



Nikon Eclipse T12-E Widefield Microscope

Standard Operation Protocol

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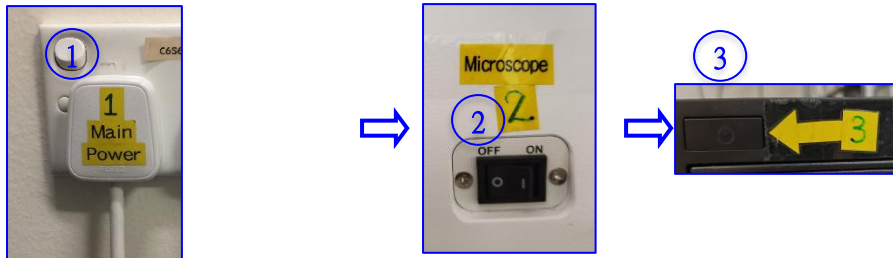
Imaging and Flow Cytometry Core

Version 2.0 2023

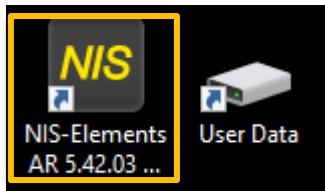
Turn on system

Please sign on the log sheet before switching on system.

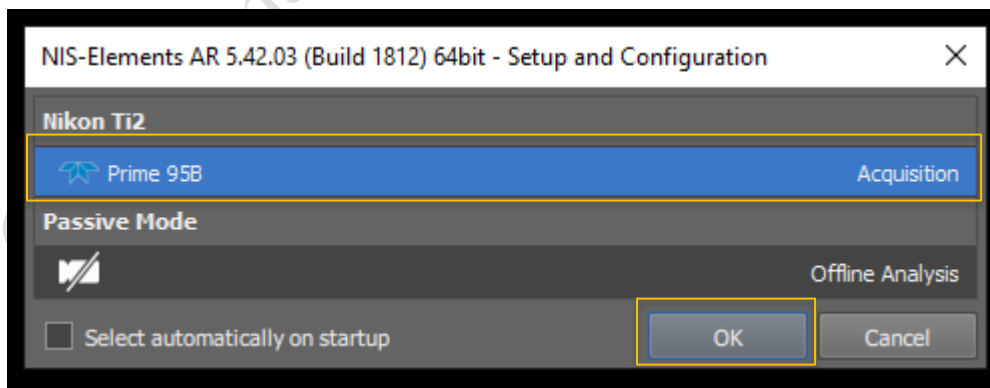
1. Switch on main power control ① wait for at least 5 sec before next step
2. Switch on microscope controller ② wait for at least 10 sec until the stage stop moving before next step
3. Turn on computer power ③



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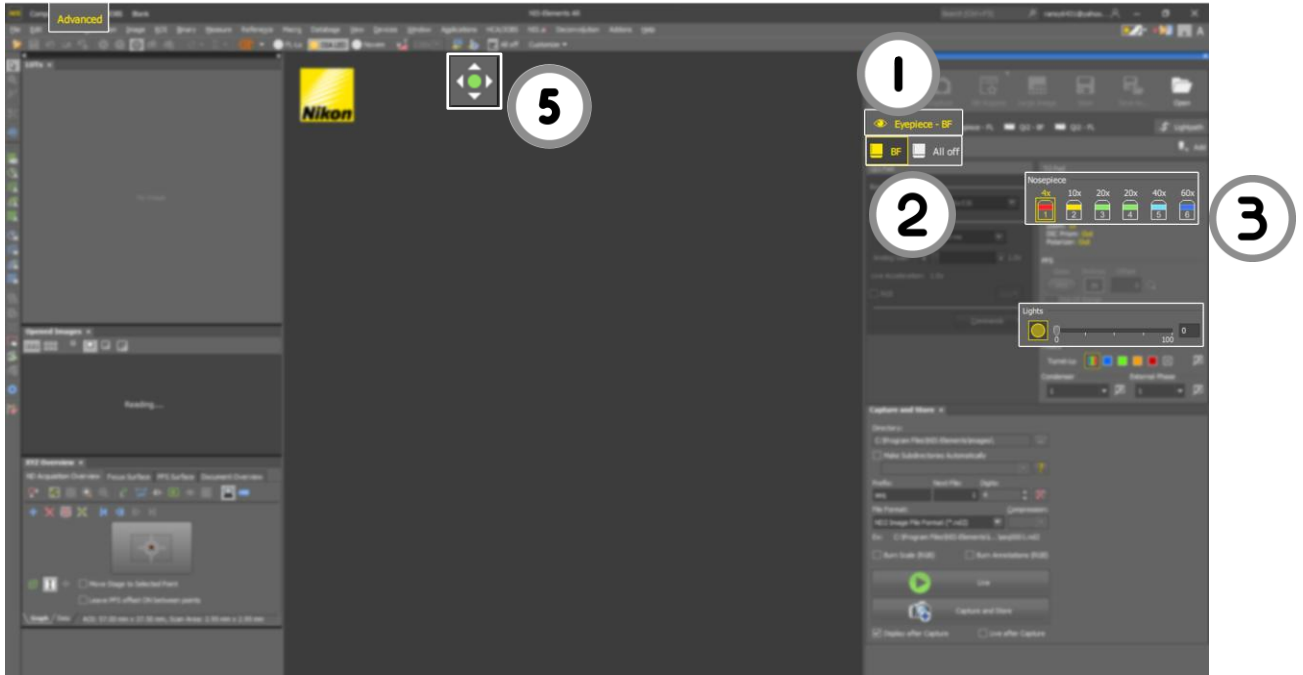
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(Optional) Set the temperature and CO₂ control for live cell imaging

