Primo Micropatterning/Color Imaging System Standard Operation Protocol

Contents

Imaging and Flow Cytometry Core

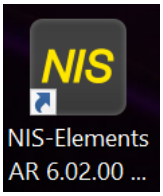
Color Imaging

Turn on System

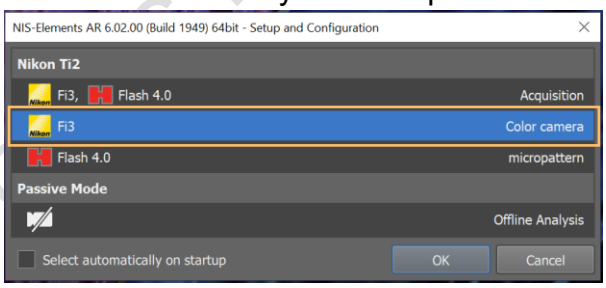
Please sign on the log sheet before switching on system.

1. Switch on main power control ① wait for at least 5 sec before next step
2. Switch on microscope controller ② wait for at least 10 sec until the stage stops moving before next step
3. Turn on computer power ③

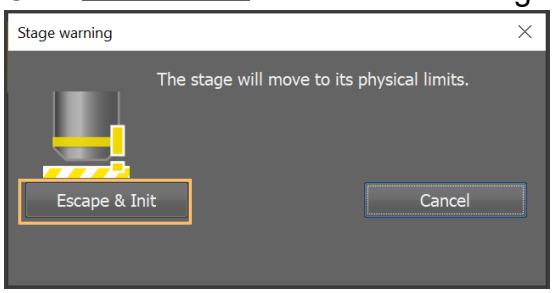
4. Click to log in **USER** account at the startup screen
5. Wait for PPMS Tracker to Pop-up, then login with your username and code
6. Double click NIS-Elements AR to start the NIS-elements software.



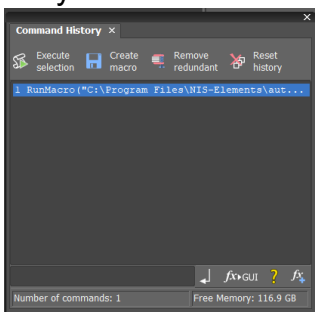
7. Choose Fi3 Color camera, click “OK” on setup and configuration, DO NOT check “select automatically on startup”.



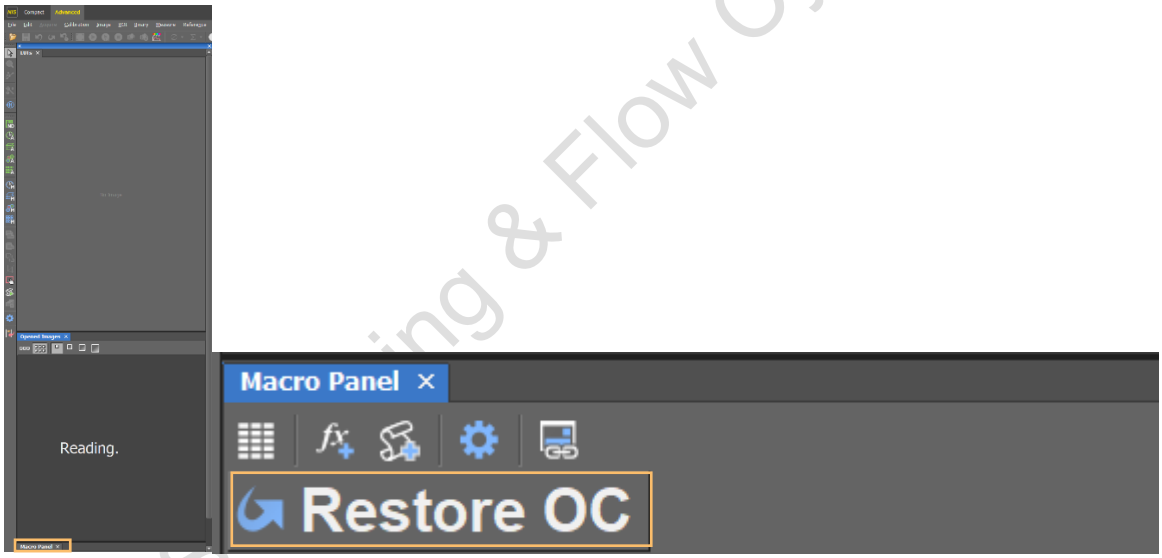
8. Click Escape & Init to initialize the stage.



9. Fully close the Command History Interface once stage has been initialized



10. Click the macro pannel, then Click Restore OC



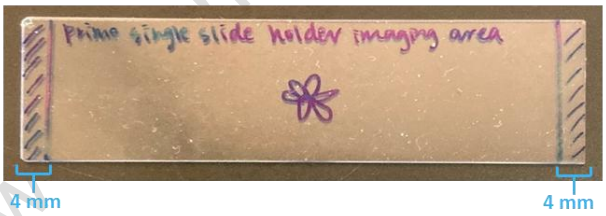
Imaging and Flow Cytometry Core

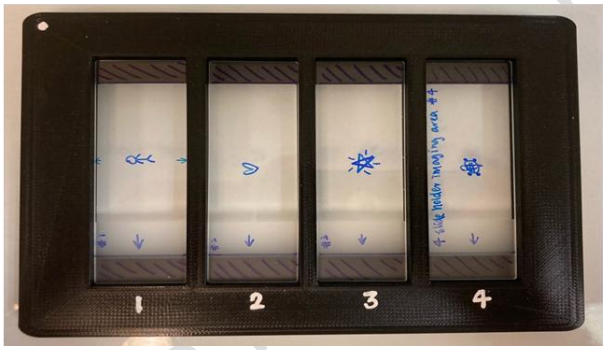
Sample Locating and Focusing

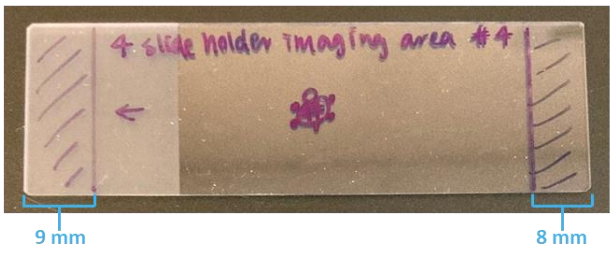
Load Sample on the Stage

1. Check sample and clean it if there is any liquid (e.g. mountant, PBS, oil, not fully dried nail polish).
2. Fix the sample(s) on the slide holder with **coverslip facing down**.
3. Put the slide holder on the stage.

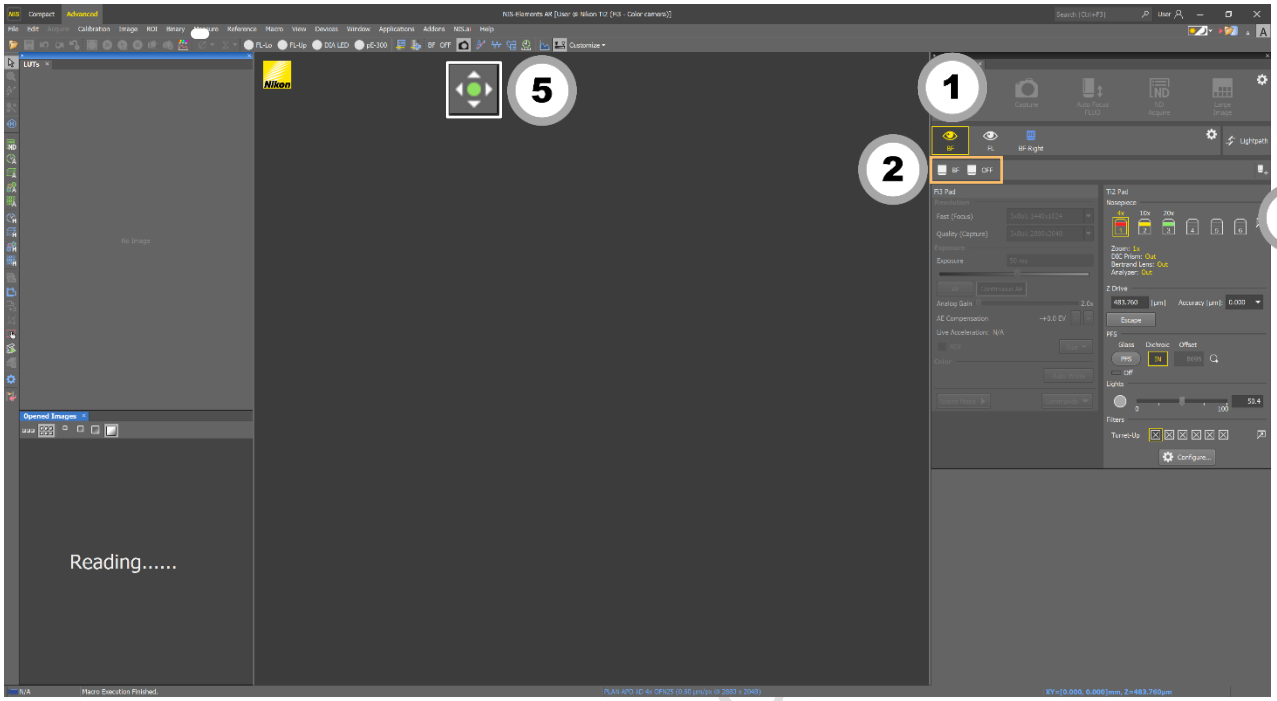
Area that will be blocked by slide holder (highlighted by blue diagonal lines)
Please do not mount coverslip on those areas as it will generate a tilted angle across the slide after fixing on the slide holder.







