Nikon Eclipse T*i*2-E Widefield Microscope Standard Operation Protocol

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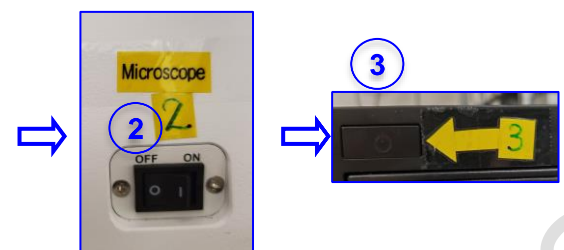
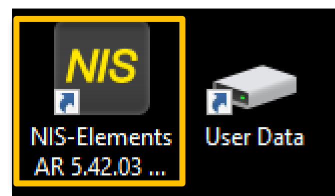
Turn on system

Please sign on the log sheet before switching on system.

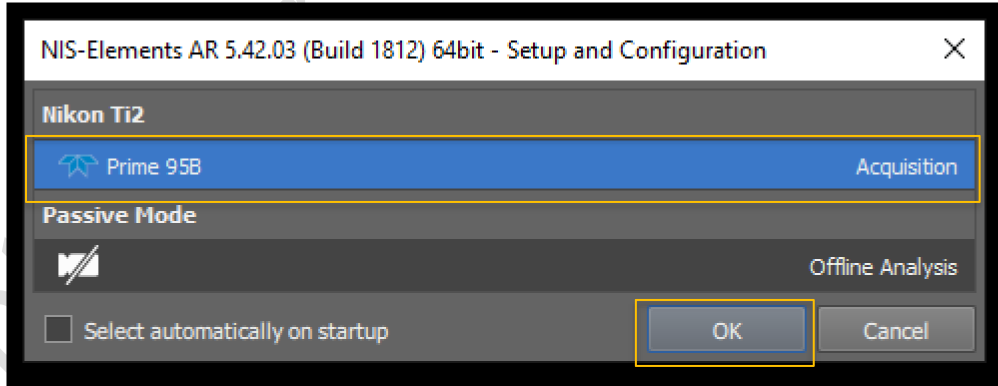
1. Switch on main power control ① wait for at least 5 sec before next step
2. Switch on microscope controller ② wait for at least 10 sec until the stage stop moving before next step
3. Turn on computer power ③



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 Prime 95B Acquisition, click "OK" on setup and configuration, DO NOT check "select automatically on startup"



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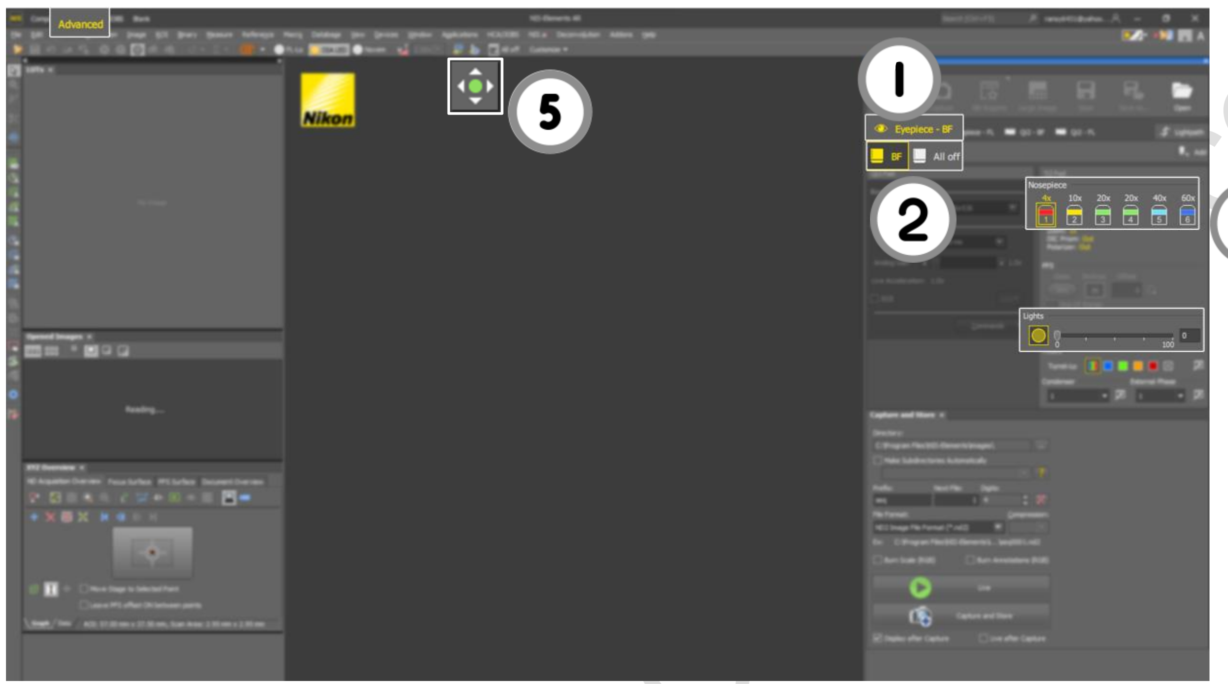
(Optional) Set the temperature and CO2 control for live cell imaging

(Only applicable for live cell imaging, please skip this step if it is not needed):

1. Switch on "Incubator" for temperature and CO_2 control.
2. Switch on the Power of Tokai Hit incubation system controller. Temperature can be altered via pressing the green button of each heating parts on the touch screen.
3. Make sure the CO_2 sliding button is turned **ON**.
4. Turn on CO_2 tank by turning the main switch anticlockwise.
5. Turn on CO_2 regulator by turning regulator clockwise to set output pressure at 100kPa.
6. Turn on tube switch for TIRF →Put on objective heater on objective if oil objective is used.
7. Metal ball floats is an indication of the presence of CO_2 gas.
8. MilliQ water has to be added into the water chamber and covered if overnight(s) acquisition is required.

