Overview panel, then tick on 'Use Focus Surface'. You can see the green points where you've defined your scanning area earlier." A yellow circle with the number '1' highlights the 'XYZ Overview' button.

Step 6 – Use Focus Surface

Now go to each green point and set your focus, add more focus points if you need to.

To add more focus points, just double click within your scanning area and then press this 'Add Point' button.

Once you are in focus, press this 'Z' button to redefine the focus, before double clicking on the next green point.

Tick 'Move Stage to Selected Point' and click on  button.

Now you can double click on a green point, then press the LIVE button to bring up your live screen and focus using your mouse wheel as usual.

Scan Large Image

Macro Image: [None] Objective: 1: 4x

Scanning: [ABR...Conf] Objective: 3: 20x

Area: [Left, top, right and bottom limits] [14.7209, -9.3111] Z: 2768.29

Stitching: [Overlap: 15] [Stitching via: Blending] [Image registration]

Focus: [Focus manually at start] [Use Focus Surface] [Use step-by-step focus]

LIVE

Step 6 – Use Focus Surface

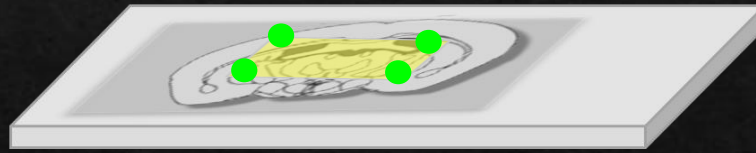
You can add focus points anywhere within your scanning area, these are some examples.

Flat but tilted sample.
You want to image the whole sample.



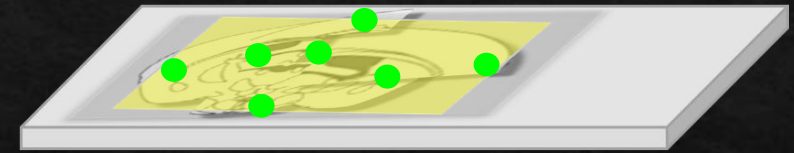
Set left, top, right and bottom limits, because you are unable to see your sample (and therefore set focus at) each corner.

Flat but tilted sample.
You want to image a smaller area within your sample.



Best way to compensate for a tilted sample is to set the points at each corner.

Folded or broken sample.



You can set additional points in the middle of your scanning area for more problematic samples.

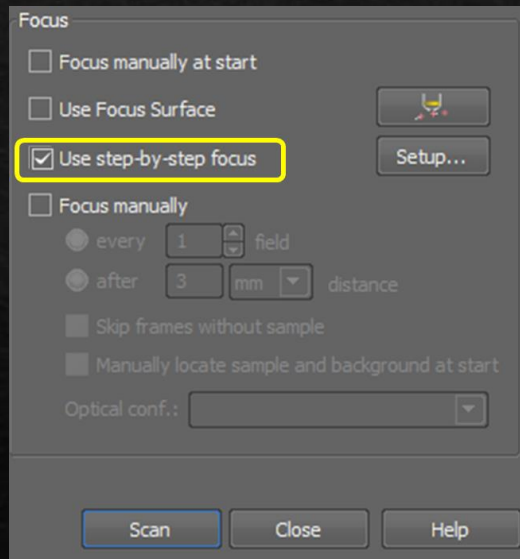
OR

Use step-by-step focus
(more info on next page)