Eyepiece Observation



1. Select "**Eyepiece - BF**"for Brightfield imaging.
2. Select the desired optical configuration (OC).

Eyepiece - BF

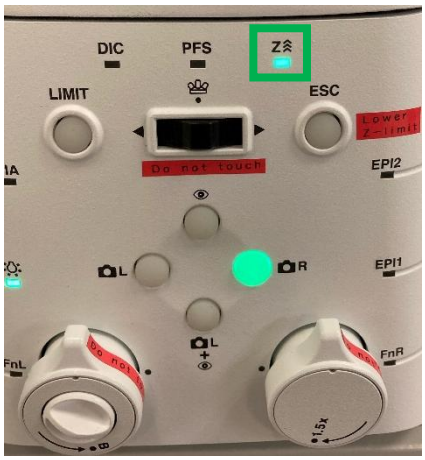
BF (Bright-field)	**OFF**
Brigh-field light is turned on	Brigh-field light is turned off

3. Select the desired objective (low magnification is recommended).
4. Change the light intensity if necessary.
   Brightfield – light intensity is adjusted using the knob on the side of the microscope.

5. Press  to adjust XY position by moving mouse.

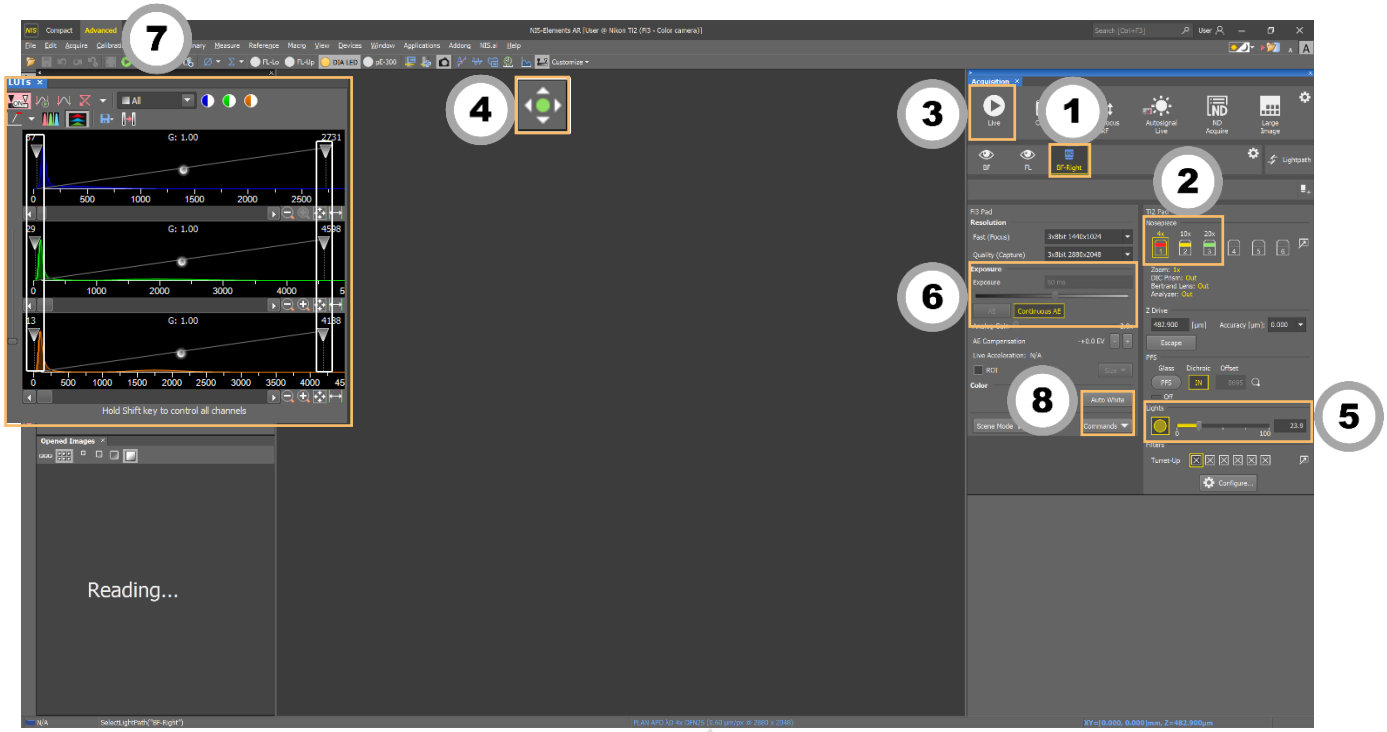
6. OR Move the Stage Controller to adjust XY position.
7. Focus the sample with the focusing knob.

   (Focusing speed can be adjusted by the Z^^^ button. Maximum focusing speed is reached when the indicator light is ON.)



Imaging and Flow Cytometry Core

Bright-Field Imaging



1. Select “**Camera - BF**”.
2. Select the desired objective.
3. Click Live to display the image.
4. Click to allow moving your sample with your mouse or use focusing knob.
5. Adjust the light intensity if necessary.
6. Click AE to adjust the exposure time automatically if necessary, or adjust exposure time manually. Continuous AE can be used to adjust exposure automatically during Live mode.
7. Click to adjust the LUTs automatically if necessary.
   *** Pulling the left arrow toward the spectrum can eliminate the background.**
   *** Pulling the right arrow toward the spectrum can make the image brighter.**
8. Move to empty background and click Auto White to correct the white balance automatically if necessary.

   If manual white balance correction is needed, click Commands → Advanced Camera Settings to adjust the R/B ratio.

Imaging and Flow Cytometry Core

Save Images

1. Click 

to capture the image.
2. Click 

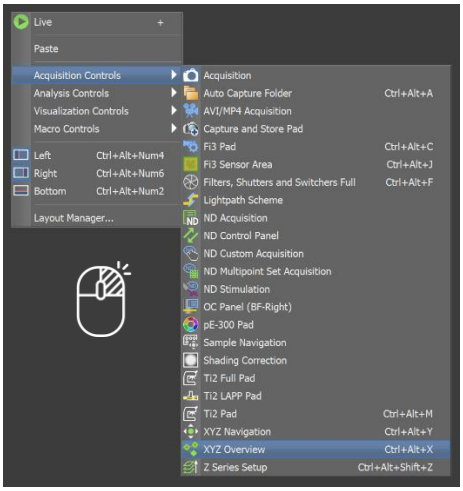
to save the image (all data should be saved to D drive/User Data under your name) .
3. Change the file name and select the file format.