Sample locating and focusing

Eyepiece Observation



1. Select “**Eyepiece - BF**”for Brightfield imaging, “**Eyepiece - FL**”for Fluorescent imaging.
2. Select the desired optical configuration (OC).

Eyepiece - BF

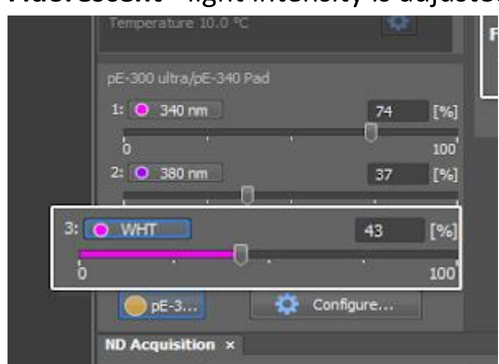
BF (Bright-field)	All off
Brigh-field light is turned on	Brigh-field light is turned off

Eyepiece - FL

DAPI	GFP	RFP	Cy5

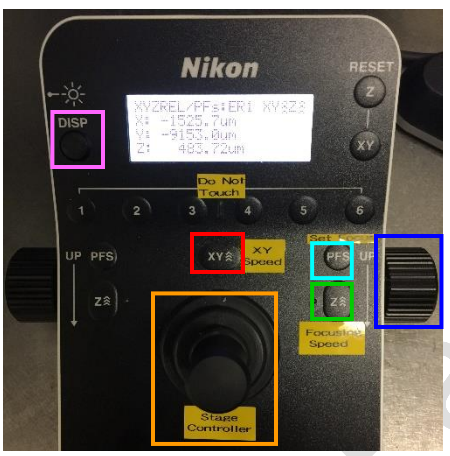
3. Select the desired objective (low magnification is recommended).
4. Change the light intensity if necessary.
   Brightfield – light intensity is adjusted using the knob on the side of the microscope.

Fluorescent - light intensity is adjusted by dragging the intensity bar.



5. Press to adjust XY position by moving mouse.
6. OR Move the Stage Controller to adjust XY position (XY speed can be adjusted: ^^^ > ^^ > ^)
7. Focus the sample with the focusing knob → Clockwise_Down; Anti-clockwise_Up

   (Focusing speed can be adjusted: ^^^ > ^^ > ^



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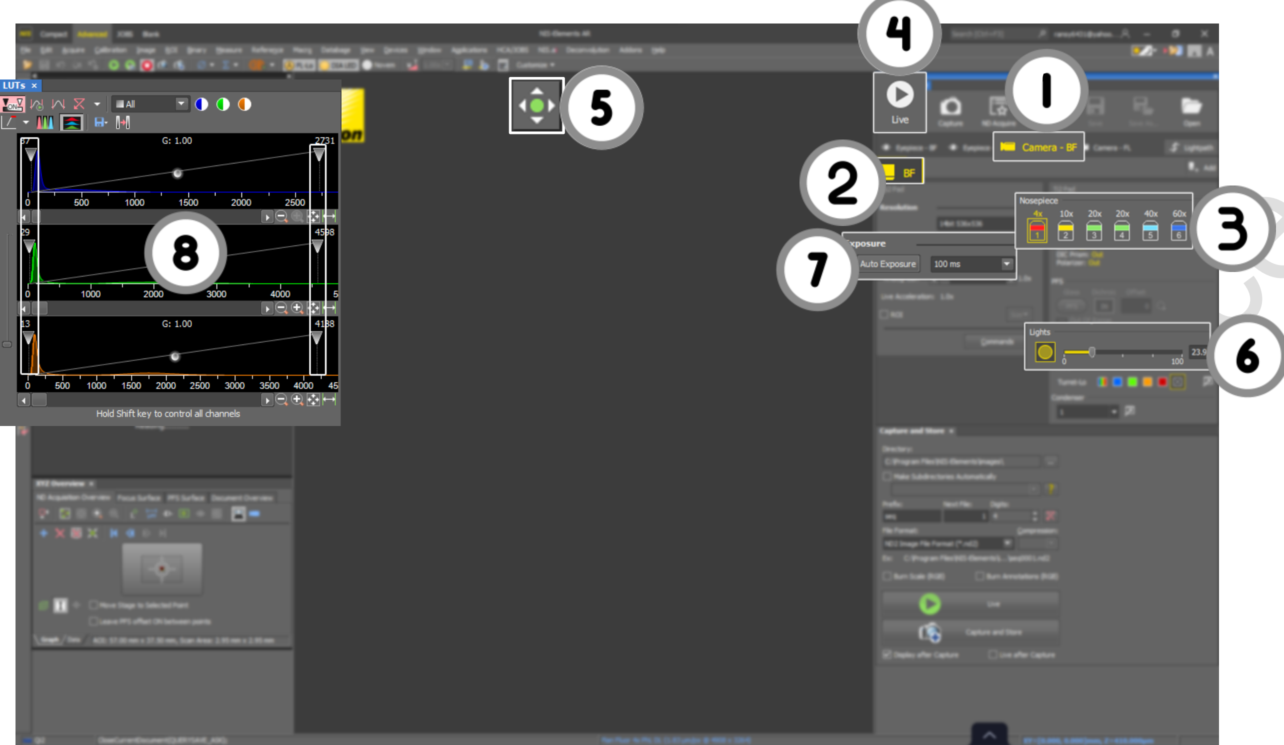
Bright-Field Imaging