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

1. Switch on "Incubator" for temperature and CO₂ 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 CO₂ sliding button is turned **ON**.
4. Turn on CO₂ tank by turning the main switch anticlockwise.
5. Turn on CO₂ 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 CO₂ gas.
8. MilliQ water has to be added into the water chamber and covered if overnight(s) acquisition is required.





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

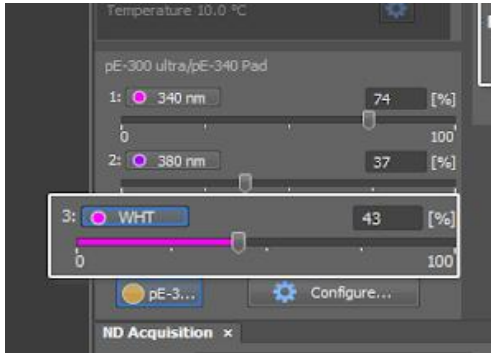
DAPI	GFP	RFP	Cy5
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

3. Select the desired objective (low magnification is recommended).
4. Change the light intensity if necessary.

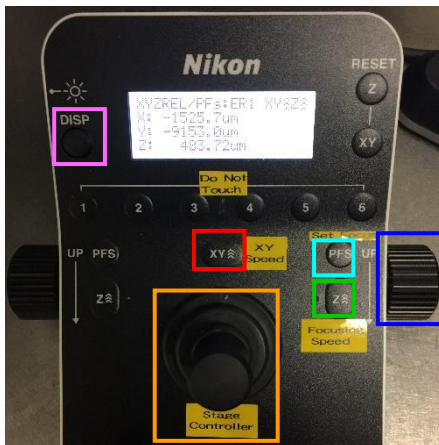
Brightfield – light intensity is adjusted using the knob on the side of the microscope.



