Primo micropatterning/color imaging system Standard Operation Protocol

Contents

Turn on system 2

Sample locating and focusing 3

Load sample on the stage....3

Eyepiece Observation....4

Bright-Field Imaging 6

Save Images....7

Stitching / Scan Large Image 8

4 slides scanning 13

Crop image 15

Batch Export of ND2 Images into Tiff 16

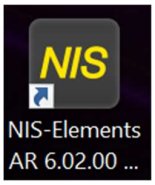
Turn off system 17

Turn on system

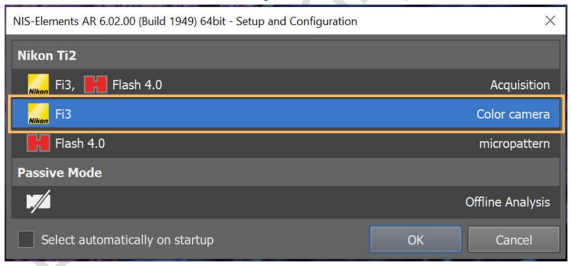
Please sign on the log sheet before switching on system.

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 stop moving before next step
3. Turn on computer power ③

4. Click to log in **USER** account at the startup screen
5. Double click NIS-Elements AR to start the NIS-elements software.

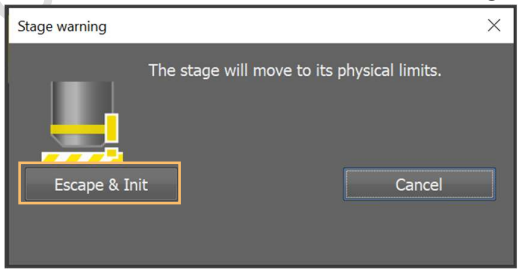


6. Choose Fi3 Color camera, click “OK” on setup and configuration, DO NOT check “select automatically on startup”.



7. Click 

to initialize the stage.

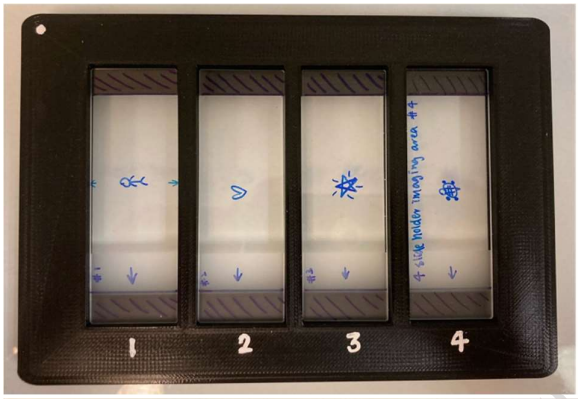


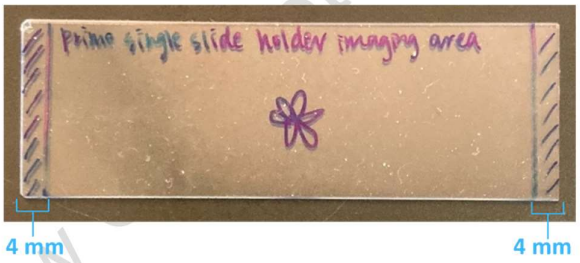
Sample locating and focusing

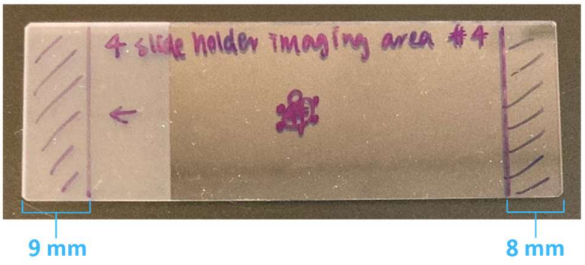
Load sample on the stage

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.

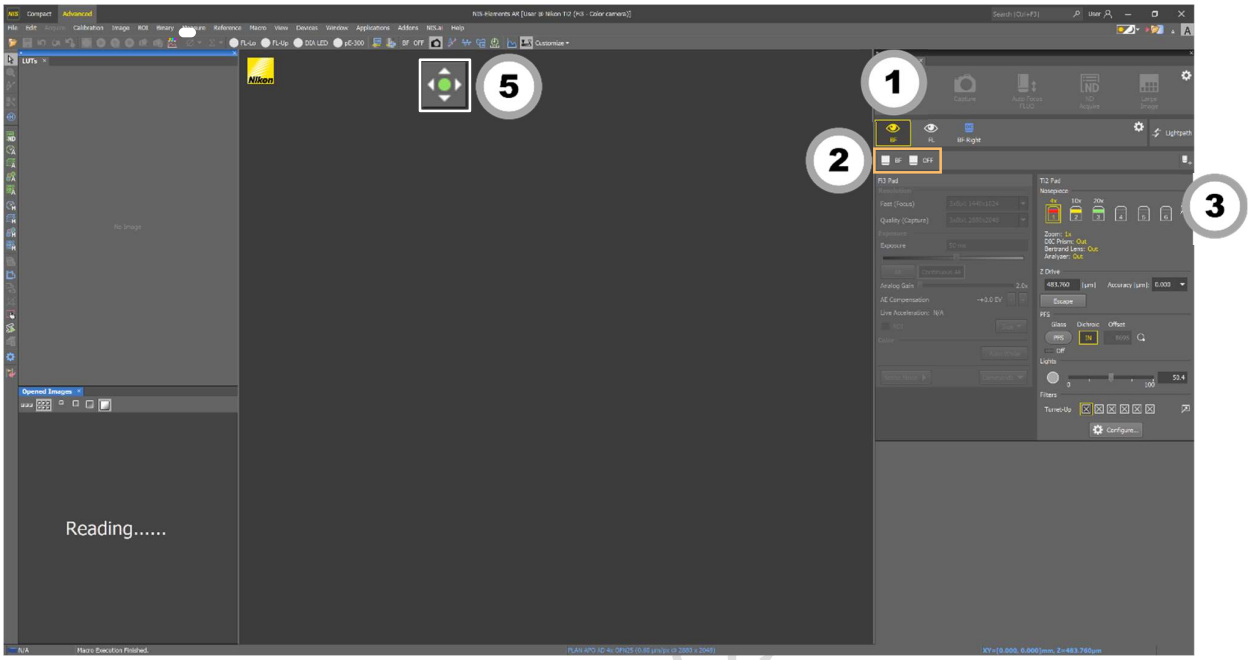
Area that will be blocked by slide holder (highlighted by blue diagonal lines)
Please do not mount coverslip on those areas as it will generate a tilted angle across the slide after fixing on the slide holder.