File format	Features
.nd2	● Recommended ● Largest file size can save all the information including the camera and device settings of your image ● Cannot be opened by Windows
.tiff	Can be opened by Windows

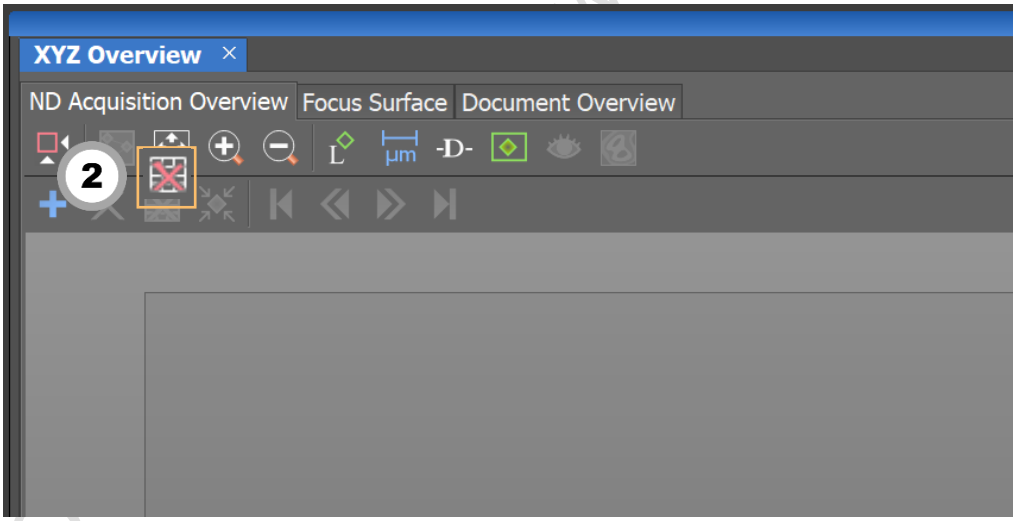
Imaging and Flow Cytometry Core

Stitching / Scan Large Image

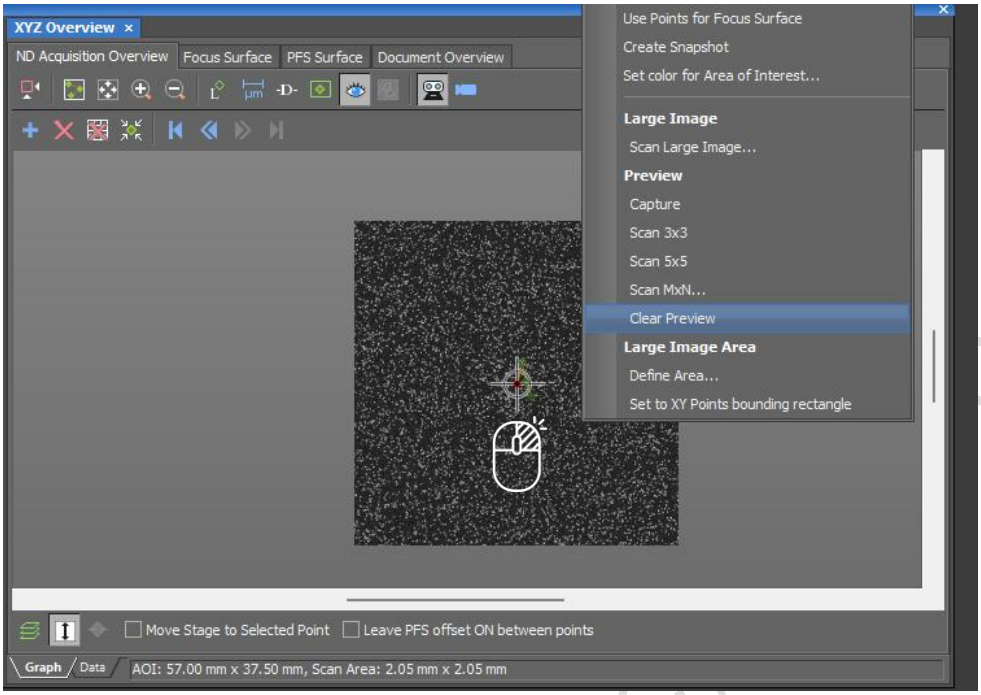
1. Right click at live window and select  "**XYZ Overview**" in Acquisition Controls.



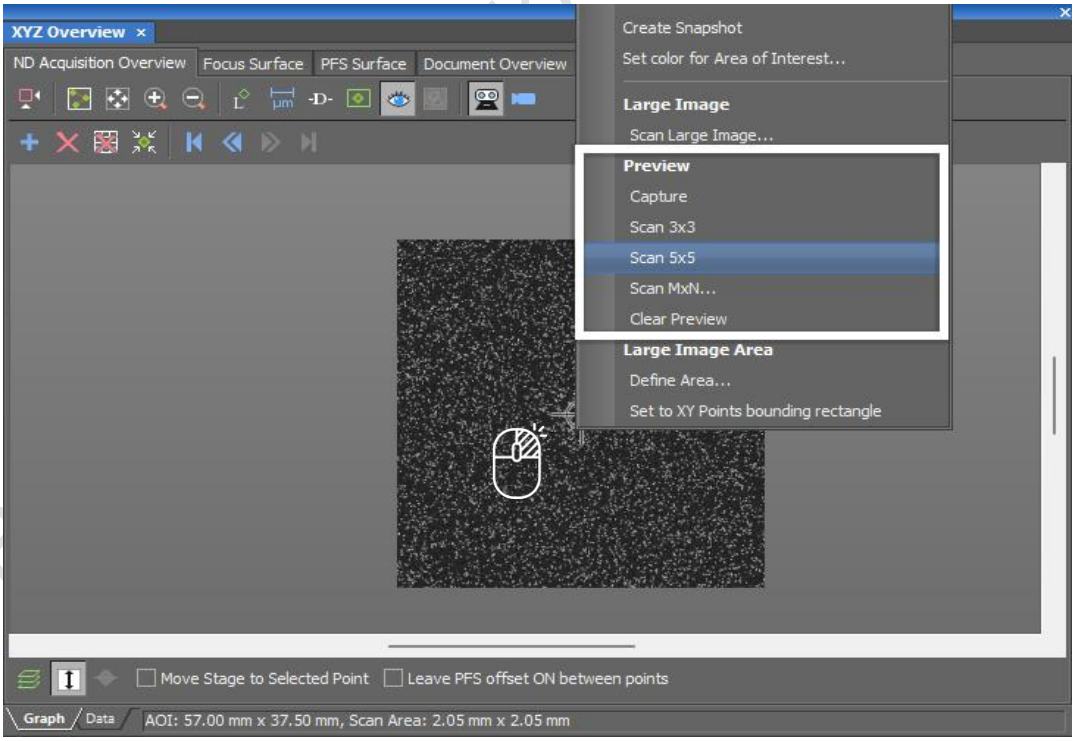
2. Click  to remove the previous data.



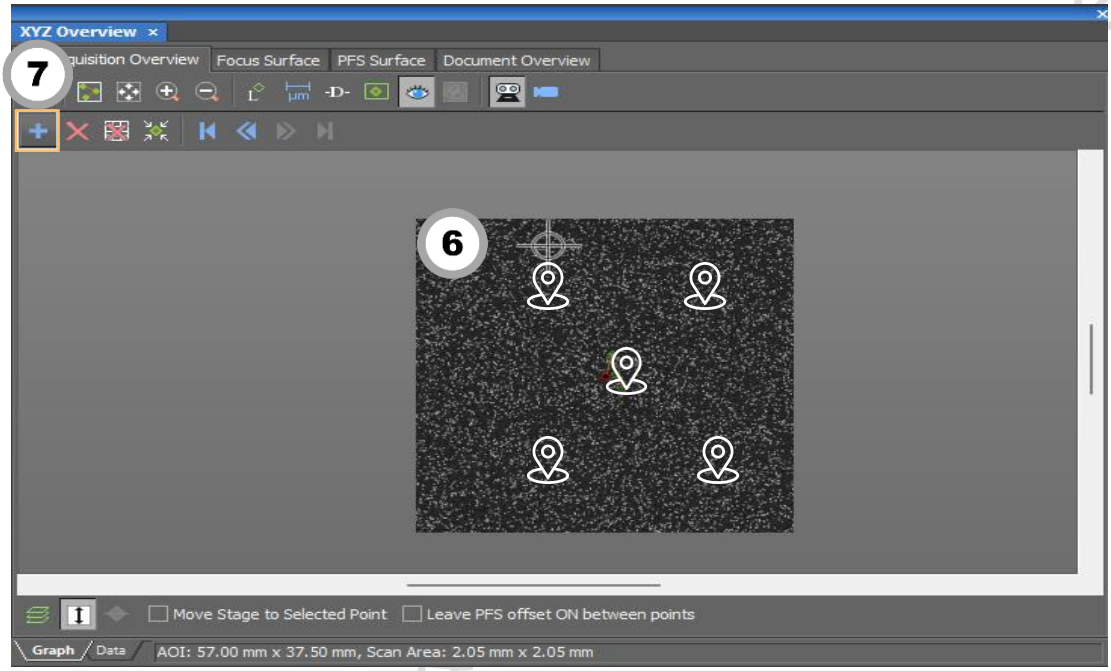
3. (Optional) Right-click on the imaging area, and select "**Clear Preview**".