1. Select “**Camera - BF**”.
2. Select“**BF**”
3. Select the desired objective.
4. Click Live to display the image.
5. Click to allow moving your sample with your mouse or use focusing knob.
6. Adjust the light intensity if necessary.
7. Click Auto Exposure to adjust the exposure time automatically if necessary, or adjust exposure time manually.
8. 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

Fluorescent Imaging

1. Select “**Camera - FL**”.
2. Choose the desired fluorescence channels (DAPI/GFP/RFP/Cy5) for channels.
3. Select the desired objective.
4. Click Live to display the image.
5. Click to allow moving your sample with your mouse or use focusing knob.
6. Adjust the fluorescent light intensity if necessary.
7. Adjust the exposure time if necessary.
8. 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

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Save Images

Capture and Store

1. Select Directory (all data should be saved in D drive/User Data under your name)
2. 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

3. Click Capture and Store

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Save Separately

1. Click Capture to capture the image.
2. Click Save As... 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

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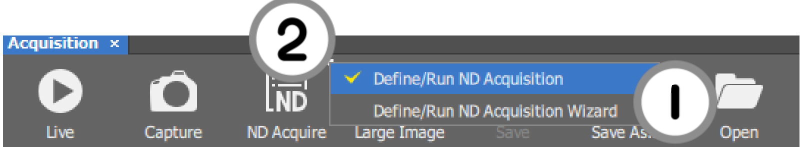
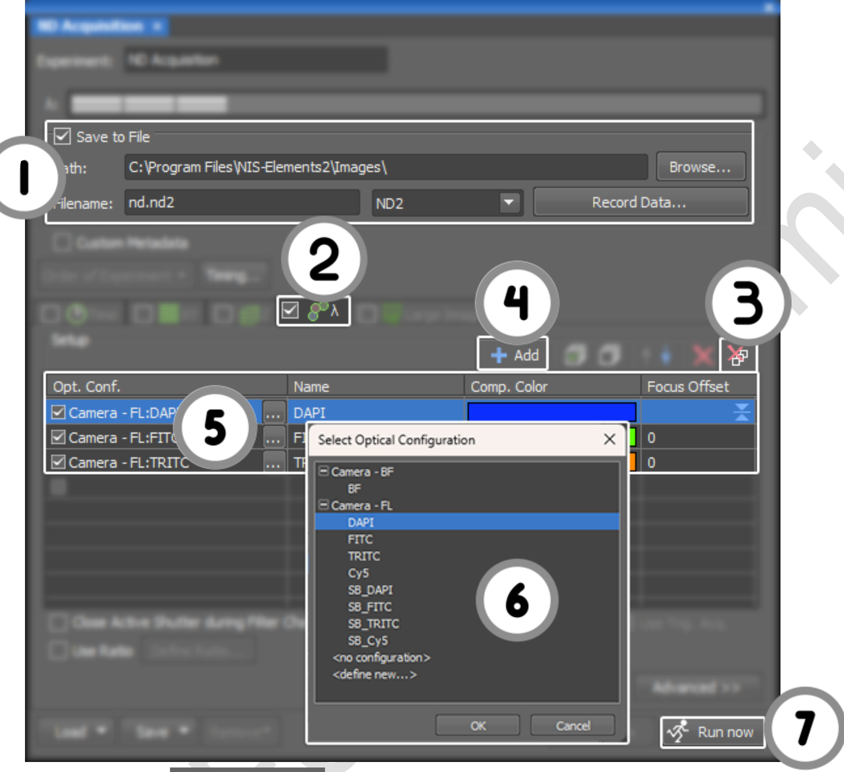
ND Acquisition

Multichannel



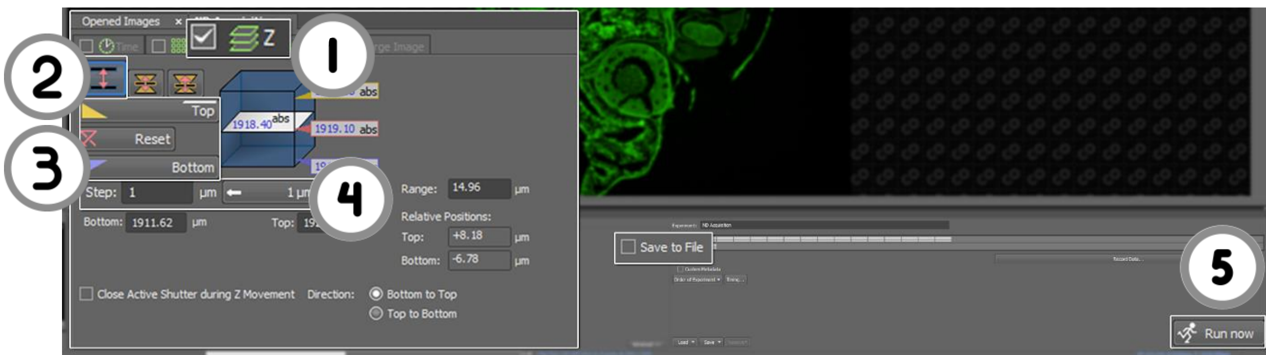
1. Right click to select "**Define/Run ND Acquisition**".
2. Left Click ND Acquire