Eyepiece Observation



4. Select “**Eyepiece - BF**”for Brightfield imaging.
5. Select the desired optical configuration (OC).

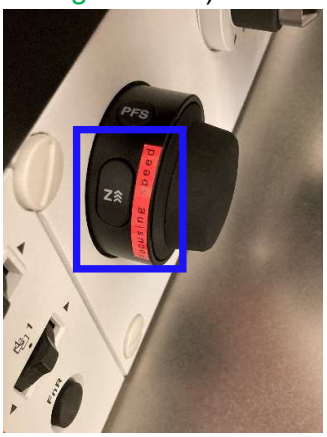
Eyepiece - BF

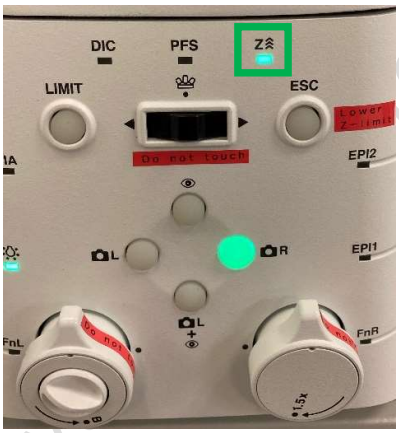
BF (Bright-field)	**OFF**
Brigh-field light is turned on	Brigh-field light is turned off

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

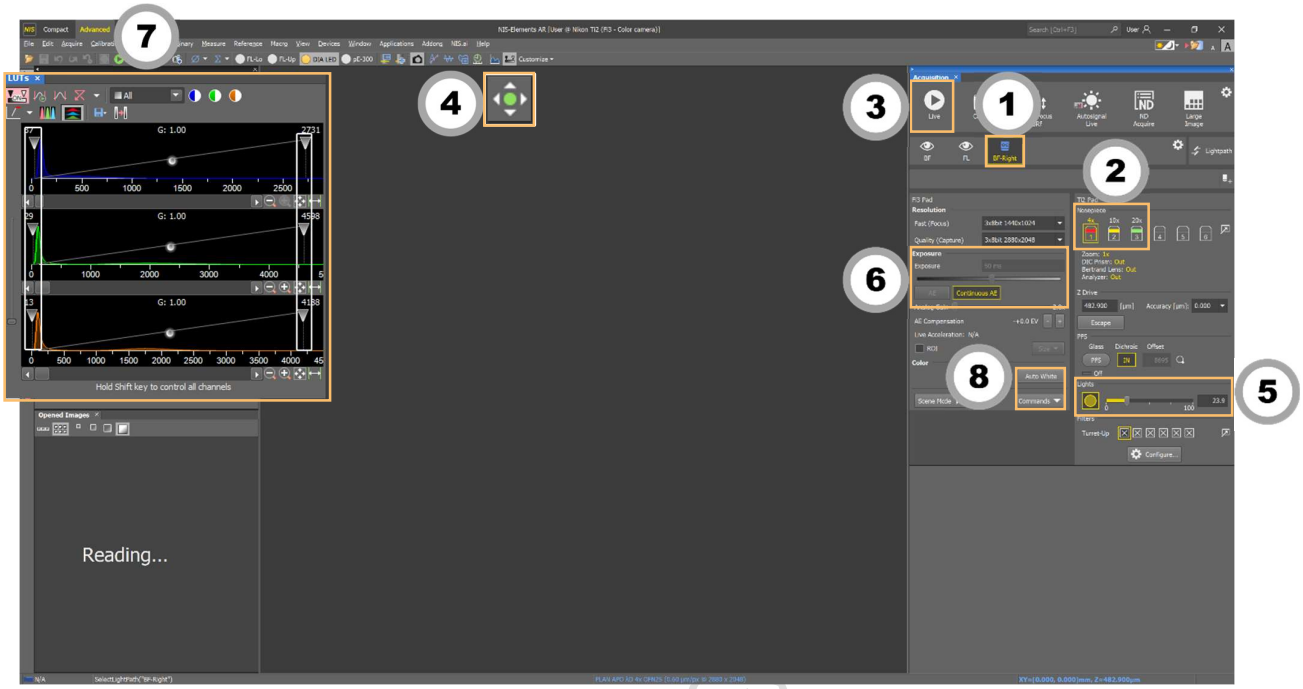
8. Press  to adjust XY position by moving mouse.
9. OR Move the Stage Controller to adjust XY position.
10. Focus the sample with the focusing knob.