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



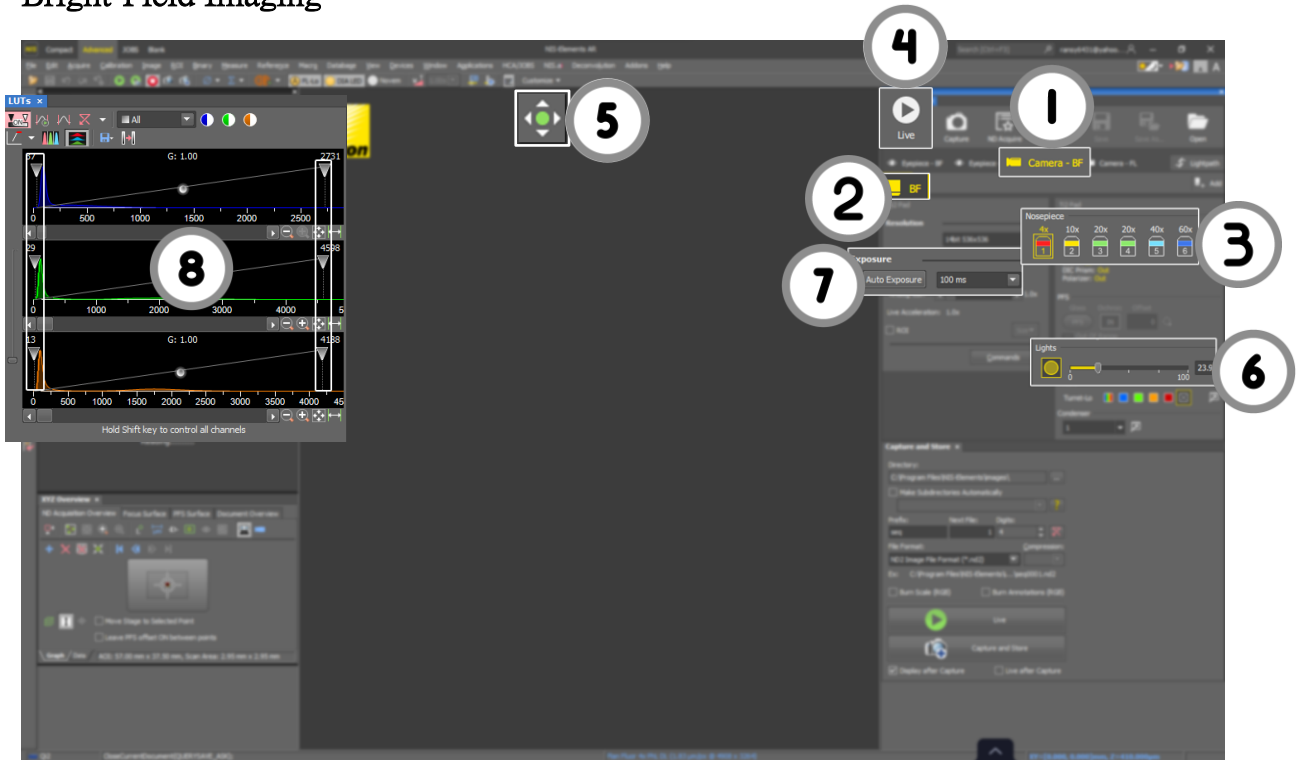
5. Press  to adjust XY position by moving mouse.
6. OR Move the **Stage Controller** to adjust XY position (**XY speed** can be adjusted: )
7. Focus the sample with the **focusing knob** → Clockwise_Down; Anti-clockwise_Up (Focusing speed can be adjusted: )






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Bright-Field Imaging

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1. Select “Camera - BF” .
 2. Select “BF”
 3. Select the desired objective.
 4. Click  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



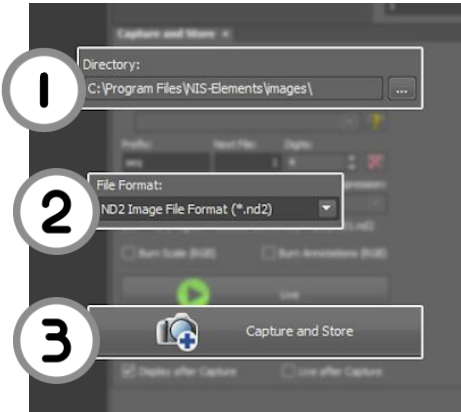
1. Select “Camera - FL” .
2. Choose the desired fluorescence channels (DAPI/GFP/RFP/Cy5) for channels.
3. Select the desired objective.
4. Click  to display the image.
5. Click  to allow moving your sample with your mouse or use focusing knob.
6. Adjust the fluorescent light intensity if necessary.
7.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

Imaging and Flow Cytometry Core

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Save Images

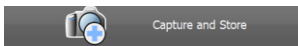
Capture and Store



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	<ul style="list-style-type: none">• 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







Imaging and Flow Cytometry Core

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Save Separately

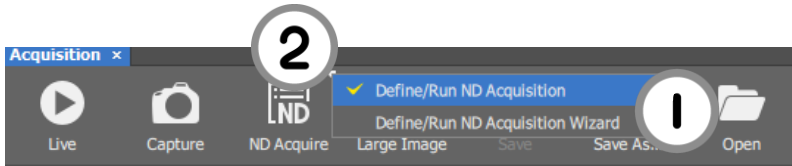
1. Click  to capture the image.
2. Click  to save the image (all data should be saved to D drive/User Data under your name).
3. Change the file name and select the file format.