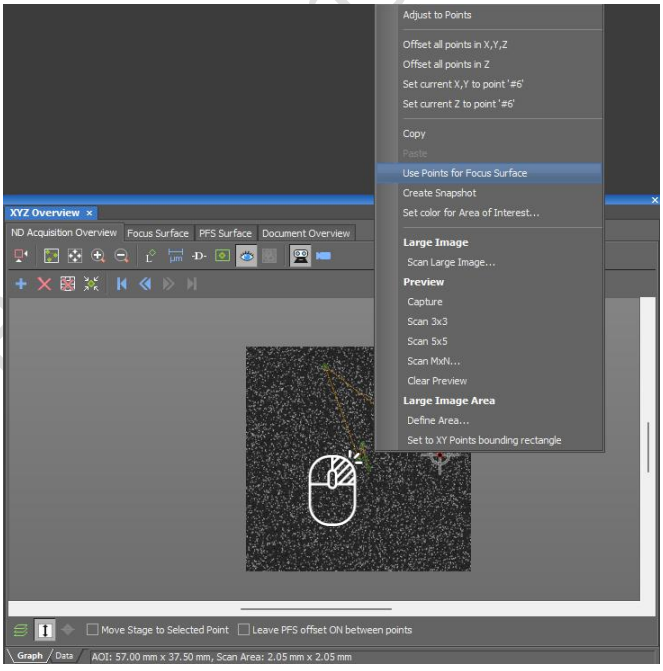
4. Click "Live" and adjust the light intensity and exposure time if necessary.
5. Right-click on the imaging area, and decide the preview area to scan a preview image.



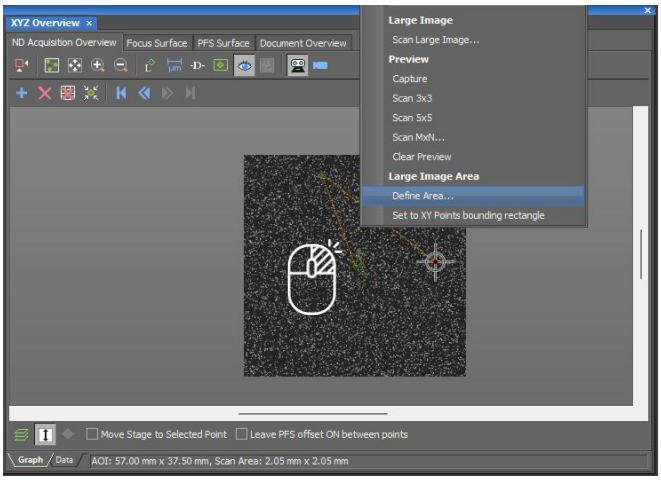
6. Double-click on the image to move the stage to a specific point.
   If your specimen is even and doesn't need to add focus points. You could skip step6-9 and directly go to step10.
7. Adjust the focus and press  to add the points.
8. Repeat steps 6-7 until all points are added.
   *points can be added at the edges of your sample and center (as shown in above picture).



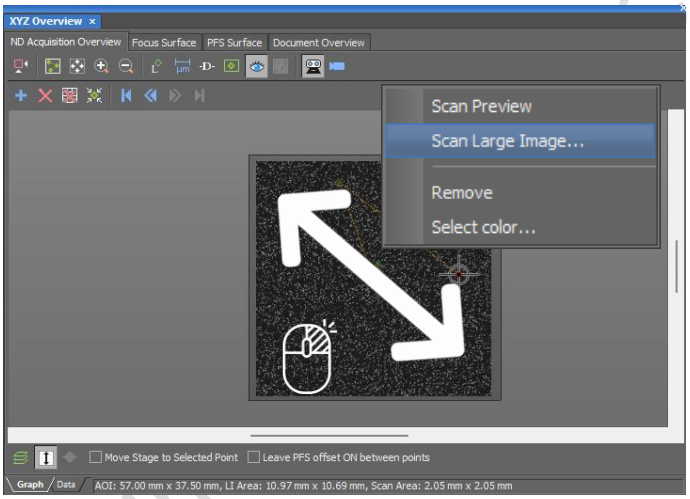
9. Right-click, and select "**Use Points for Focus Surface**".



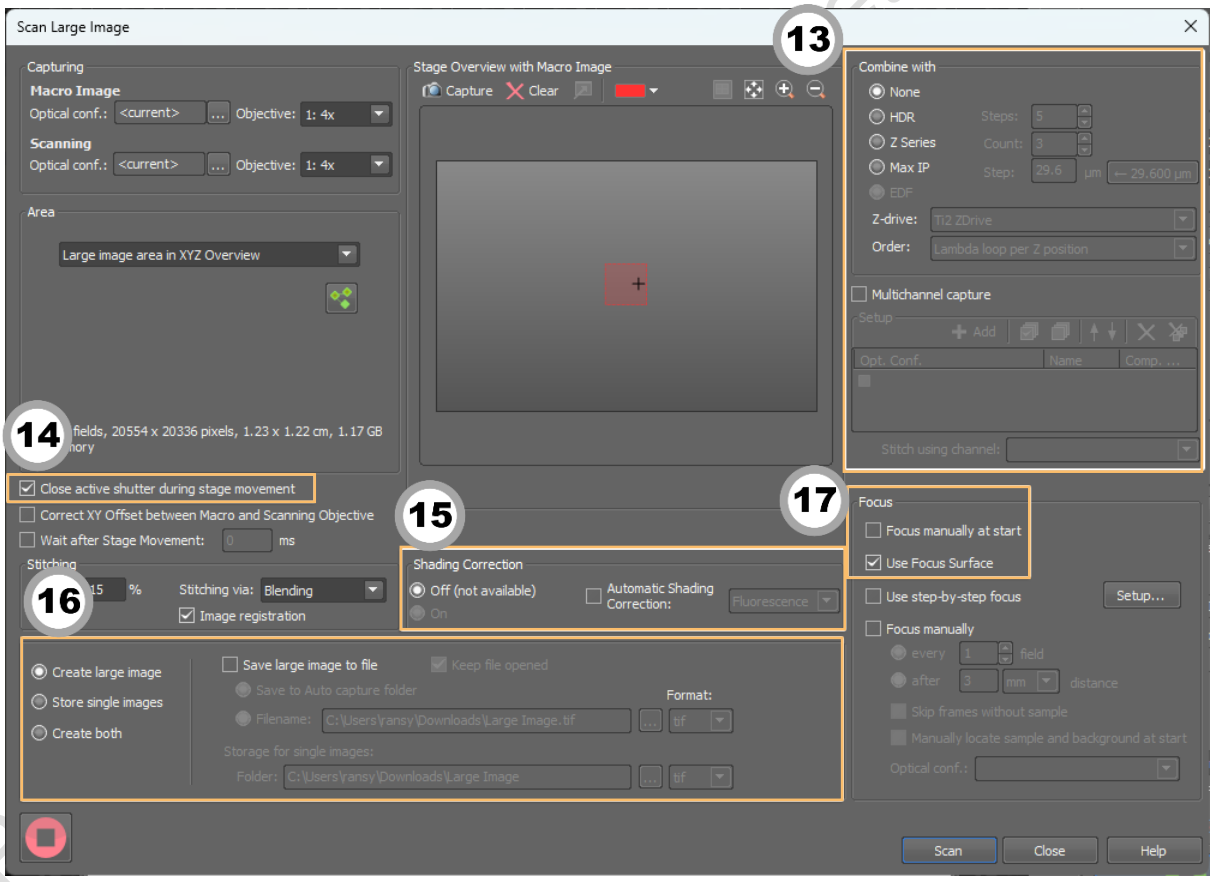
10. Right-click, and select "**Define Area**



11. Drag to set the scan area. Adjust the scan area by dragging the boundary if necessary.
12. Right-click, and select "**Scan Large Image…**".