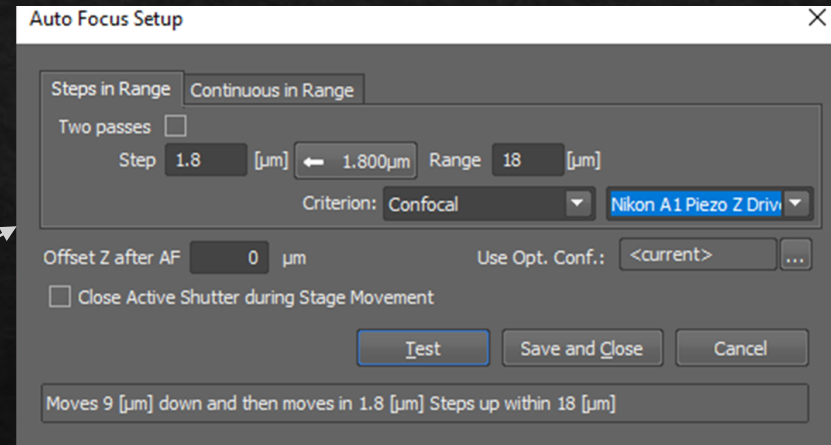
Step 7 – Use step-by-step focus

If Step 6 - 'Use Focus Surface' isn't sufficient, then this applications might be a better option. This is more time consuming, before imaging at each location, the machine takes a very rough z-stack, it automatically looks through the stack and uses the most in focus layer as your focus.

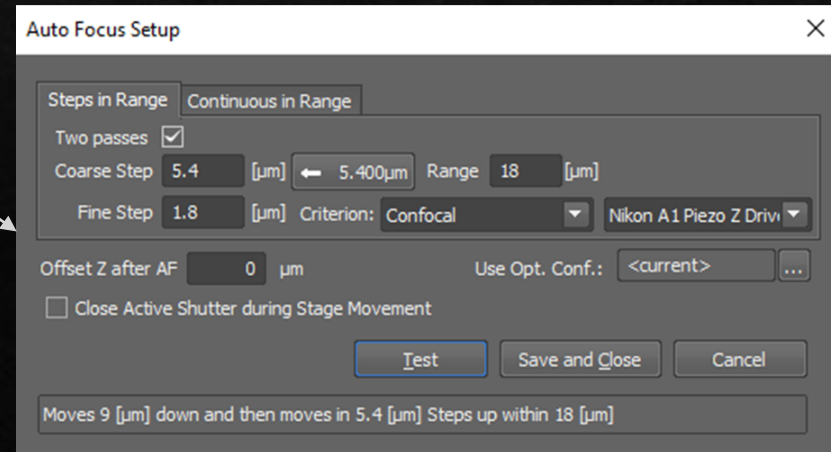
Tick on 'Use step-by-step focus' then press the 'Setup' button.