File format	Features
.nd2	<ul style="list-style-type: none">• 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

CPOS Imaging and Flow Cytometry Core

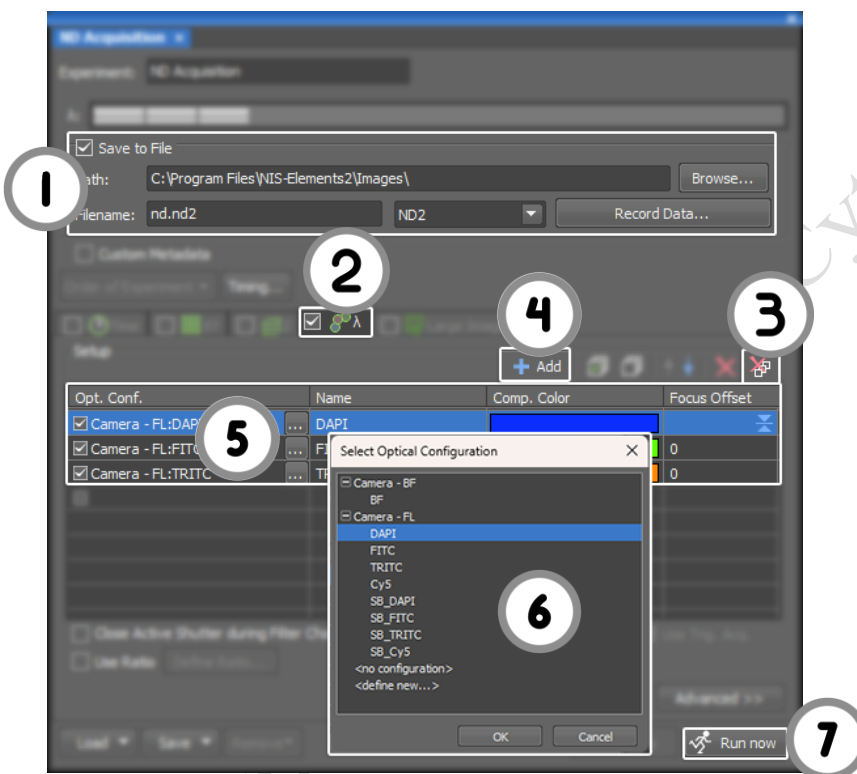
ND Acquisition

Multichannel

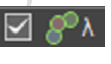


1. Right click to select “Define/Run ND Acquisition” .

2. Left Click 



1) Select Save to File , and select the appropriate path to save the images automatically if necessary (all data should be saved to D drive/User Data under your name).

2) Click  for Multichannel imaging , and ensure unwanted acquisition such as z-stack, time-lapse, etc. is not ticked.

3) Click  to delete the previous settings.

4) Click  to add channels.

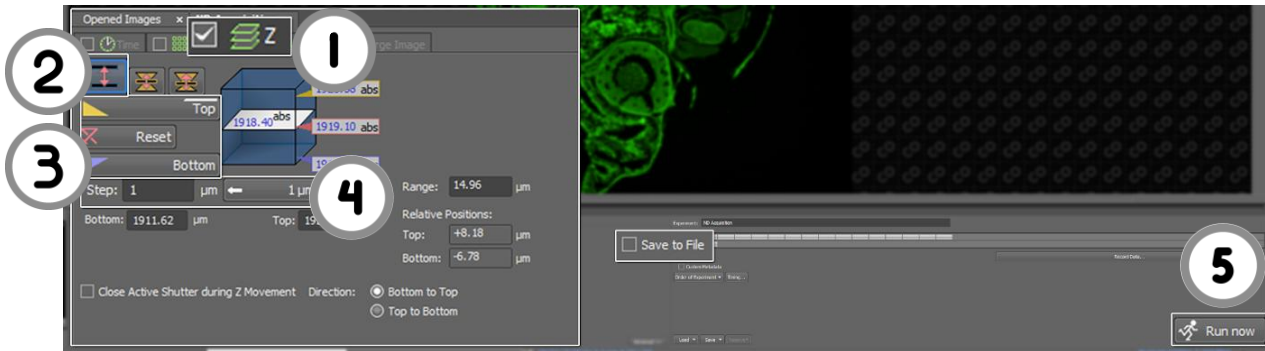
5) Click 



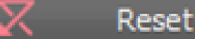



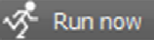
6) Select the desired channel.