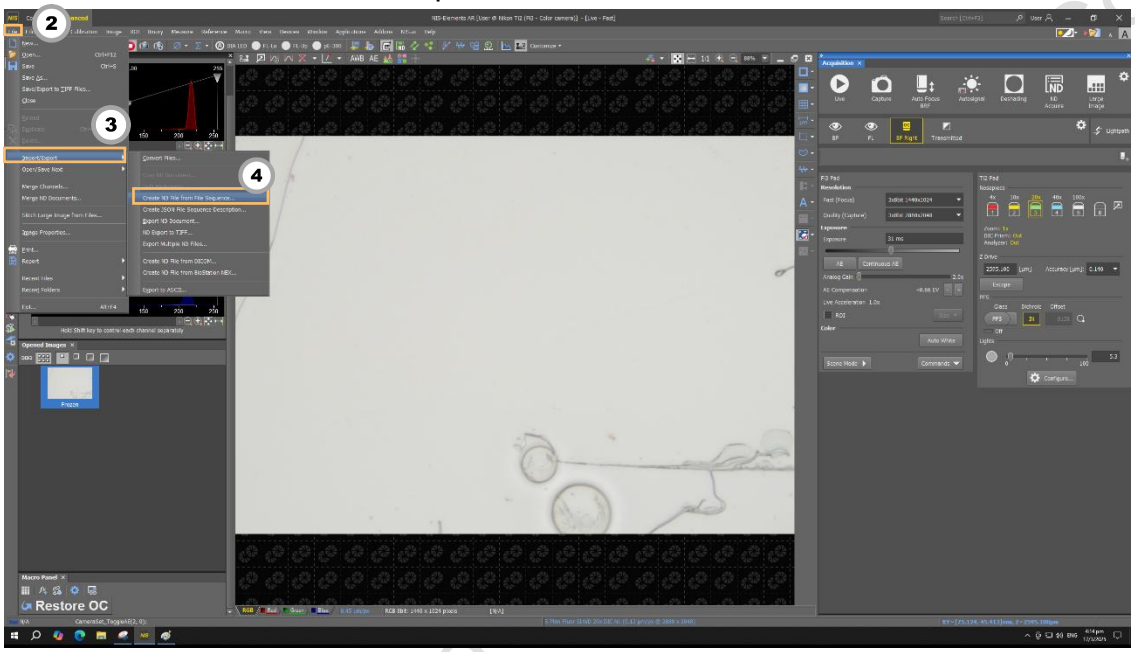


13. Combine with other acquisitions if necessary.
14. Uncheck "**Close active shutter during stage movement**", which will save scanning time.
15. Set the overlap percentage no less than 10%. Stitching via " **Blending**" with **"Image Registration**" checked.
16. Tick "**Automatic Shading Correction**" and select the method for "**Brightfield**"
17. Select the file save format.
    "**Create Large image**" is to save the stitched image
    "**Store single images**" is to save the raw tiles in TIFF
    "**Create both**" is to save both stitched image and raw tiles
18. Ensure "**Use Focus Surface**" is ticked.
    If focus points are not added, please select "**Focus manually at start**".
19. Click "**Scan**".

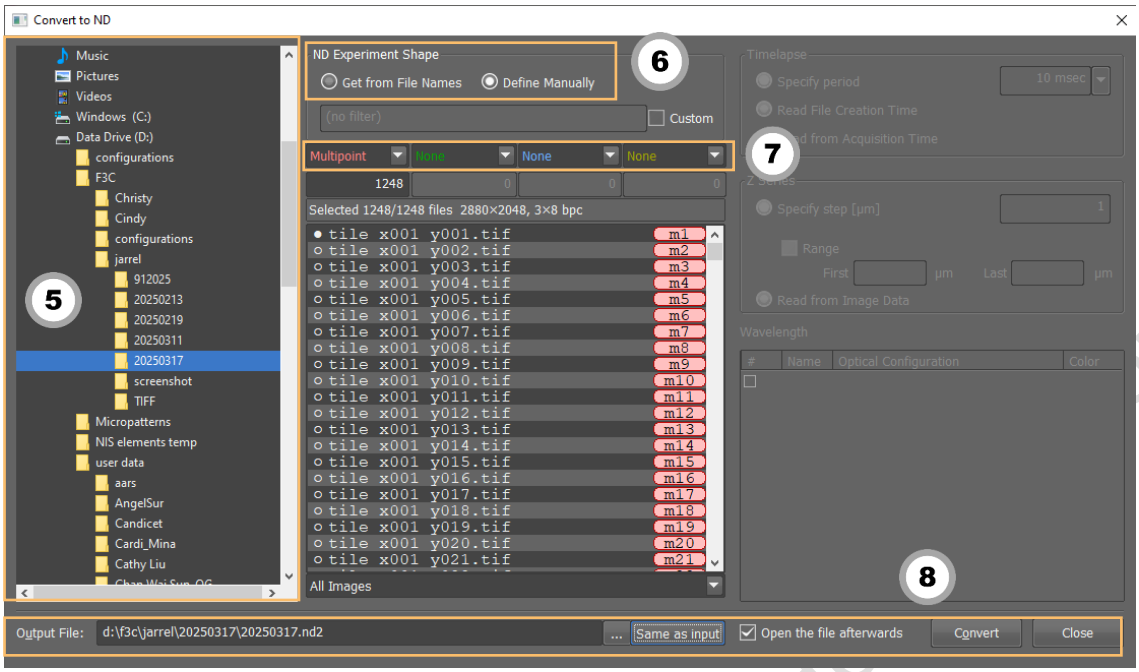


Optional (ONLY If you are scanning lots of tile more than ~700)

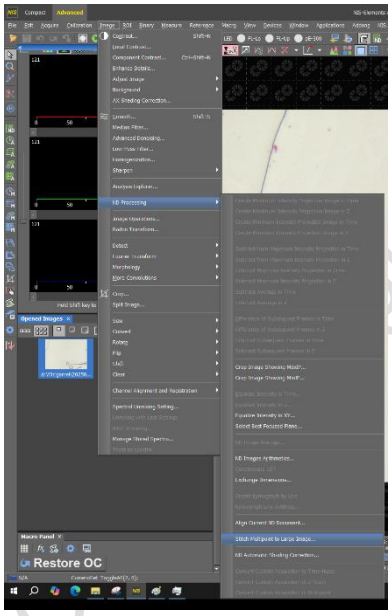
1.Choose “Store Single Images” instead of “Create Large Image” in step 16 page 13.
2.Once progress finished, click File
3.Import/Export
4.Create ND File from File Sequence



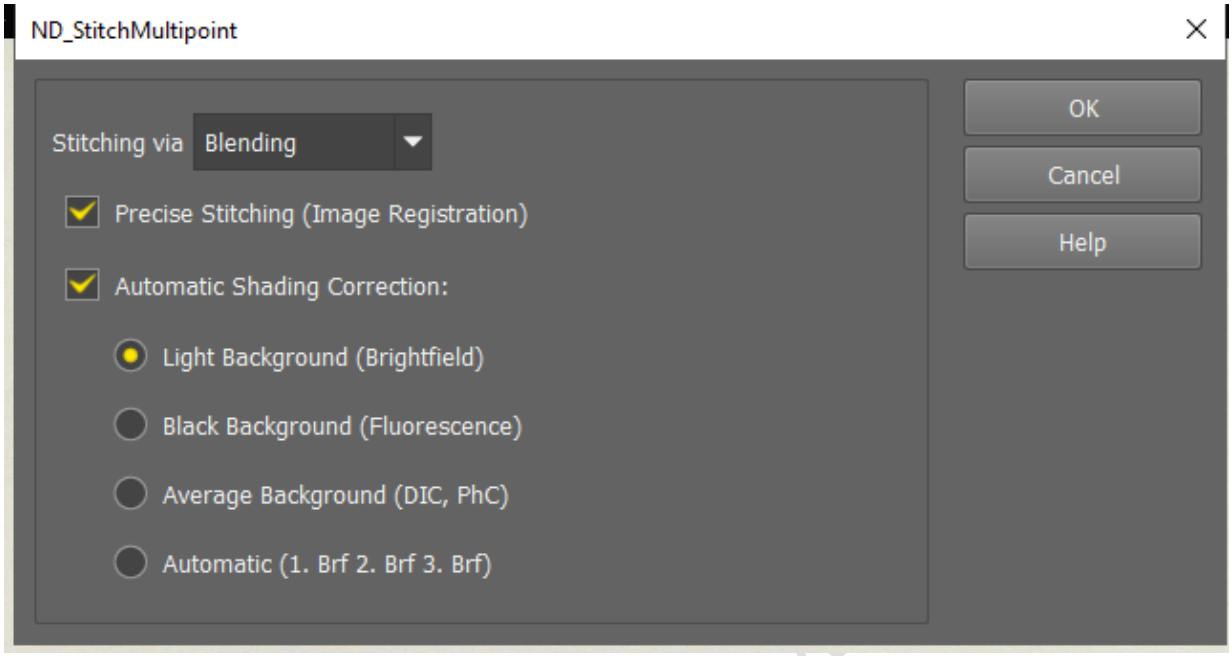
5.Locate your Folder
6.Choose Define Manually
7.Choose Multipoint/None/None/None setting, then select all your files in multipoint
8.Choose the location for the output files then click convert