1) Select Save to File, and select the appropriate path to save the images automatically if necessary (all date should be saved to D drive/User Data under your name).
2) Click λ for Multichannel imaging , and ensure unwanted acquisition such z-stack, times, etc. is not ticked.
3) Click to delete the previous settings.
4) Click Add to add channels.
5) Click ...
6) Select the desired channel.
7) Click Run now

Z-Stack

Define by top and bottom



1. Click Z for z stack imaging, and ensure unwanted acquisition such as time, λ is not ticked.
2. Select
3. Click Reset to remove the previous data if necessary. Change the focus and define the top and bottom of your sample by clicking Top , and Bottom.
4. Click to use the recommended steps or type the desired steps.
5. Click Run now

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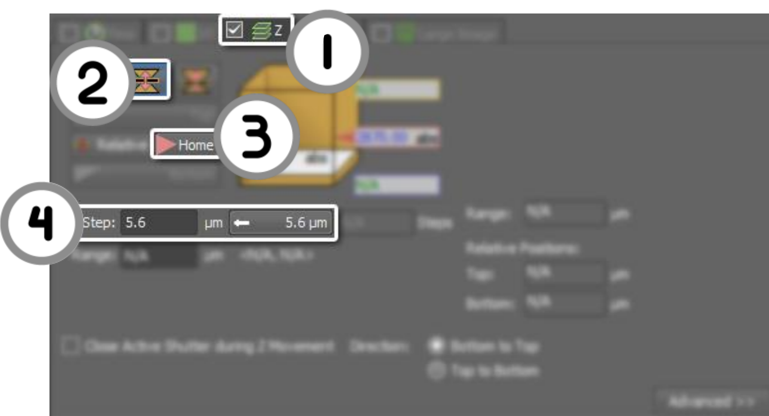
Symmetric mode defined by range



1. Click Z for z stack imaging, and ensure unwanted such as time, λ is not ticked.
2. Select
3. Adjust the focus and click Home
4. Click to use the recommended steps or type the desired steps.
5. Click Run now

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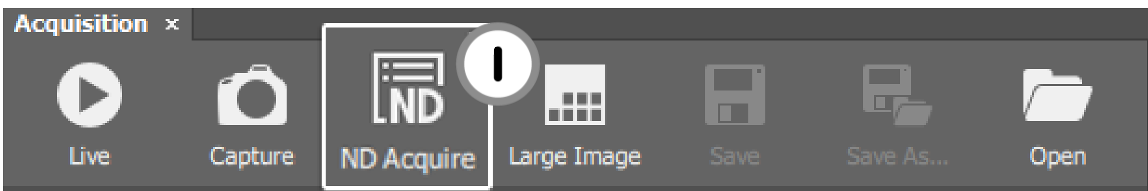
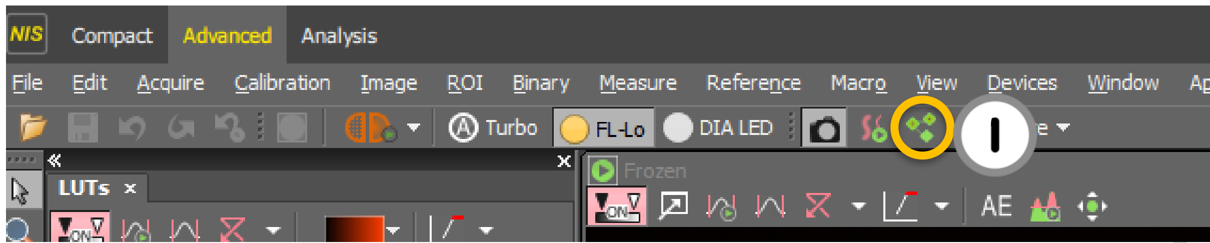
Time Lapse

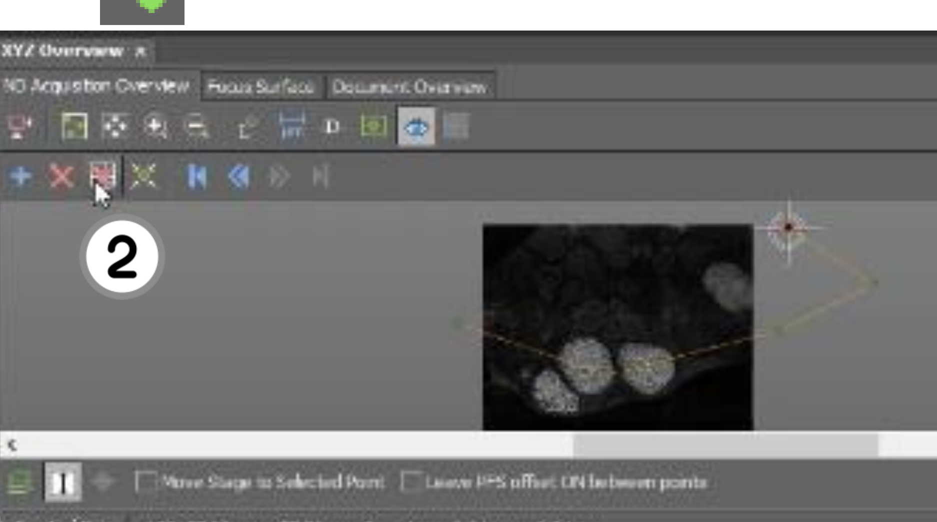


1. Click “ND Acquire”.
2. Tick “**Save to File**” and set the path to D drive/User Data under your name.
3. Tick “**Time**”.
4. Click [delete icon] to delete all previous settings.
5. Click [+ Add] to add time series.
6. Set the time-lapse detail.
7. Click [Run now]