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





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.

Save Images

1. Click 

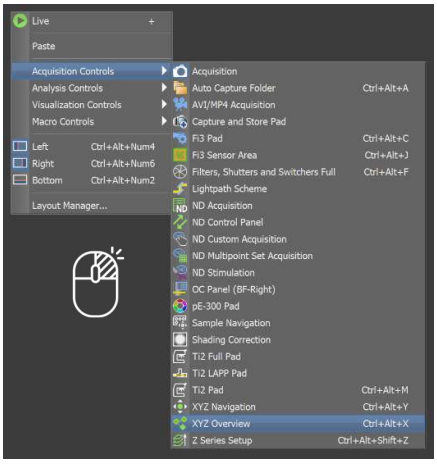
to capture the image.
2. Click 

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

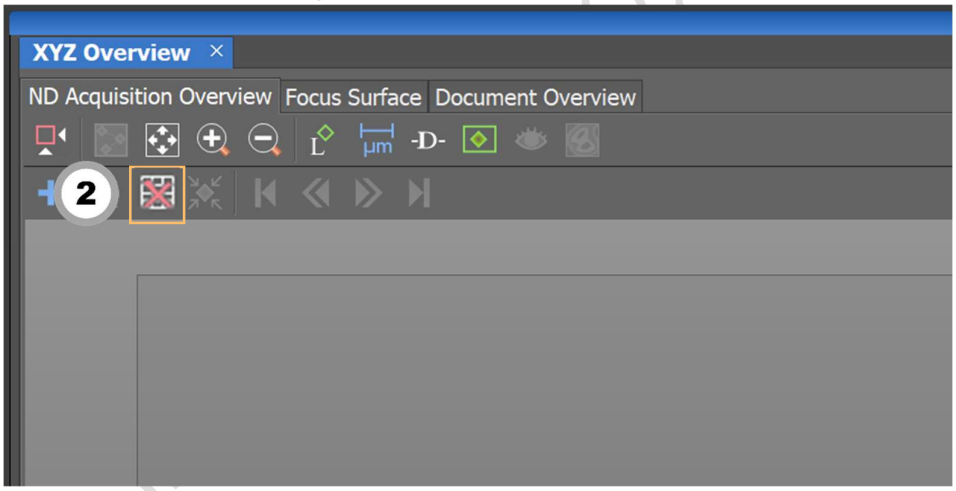
File format	Features
.nd2	● Recommended ● Largest file size can save all the information including the camera and device settings of your image ● Cannot be opened by Windows
.tiff	Can be opened by Windows

Stitching / Scan Large Image

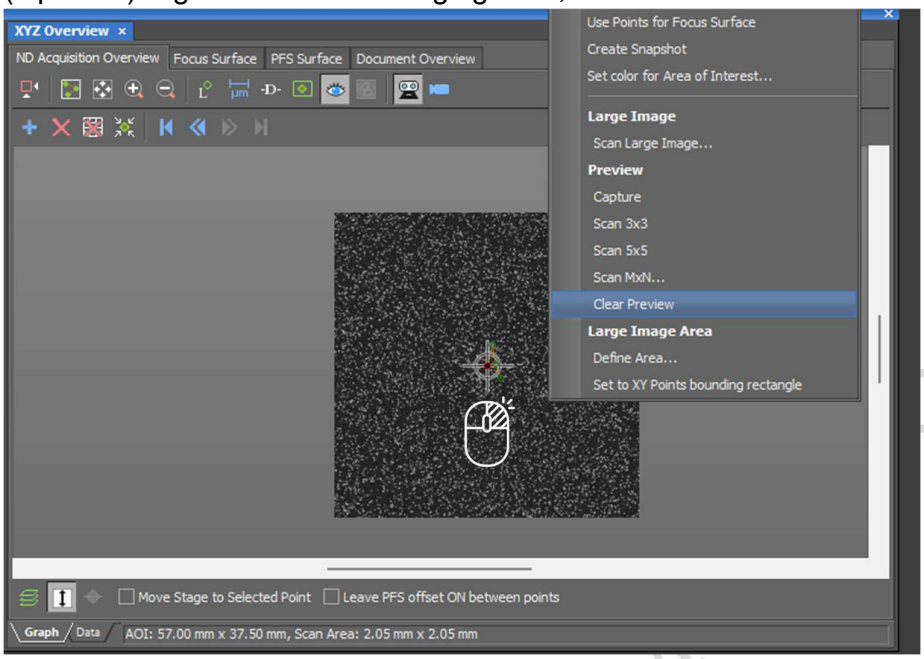
1. Right click at live window and select  "**XYZ Overview**" in Acquisition Controls.