9.Click Image -> ND Processing -> Stitch Multipoint to Large Image



10.Please choose the setting below, as it is recommended by the Vendor



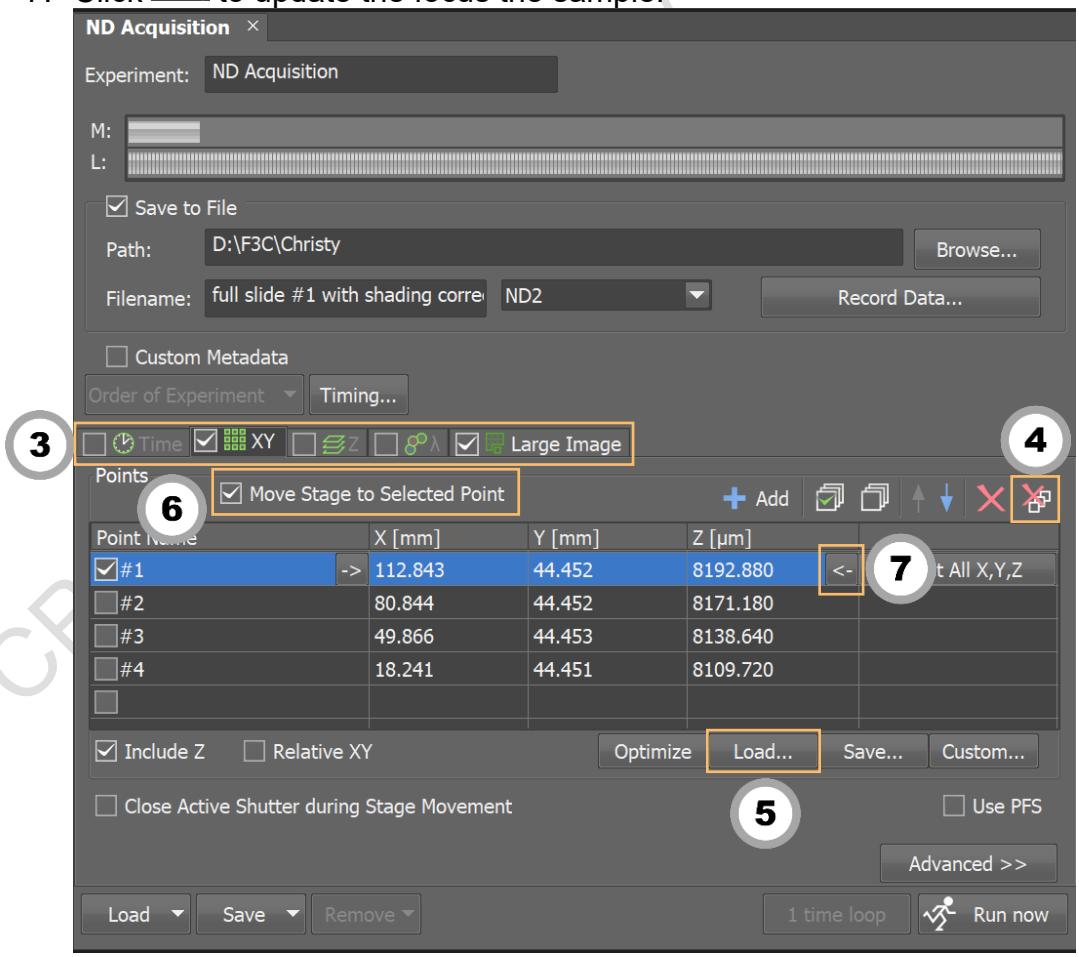
4 Slides Scanning

1. Find the focus and adjust the exposure as described in “Brightfield Imaging”. Click “**ND Acquire**”.
2. Click  to turn on shading correction.

3. Check the box next to “**XY**” and “**Large Image**”.
4. In XY tab, click  to remove all points from previous experiment.
5. Click “**Load…**” and select file from computer **D:\configurations** to import the XY coordinates for 4 slides scanning.

Full frame	XY center 4 slides holder whole slide scanning
22x40mm coverslip	XY center 4 slides holder 22x40mm

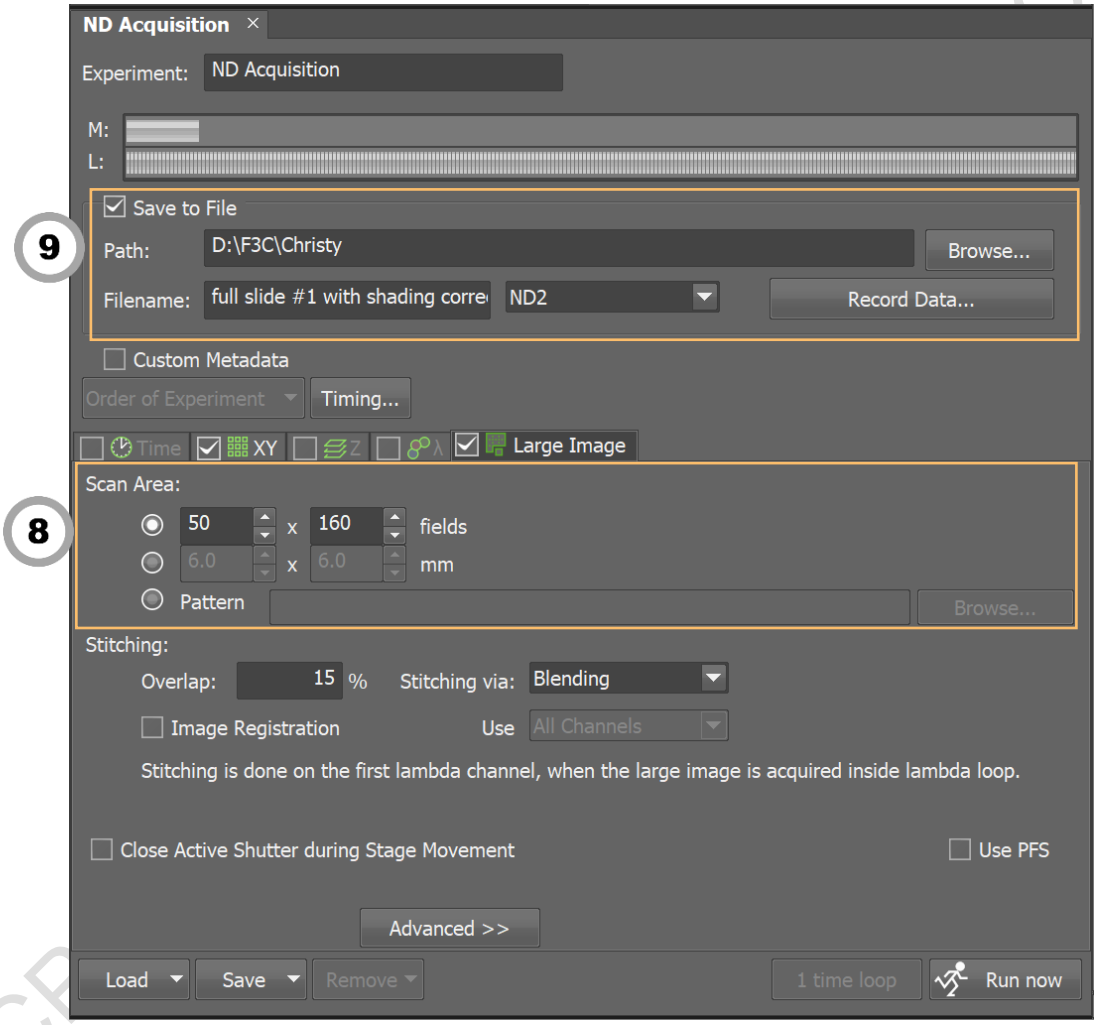
6. Enable “**Move Stage to Selected Point**” and click Point #1 to #4 to check the focus of the samples.
7. Click  to update the focus the sample.