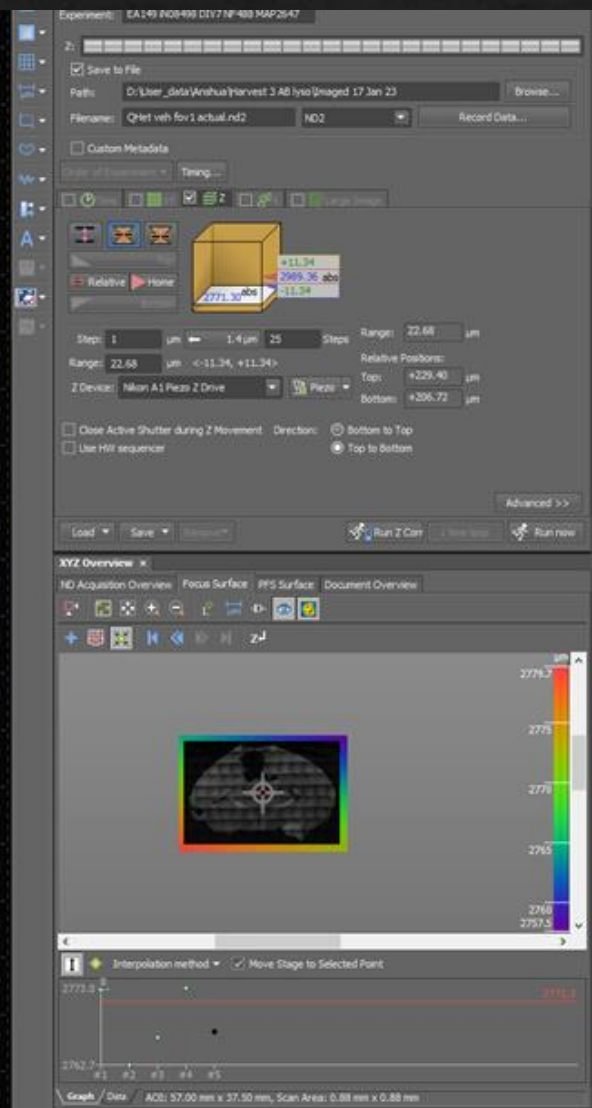
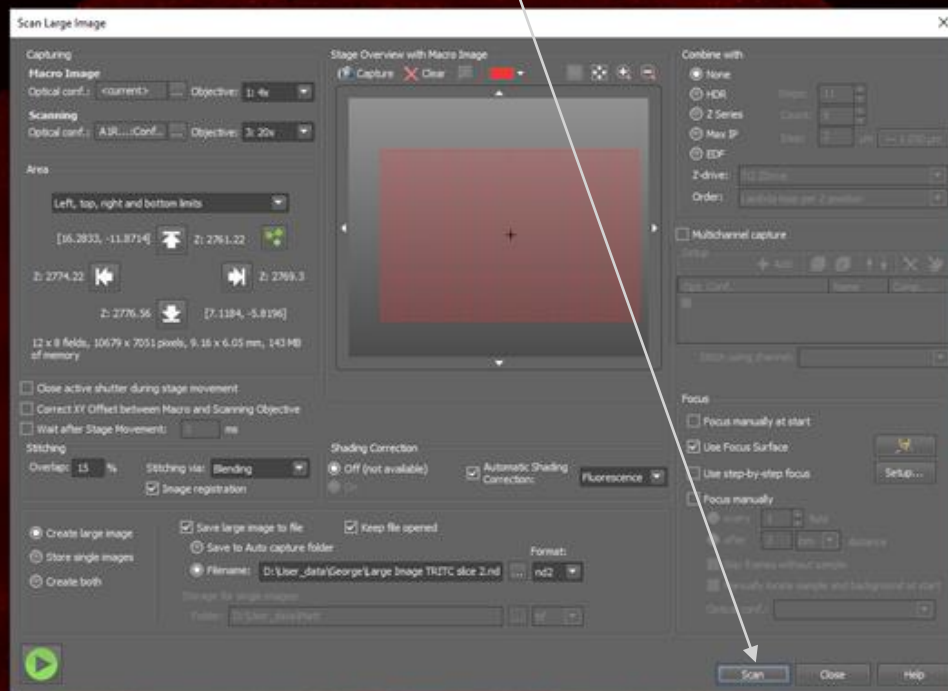
Single Pass