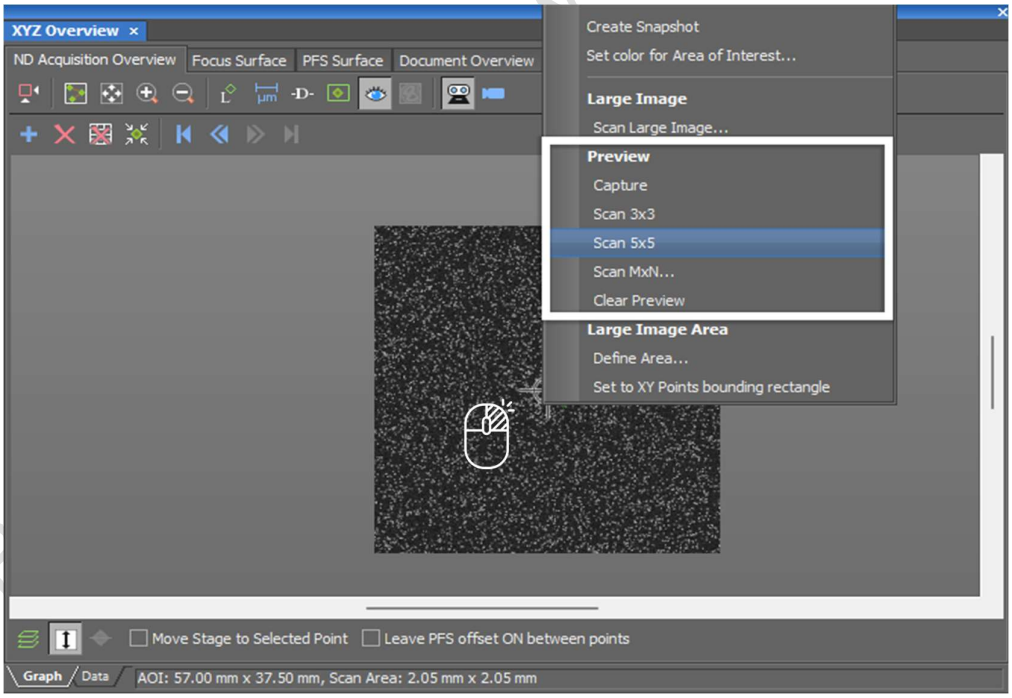
2. Click  to remove the previous data.



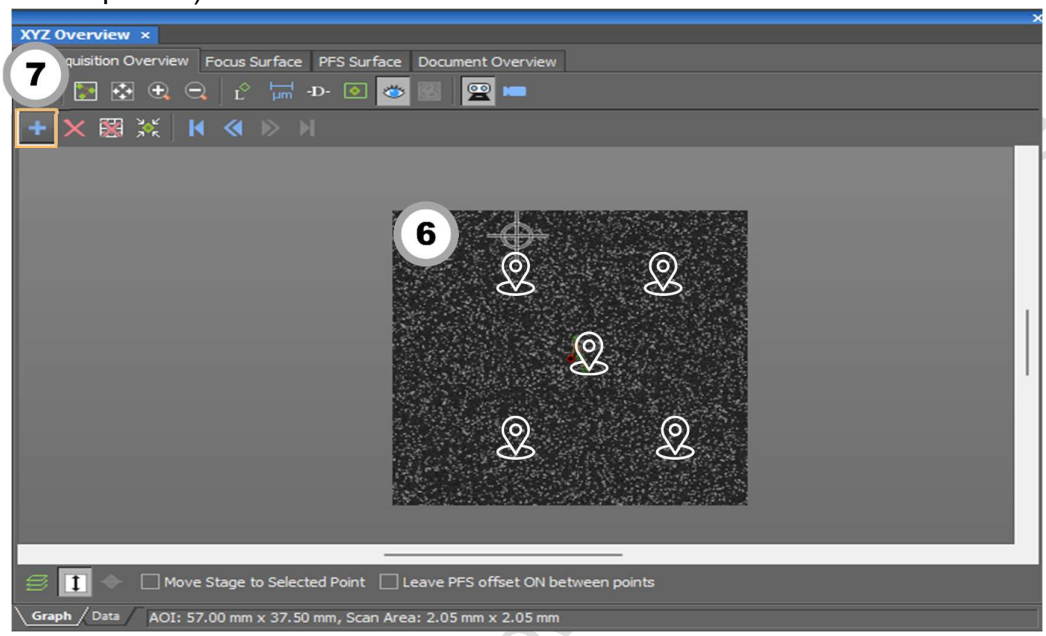
3. (Optional) Right-click on the imaging area, and select “**Clear Preview**”.



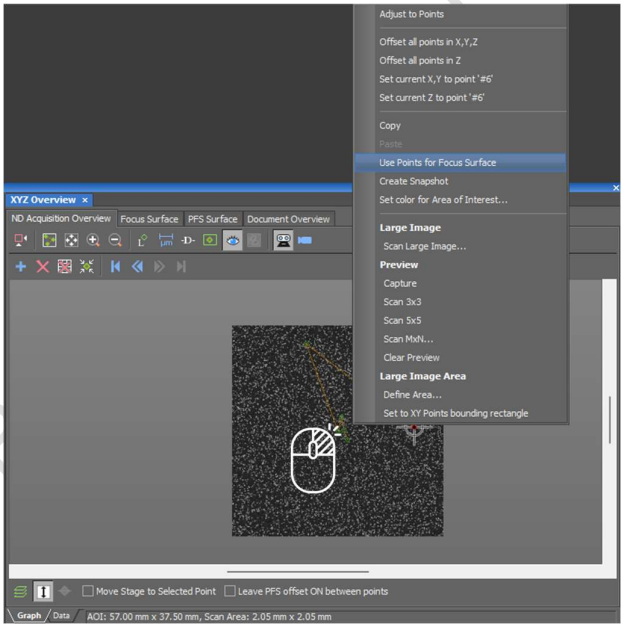
4. Click “Live”, and adjust the light intensity and exposure time if necessary.
5. Right-click on the imaging area, and decide the preview area to scan a preview image.