7) Click 

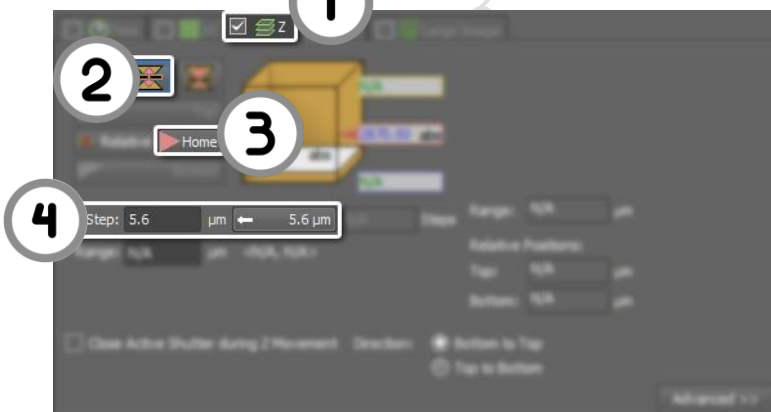
Z-Stack





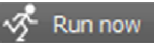
Define by top and bottom



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

Symmetric mode define by range

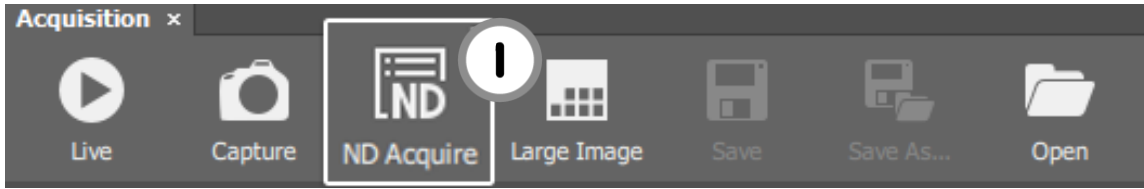


1. Click  for z stack imaging, and ensure unwanted such as time, λ is not ticked.
2. Select .
3. Adjust the focus and click .
4. Click  to use the recommended steps or type the desired steps.
5. Click .