Two Passes



Step 8 – Press Scan

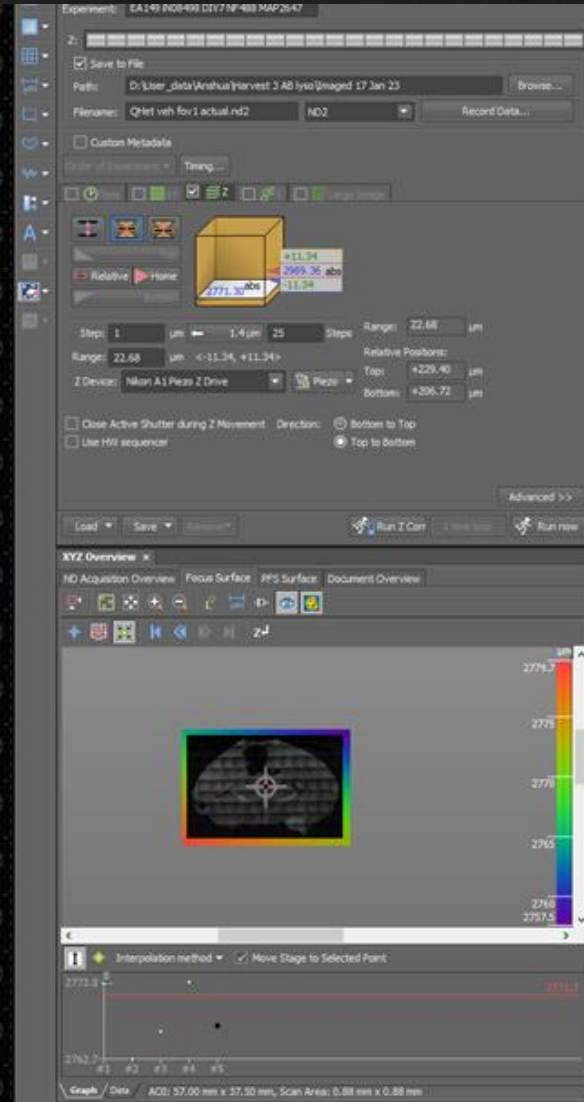
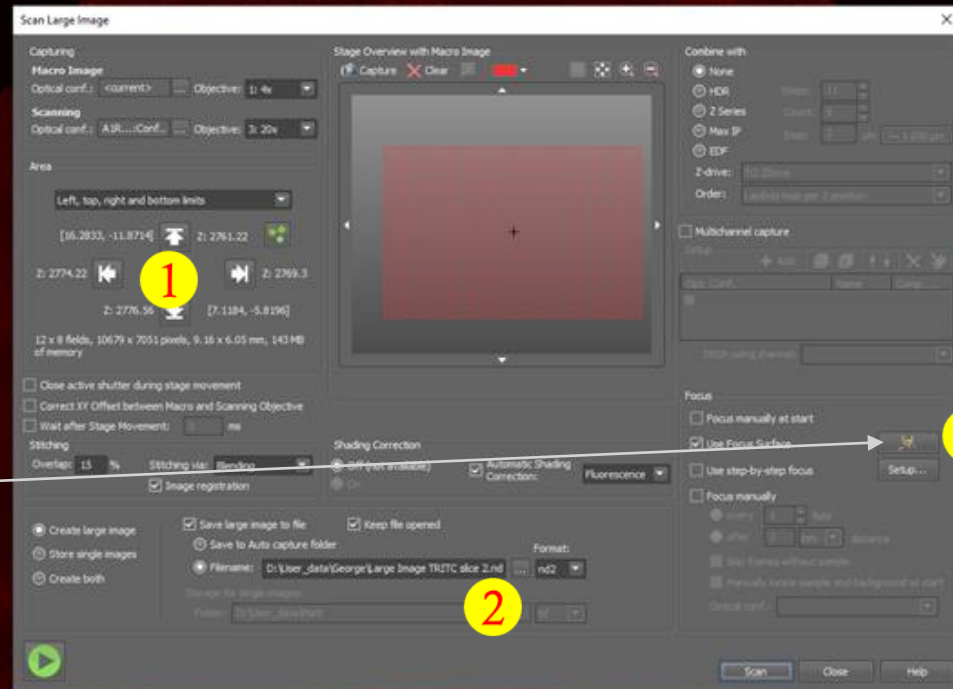


Step 9 – Ready for the next scanning area?