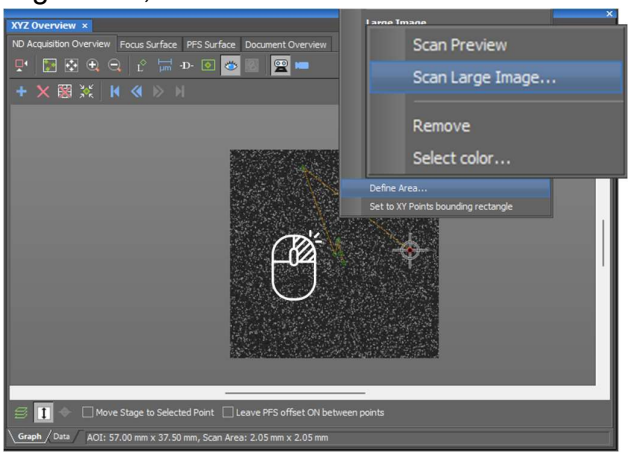
6. Double-click on the image to move the stage to a specific point.
7. Adjust the focus and press  to add the points.
8. Repeat steps 6-7 until all points are added.
   *points can be added at the edges of your sample and center (as shown in above picture).



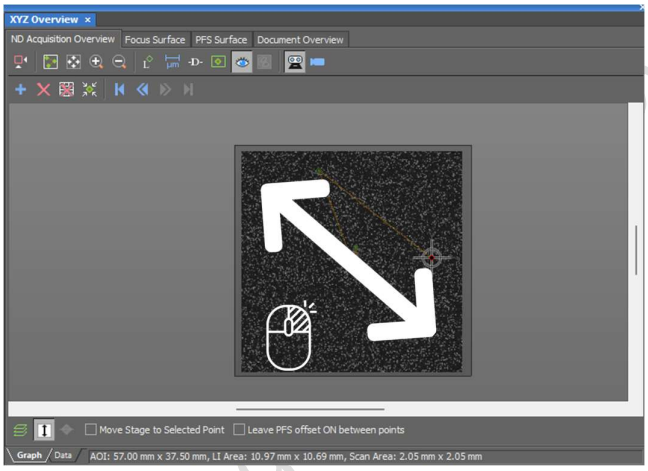
9. Right-click, and select "**Use Points for Focus Surface**".



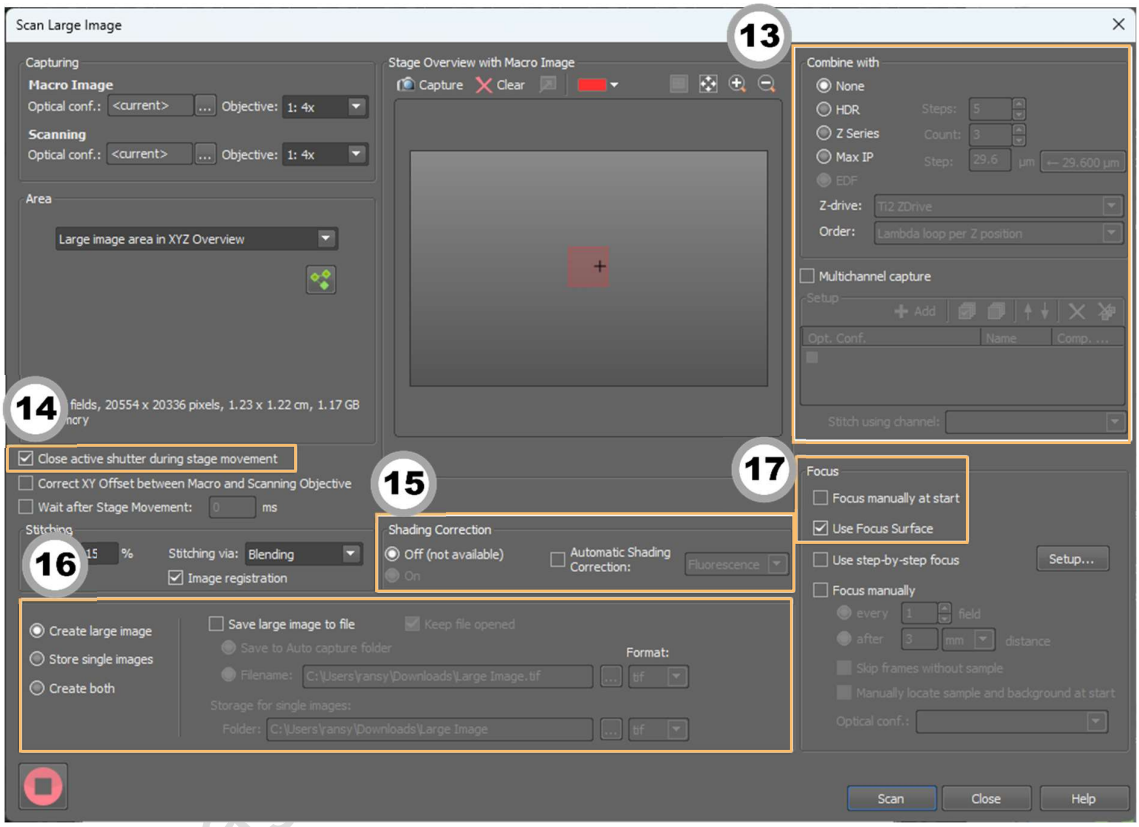
10. Right-click, and select “**Define Area**