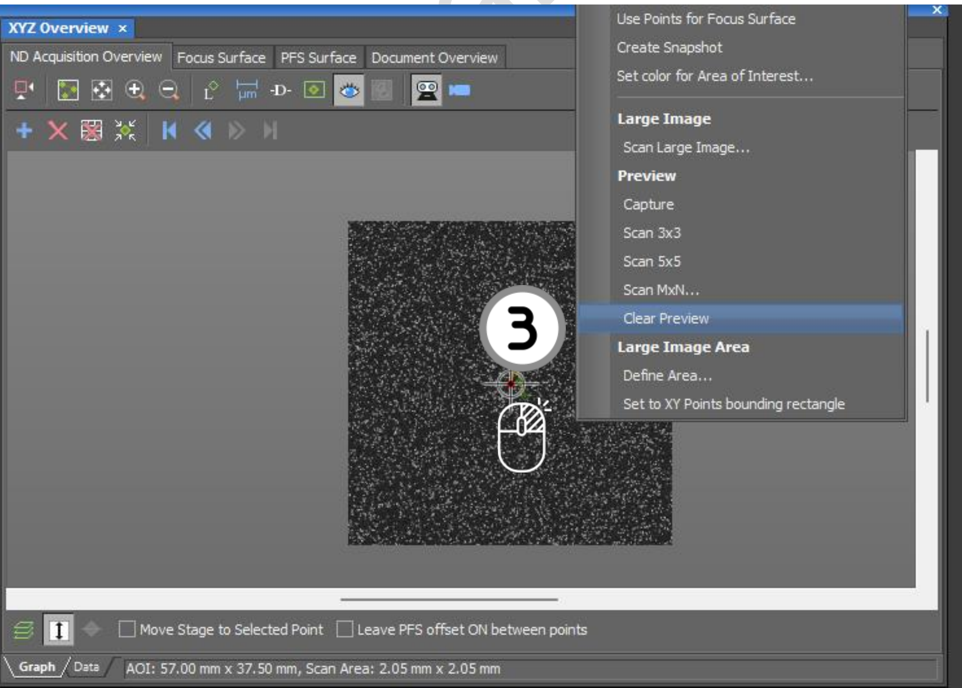
Stitching / Scan Large Image



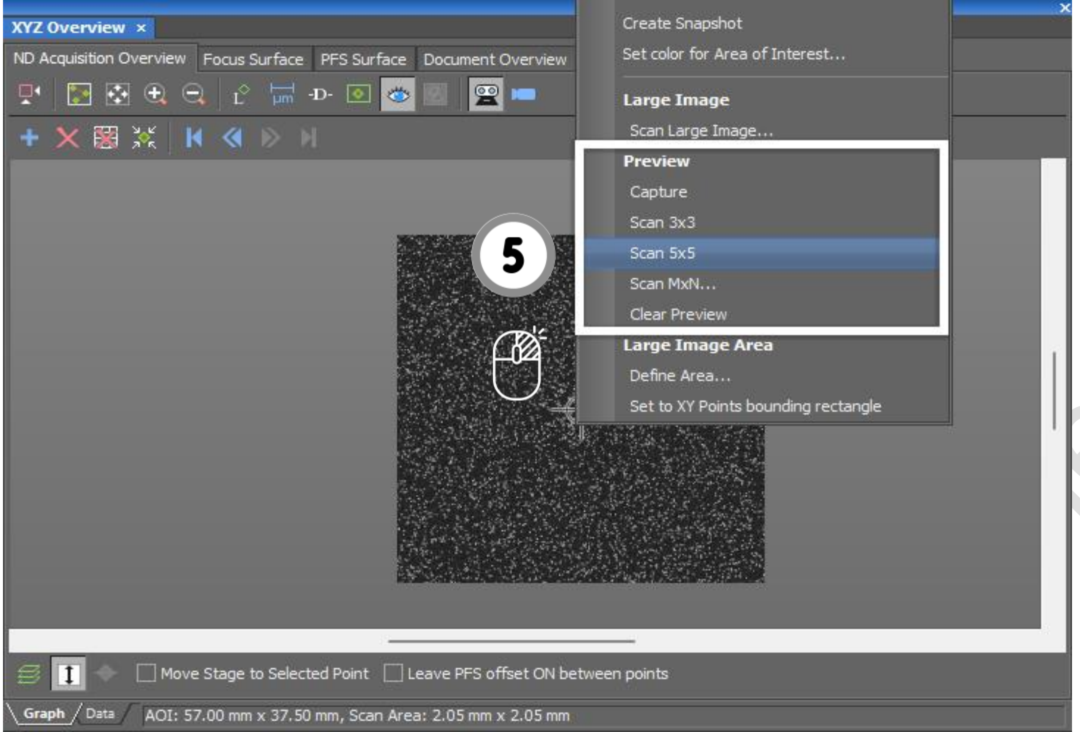
1. Select “**XYZ Overview**”.