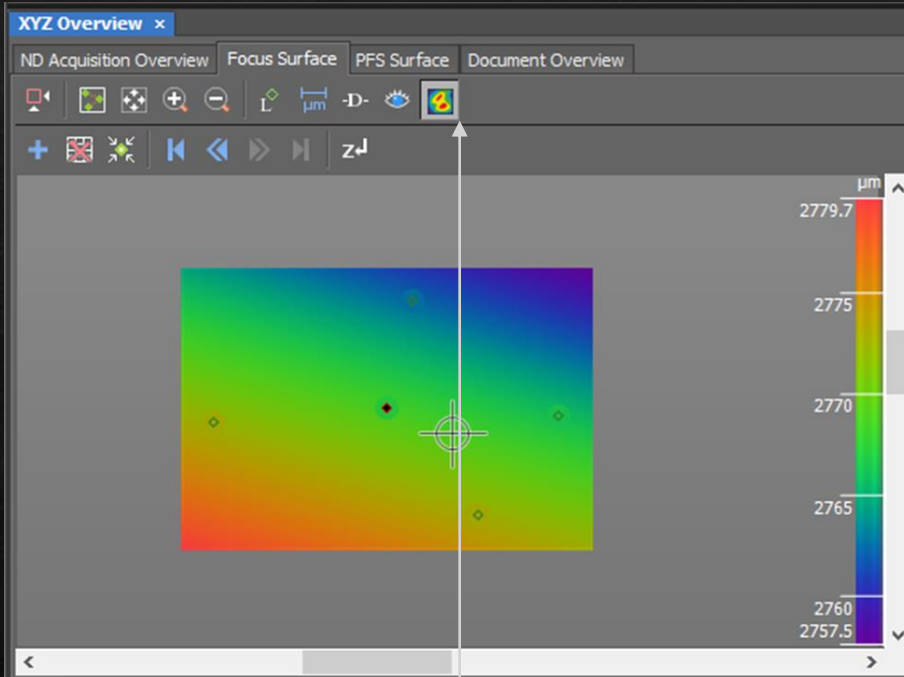
1 Go back to Eyes and set up 'Left, top, right and bottom limits' like before.

2 You need to input a new filename before taking the next image. Rename here or press on the ... button if you need to save it into a different location.

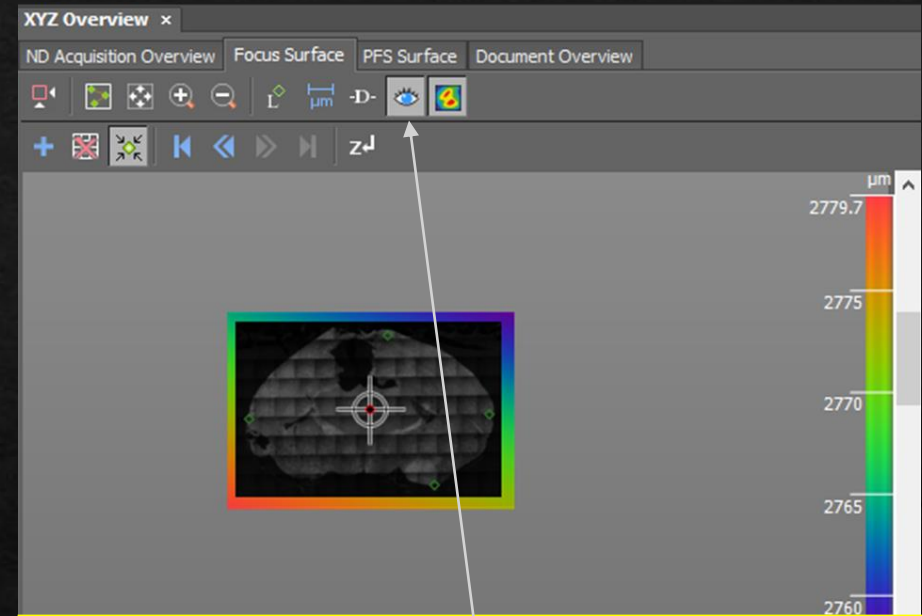
3 Then press this to update the XYZ Overview panel and set your focus like before.



Finally, a few neat tricks you can try...



After you've set your focus at multiple points, this allows you to visualise the tilt.



You can do a quick scan of your sample (without averaging, or use a 4X or 10X lens, lowering the lens rule between switches still applies, please be careful.) Then select a smaller region within your sample by double clicking to move stage to selected point, then set the 'Left, top, right and bottom limits' accordingly.