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. Tick “**Close active shutter during stage movement**” if you want to reduce the bleaching.
15. Tick “**Automatic Shading Correction**” and select the imaging method if necessary.
16. Select the file save format.
17. Ensure “**Use Focus Surface**” is ticked.
18. Click “**Scan**”.



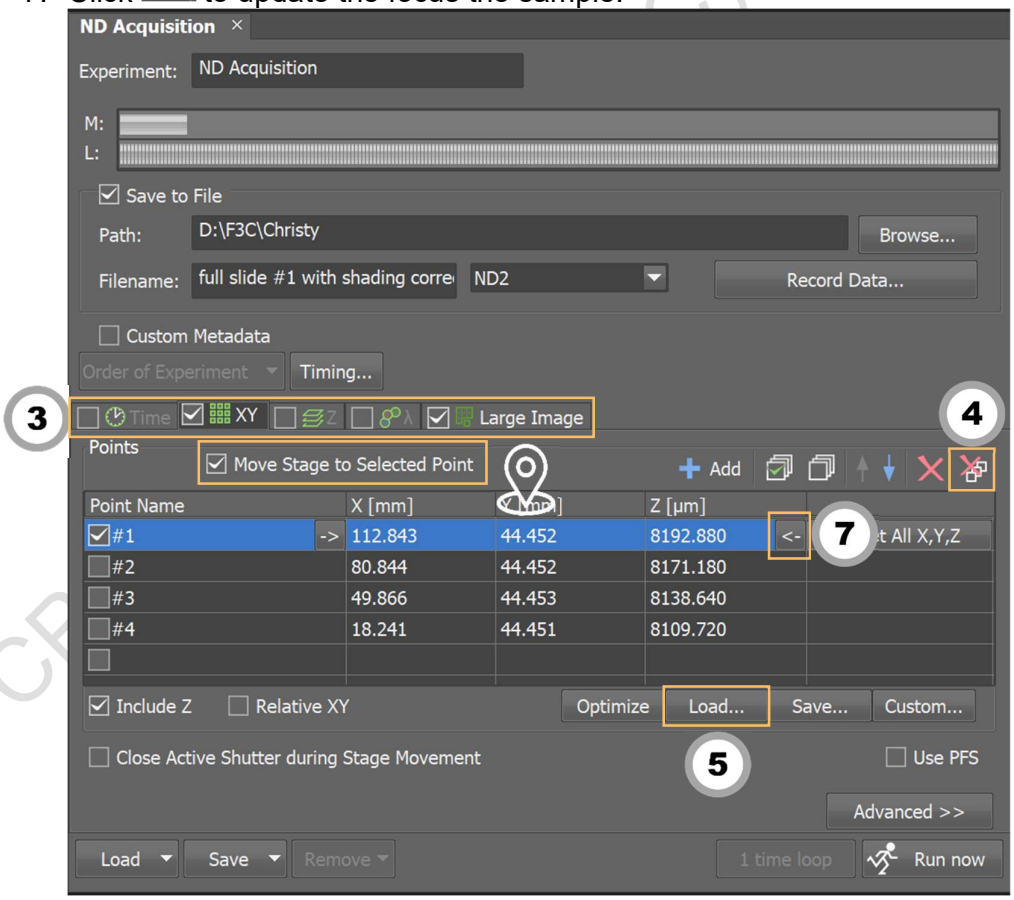
4 slides scanning

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

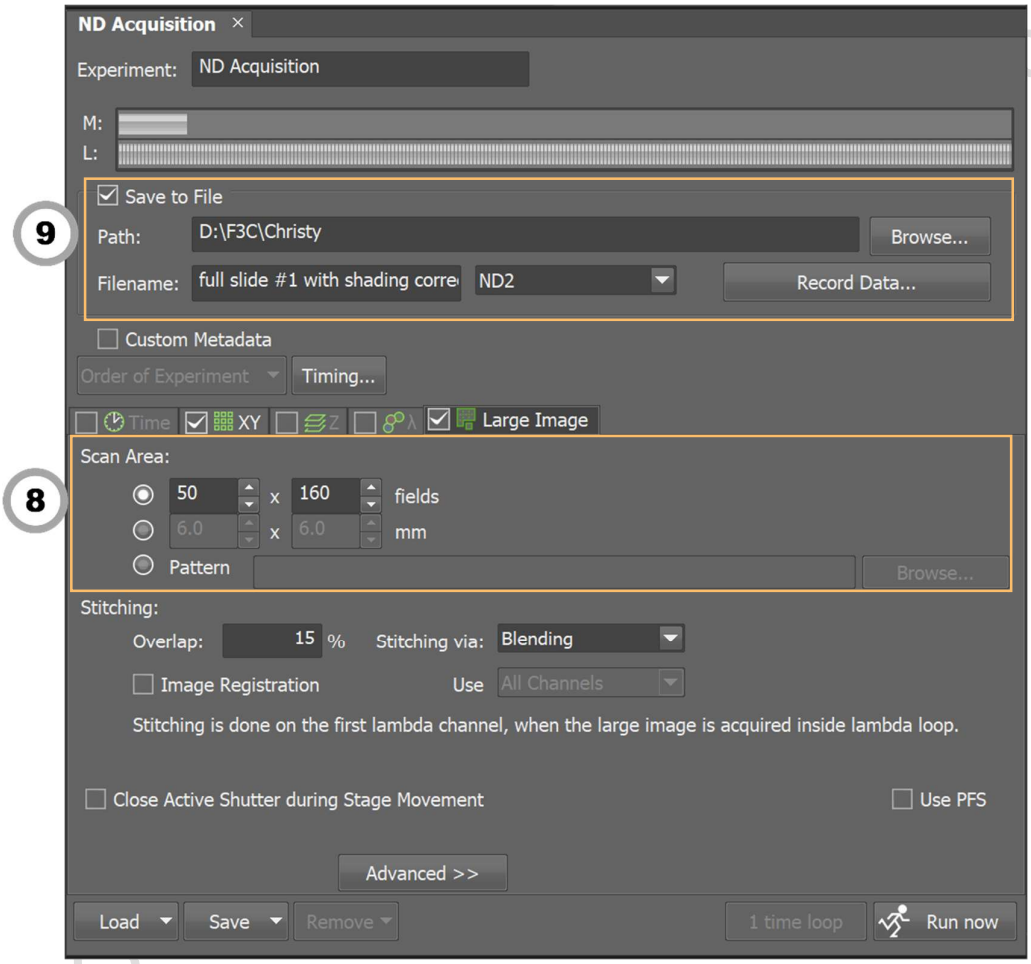
6. Enable “**Move Stage to Selected Point**” and click Point #1 to #4 to check the focus of the samples.