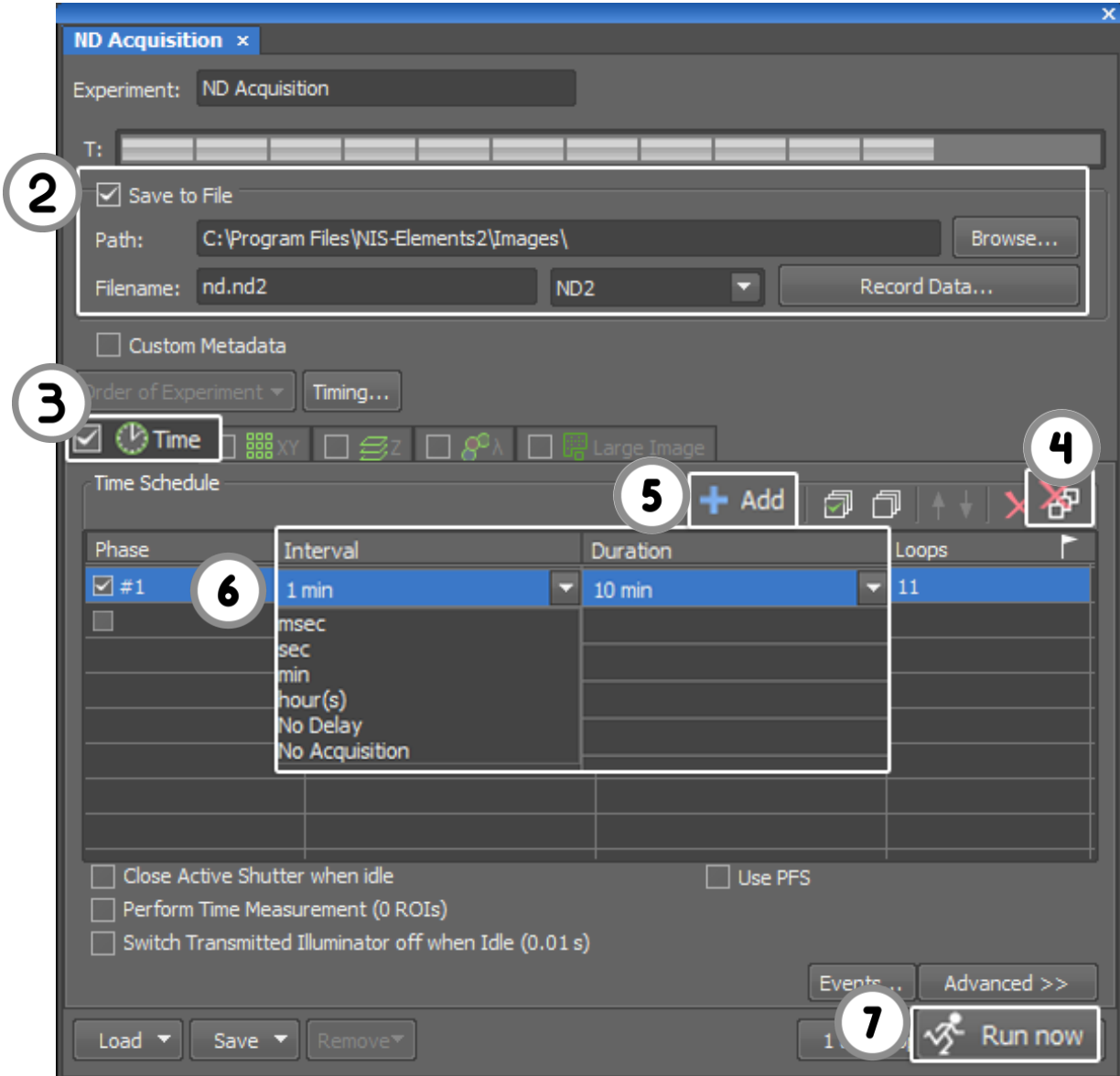
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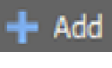
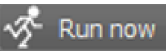
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Time Lapse



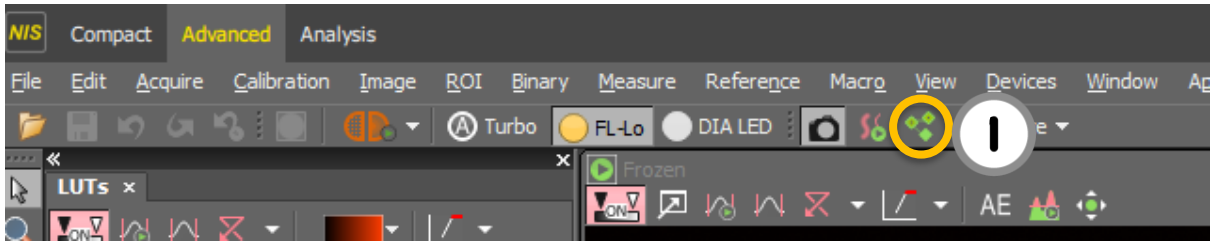
1. Click “ND Acquire” .