8. In Large Image tab, input the scan area parameters.
   For full frame scanning of 4 slides holder,

4x objective	10	x	32	fields
10x objective	25		80	

   For 22x40mm coverslip, input 22 x 40 mm.
   *For whole slide scanning with 20x objective, please refer to previous section "Stitching / Scan Large Image".
9. Select the file save format.
10. Click "**Run now**".



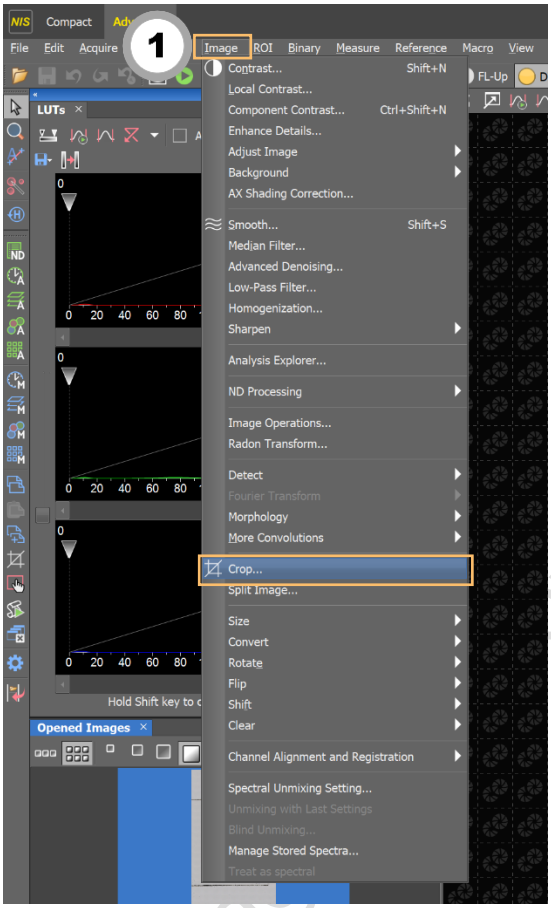
Estimated data size and imaging time for 4 slides scanning

Objective	Scanning area	Est. imaging time	Est. data size (nd.2)
4x	Full frame	~ 9 minutes	4 Gb
	22x40 mm	~ 6 minutes	2 Gb
10x	Full frame	~ 40 minutes	24 Gb
	22x40 mm	~ 21 minutes	13 Gb

20x	Not recommended for 4 slides scanning

Crop Image

1. Click "**Image**" and choose "**Crop…**" from the dropdown list.

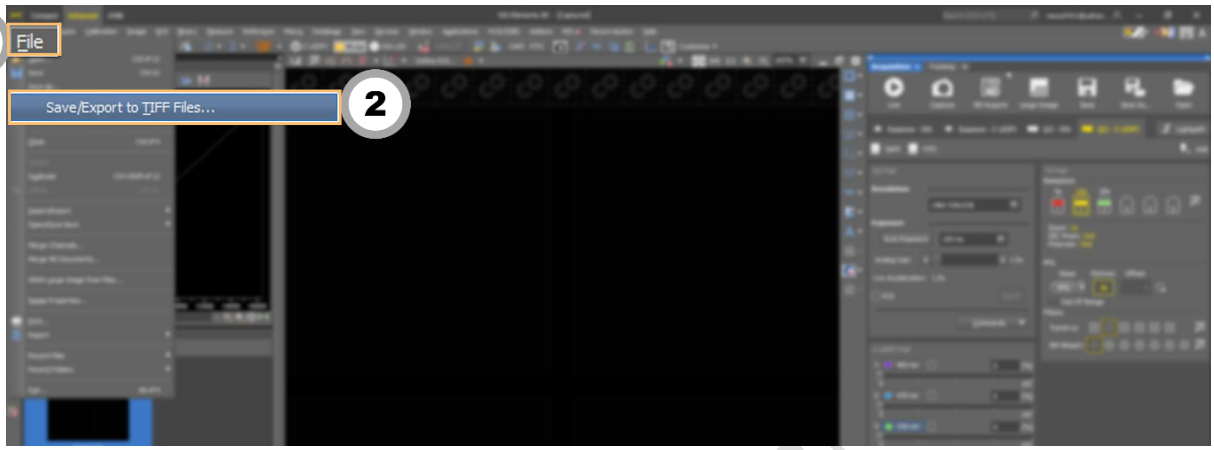


2. Drag and pull to cover the region of interest. The size of the cropped image can be manipulated in the pop-up window.
3. Click "**OK**".

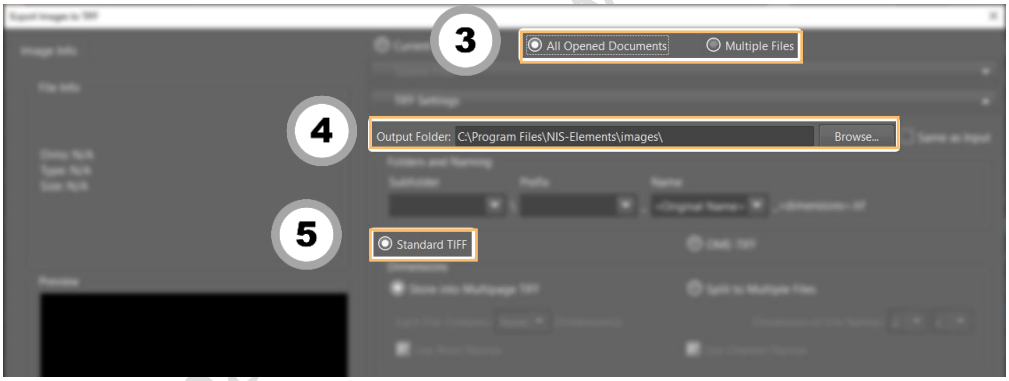
Imaging and Flow Cytometry Core

Batch Export of ND2 Images into Tiff

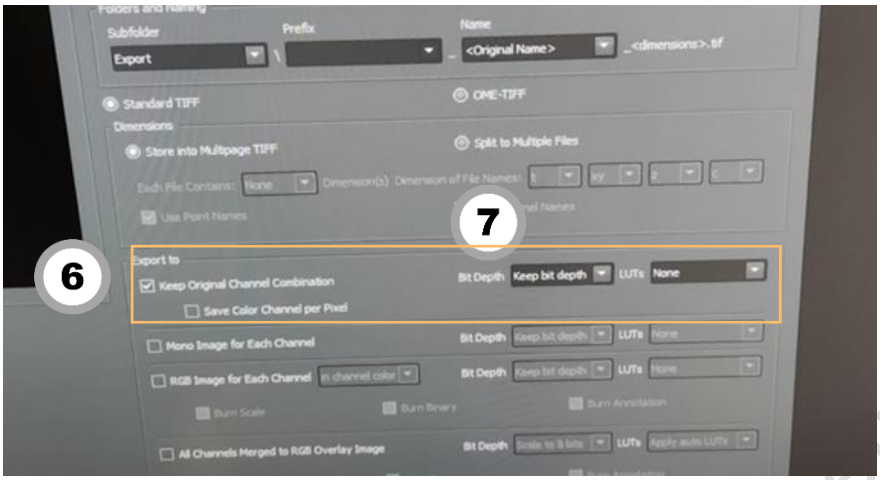
1. Click "**File**".
2. Select "**Save/Export to TIFF Files…**".



3. Select the desired files to be exported.
4. Select the path (all data should be saved to D drive/User Data under your name).
5. Select "**Standard TIFF**".



6. "**Keep Orginial Channel Combination**", "**Keep bit depth**", "**None**" LUT, No "**Save Color Channel per Pixel**".