7. Click  to update the focus the sample.



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 “Stitching / Scan Large Image”.
9. Select the file save format.
10. Click “**Scan**”.

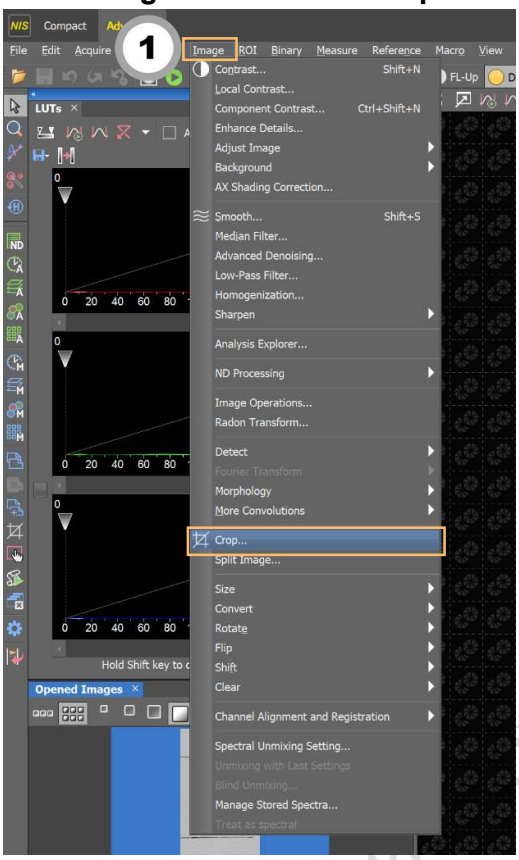


Estimated data size and imaging time for 4 slides scanning

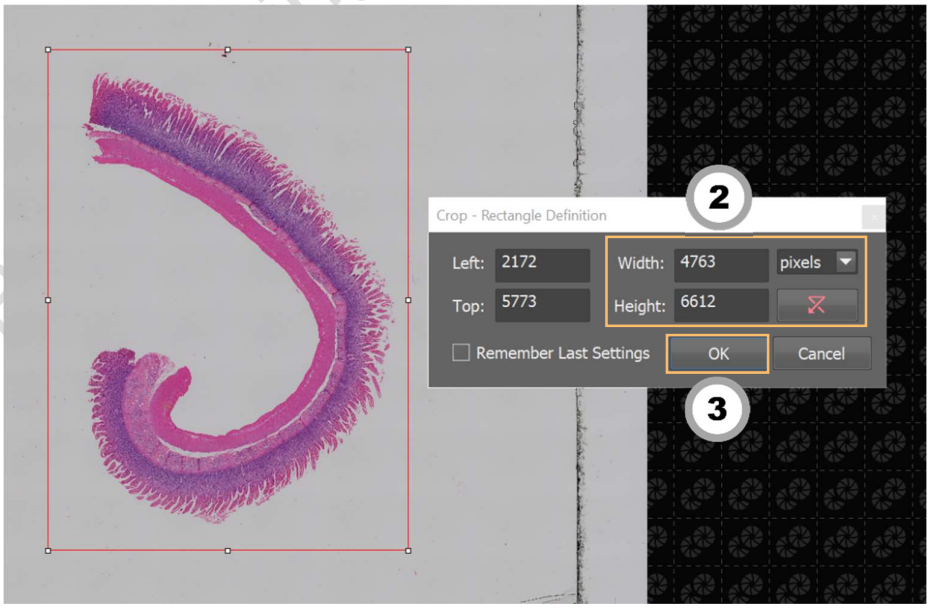
Objective	Scanning area	Est. imaging time	Est. data size (nd.2)
4x	Full frame	~ 9 minutes	4 Gb
	22x40 mm	~ 6 minutes	2 Gb
10x	Full frame	~ 40 minutes	24 Gb
	22x40 mm	~ 21 minutes	13 Gb
20x	Not recommended for 4 slides scanning		