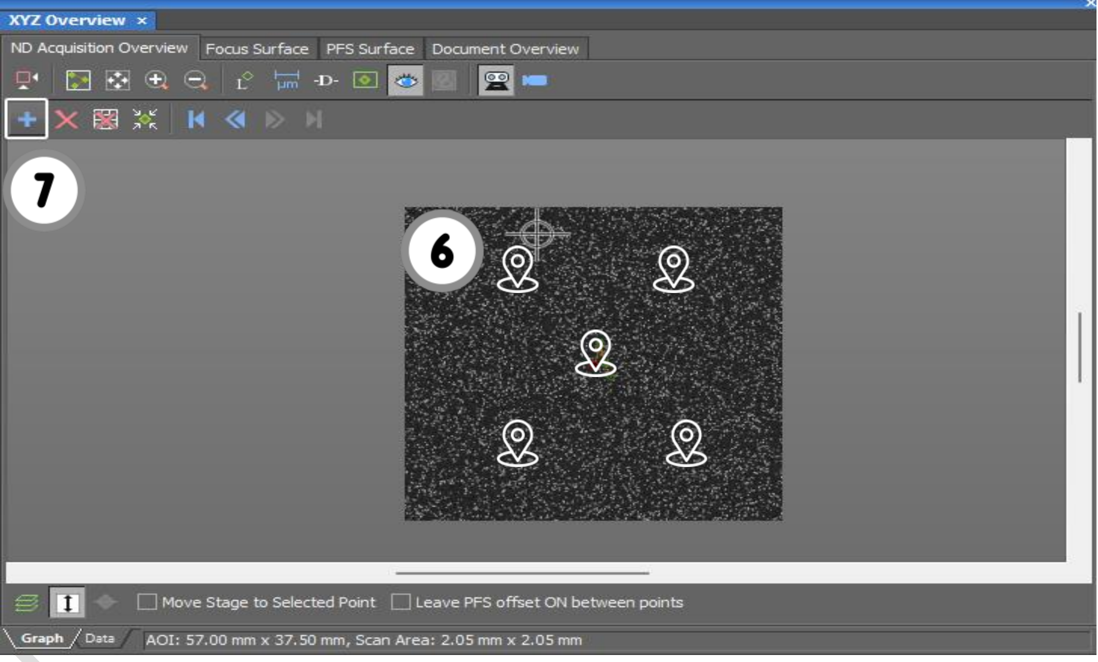
2. Click to remove the previous data.



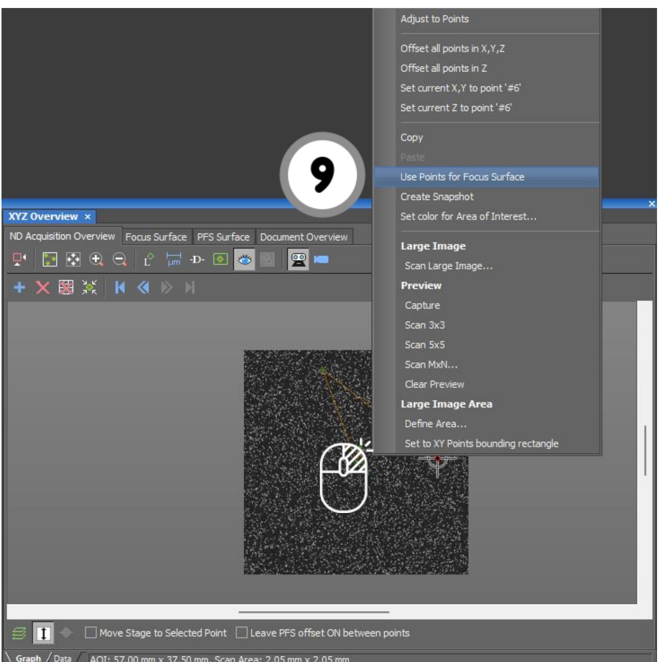
3. (Optional) Right-click on the imaging area, and select “**Clear Preview**”.
4. Click “Live”, and adjust the light intensity and exposure time if necessary.



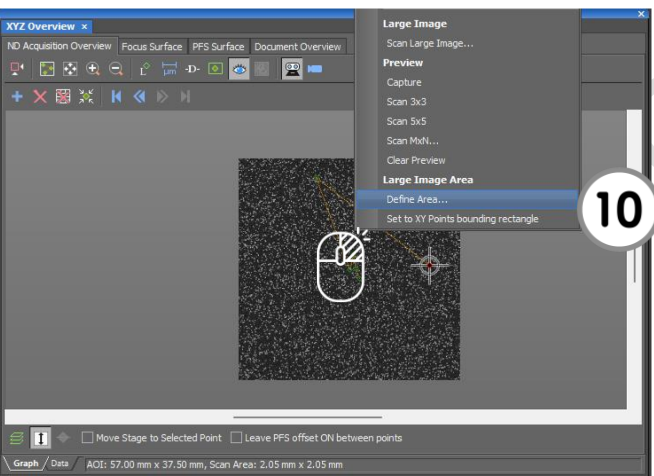
5. Right-click on the imaging area, and decide the preview area to scan a preview image.
6. Double-click on the image to move the stage to a specific point.



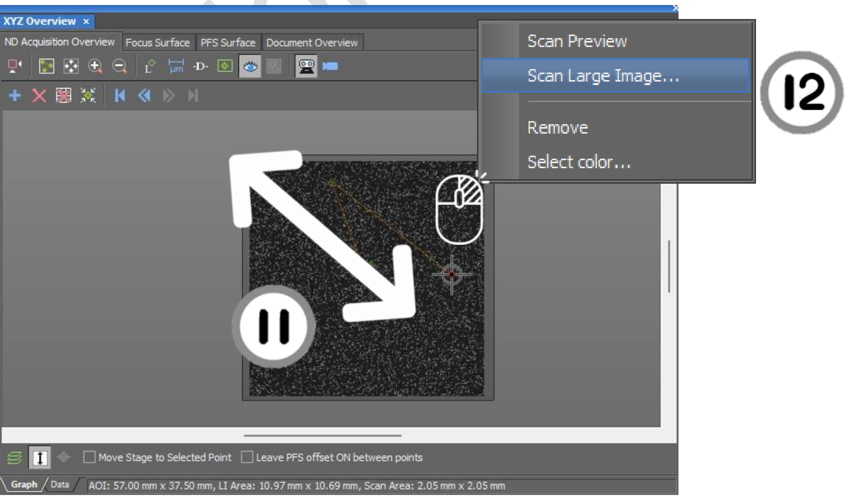
7. Adjust the focus and press + to add the points.
8. Repeat steps 8-9 until all points are added.
   *points can be added at the edges of your sample and center (as shown in above picture).