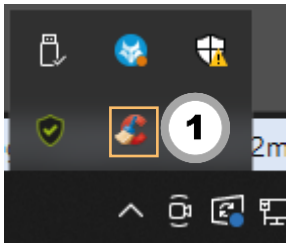
7. Optional: **Apply Saved LUTs**" if you adjusted the LUTs(Do not recommend if for intensity quatification).
   Tick "**Burn Scale**" if the scale bar is needed
   But for large data size tiff, if you select "**Apply Saved LUTs" or "Burn Scale"** will lead to export tiff failure. There will be an error "System was unable to create RGB bitmap in mrmory. Document dimensins may be over the OS bitmap size limit."
8. Click "**Export**".

Imaging and Flow Cytometry Core

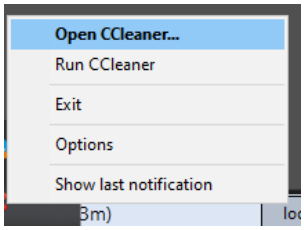
Turn off System

Please check if the equipment will be used by other users. Please switch off system if no one books equipment over two sessions (1h) after you

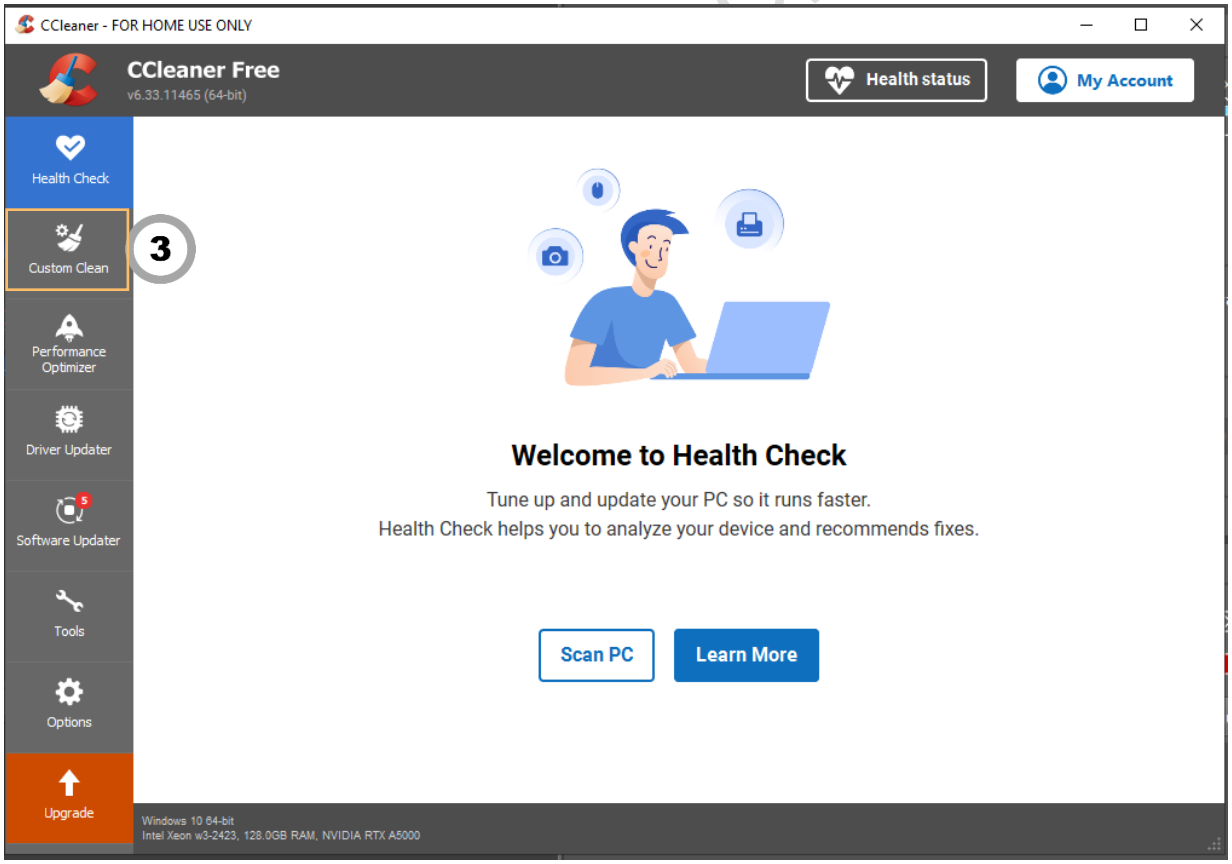
1. Right Click the CCleaner Icon



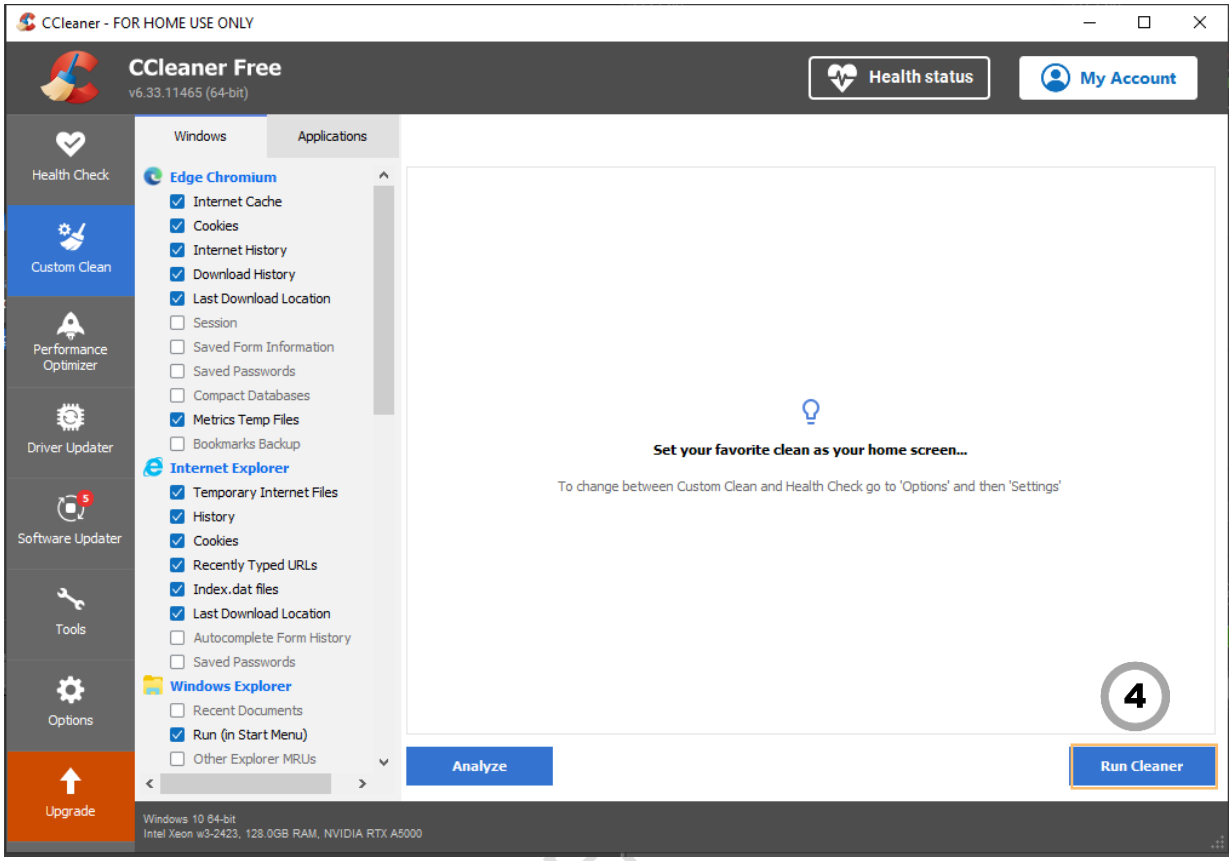
2.Click Open CCleaner



3.Click Custom Clean



4.Click Run Cleaner



5. Switch objective to lowest magnification (4x) in the software and press “ESC” to reach the Lower Z-limit.

6. Exit NIS-elements software.
7. Transfer data to your data transfer server.

Please switch off system if no one books equipment over two sessions (1h) after you

8. Shut down the computer ③, wait until the PC is completely off.
9. Switch off microscope controller ②, wait for 5 seconds.
10. Switch off main power control ①.