Crop image

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

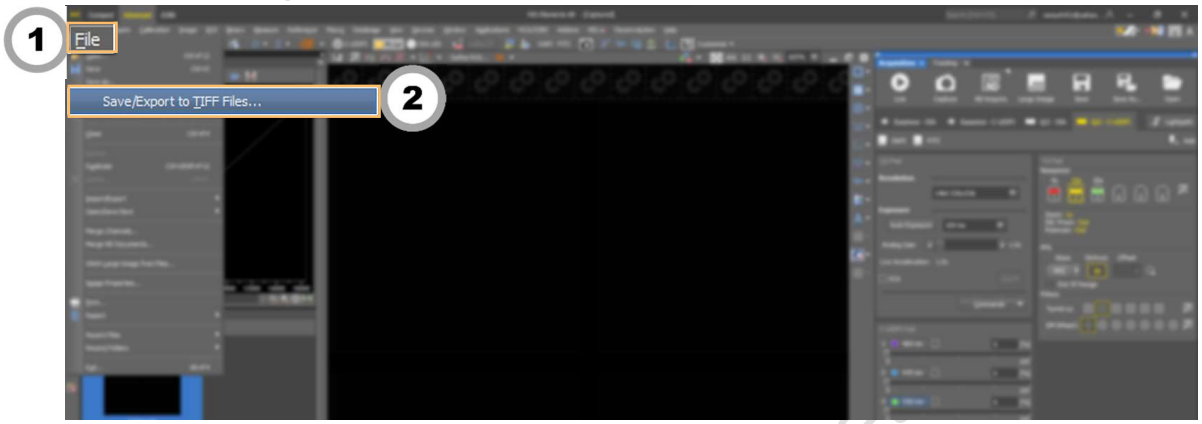


2. Drag and pull to cover the region of interest. The size of the cropped image can be manipulated in the pop-up window.
3. Click “**OK**”.

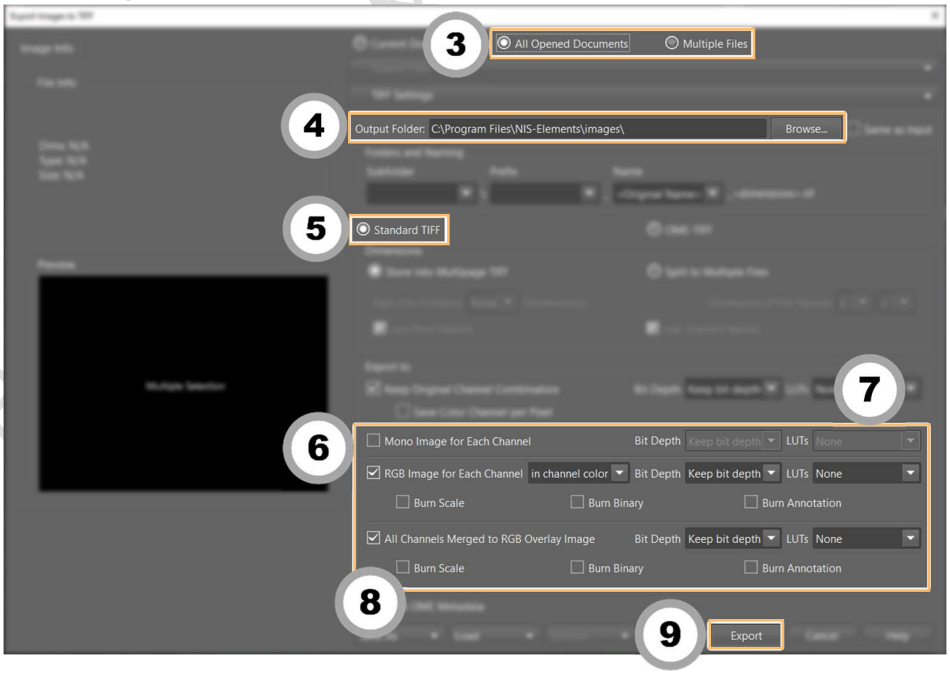


Batch Export of ND2 Images into Tiff

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. Select the desired color to be exported.
7. Select “**Apply Saved LUTs**” if you adjusted the LUTs(Do not recommend if for intensity quatification).
8. Tick “**Burn Scale**” if the scale bar is needed
9. Click “**Export**”.



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. Switch objective to lowest magnification (5x) in the software and press "**ESC**" to reach the Lower Z-limit.
2. Exit NIS-elements software
3. Transfer data to Imaging & Flow Cytometry Core storage server
4. Shut down the computer ③, wait until the PC is completely off.
5. Switch off microscope controller ②, wait for 10 seconds.
6. Switch off main power control ①.