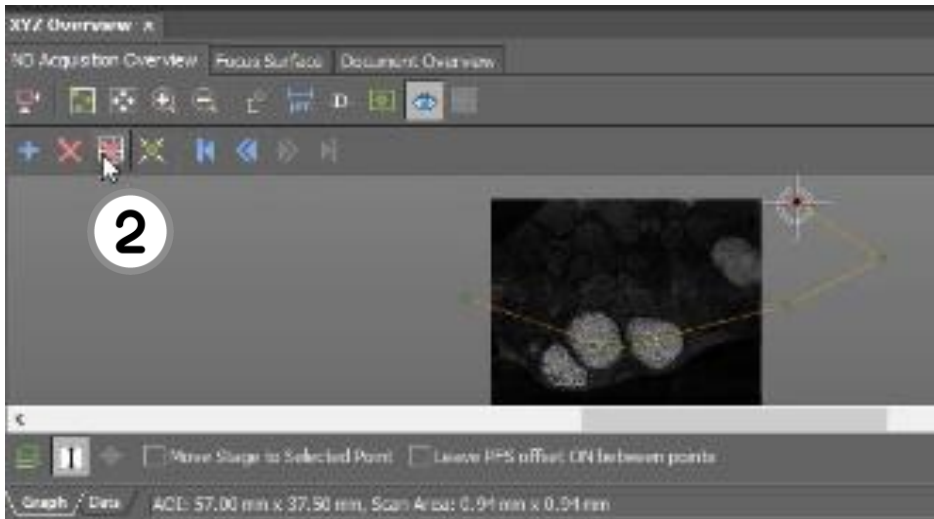
2. Tick “Save to File” and set the path to **D drive/User Data under your name**.
3. Tick “Time” .
4. Click  to delete all previous settings.
5. Click  to add time series.
6. Set the time-lapse detail.
7. Click 