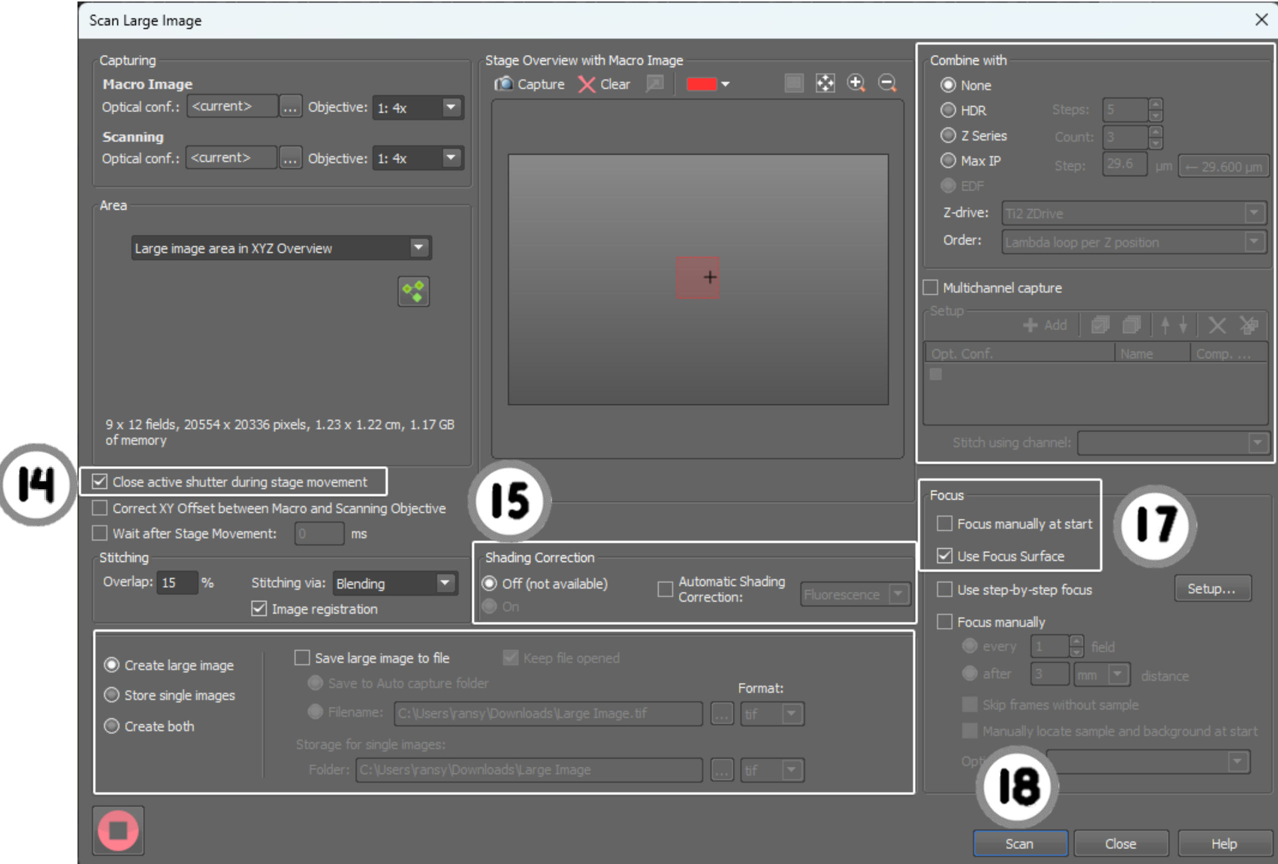
9. Right-click, and select “**Use Points for Focus Surface**”.



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**”.

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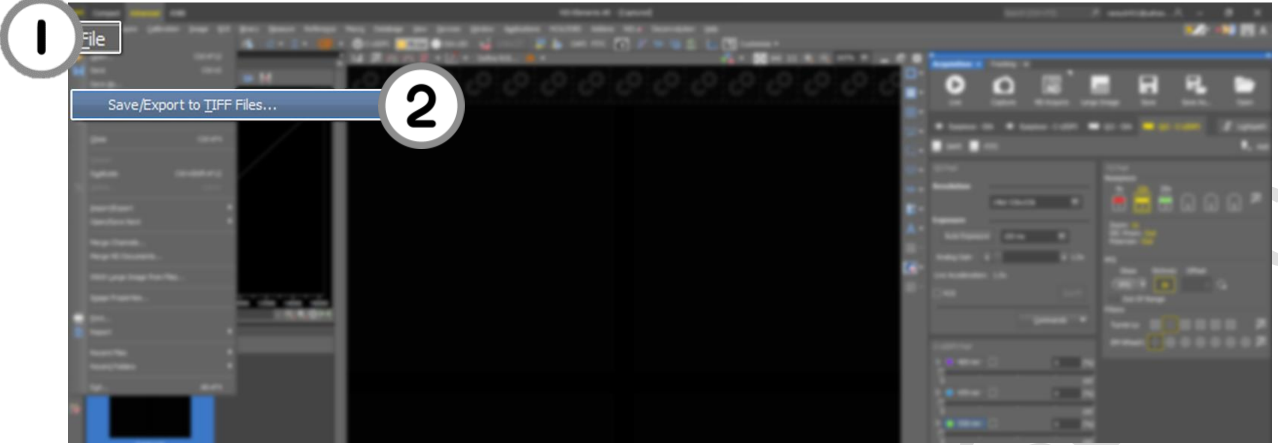
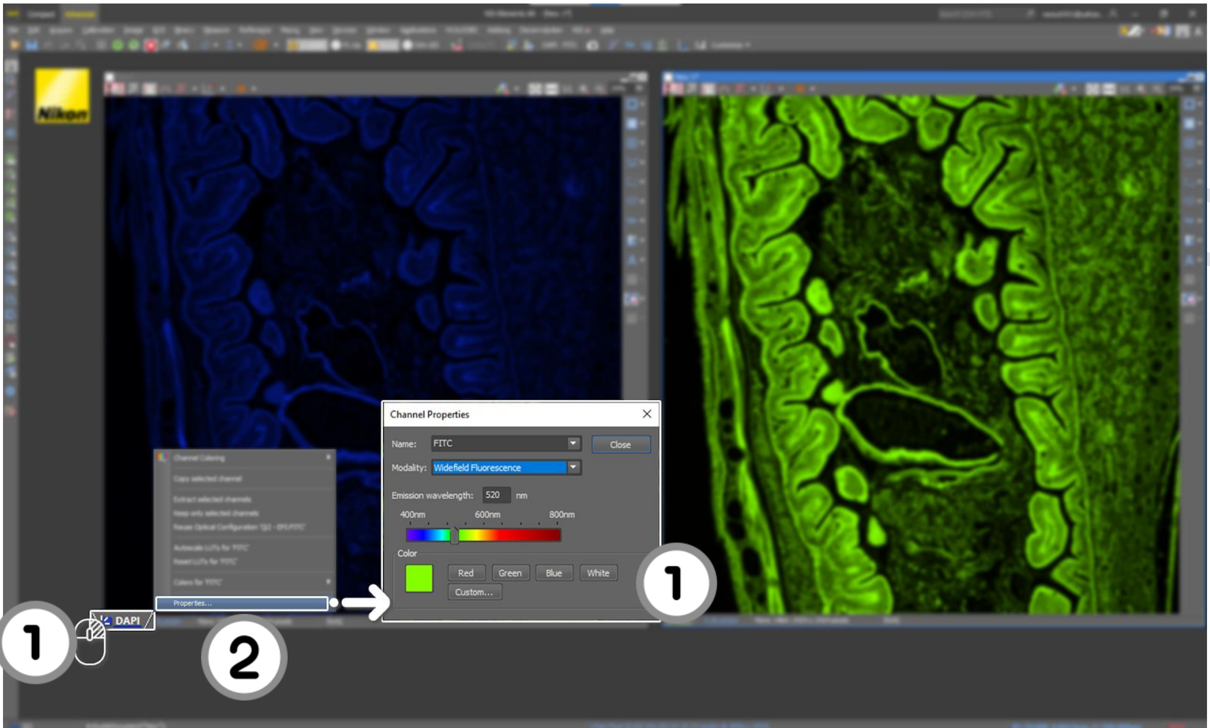
Batch Export of ND2 Images into Tiff



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**”.

Change the Channel Color



1. Right-click on the tag.
2. Select “**Properties...**”.
3. Select the desired color.

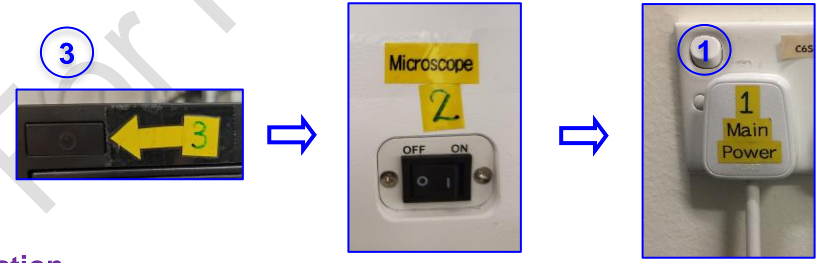
Turn off system

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. IF 100x/60x oil objective lens(es) is/are USED, it must be cleaned thoroughly with the LENS PAPER instead of Kimwipes.
2. Oil residue from the objective lens should firstly be removed using DRY lens tissue.
3. Repeat this step with a new area/piece of the lens cleaning tissue until no oil streaks are seen on the tissue.
4. Switch objective to lowest magnification (4x) in the software and press "**ESC**" to reach the Lower Z-limit.
5. Exit NIS-elements software
6. Transfer data to Imaging & Flow Cytometry Core storage server
7. Shut down the computer③ , wait until the PC is completely off.
8. Switch off microscope controller② , wait for 10 seconds
9. Switch off main power control①

For Live cell imaging

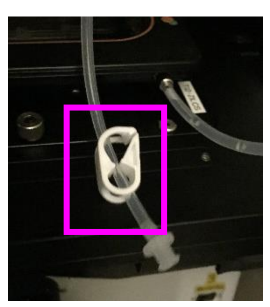
10. Switch off the Power of Tokai Hit incubation system controller.
11. Turn off CO_2 tank by turning the main switch clockwise
12. Turn off CO_2 regulator by turning regulator clockwise to the end
13. Take off objective heater on objective
14. Release the valve and remove the water from the chamber by plugging a 50ml syringe (located in the tool box) to the tube.



Incubation System controller

Main Switch

Regulator



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