Imaging and Flow Cytometry Core Stitching / Scan Large Image

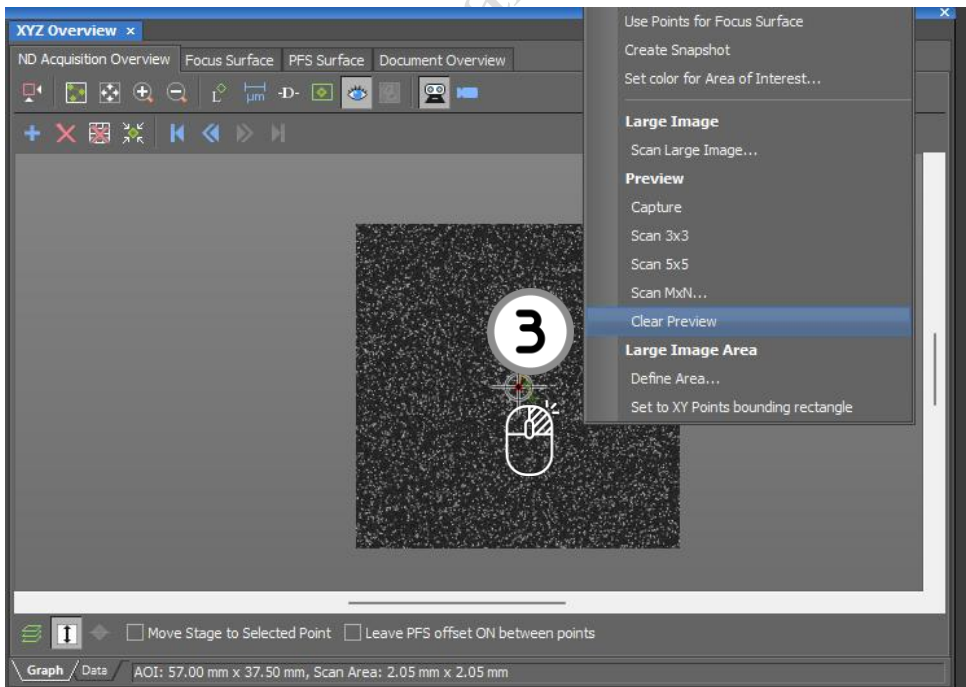
Version 2.0 2023



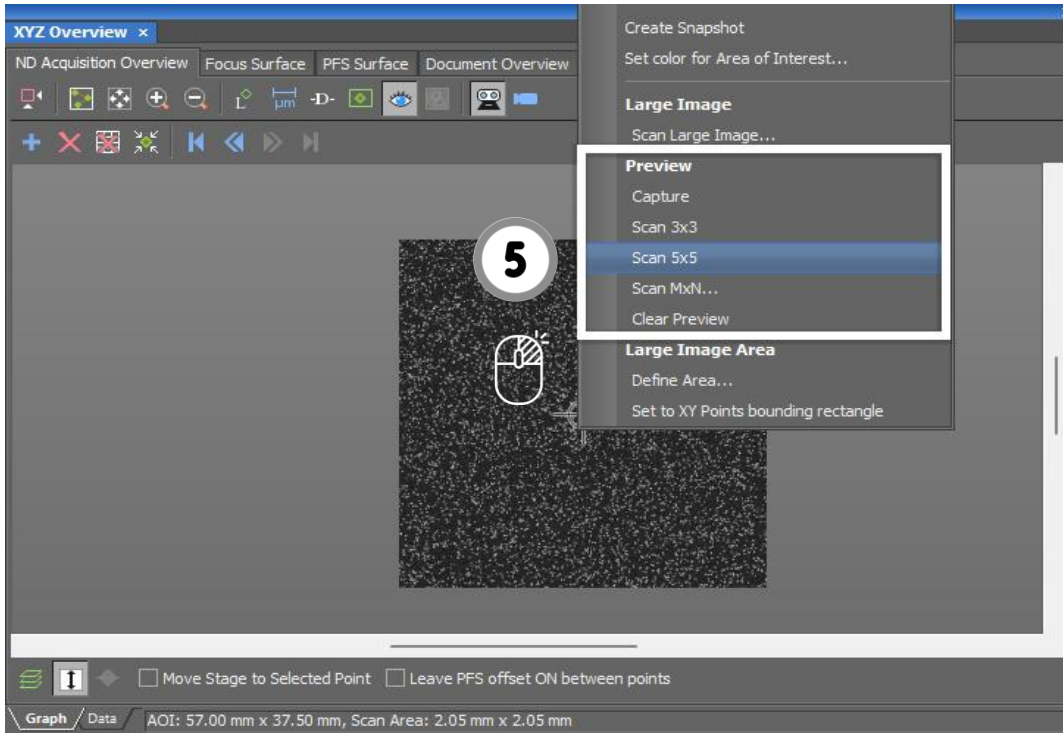
1. Select “XYZ Overview” .



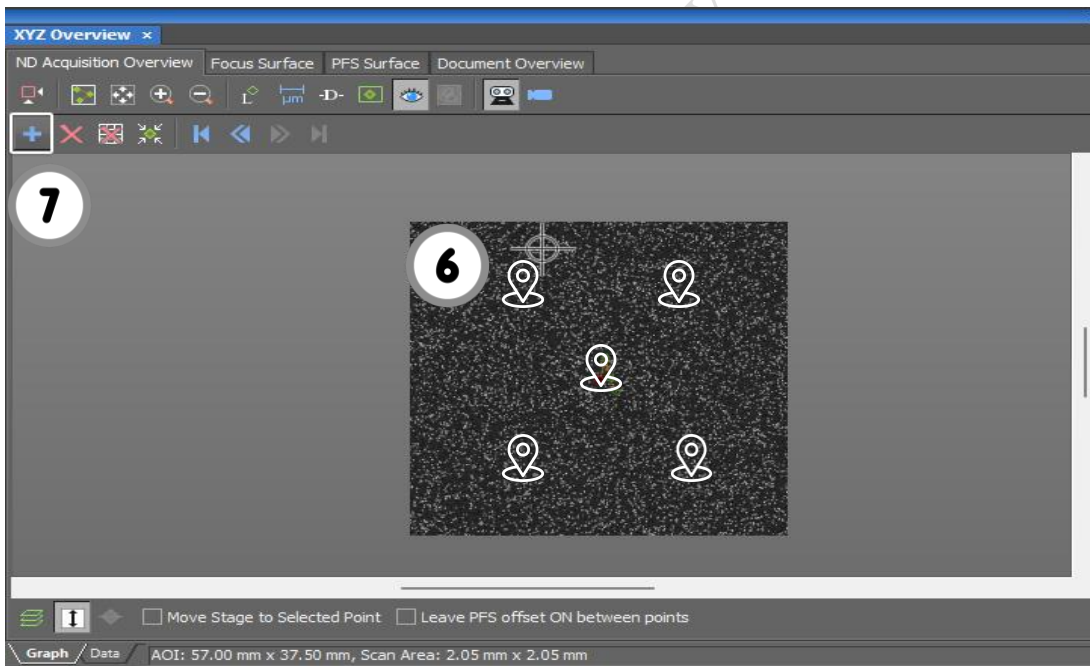
2. Click  to remove the previous data.




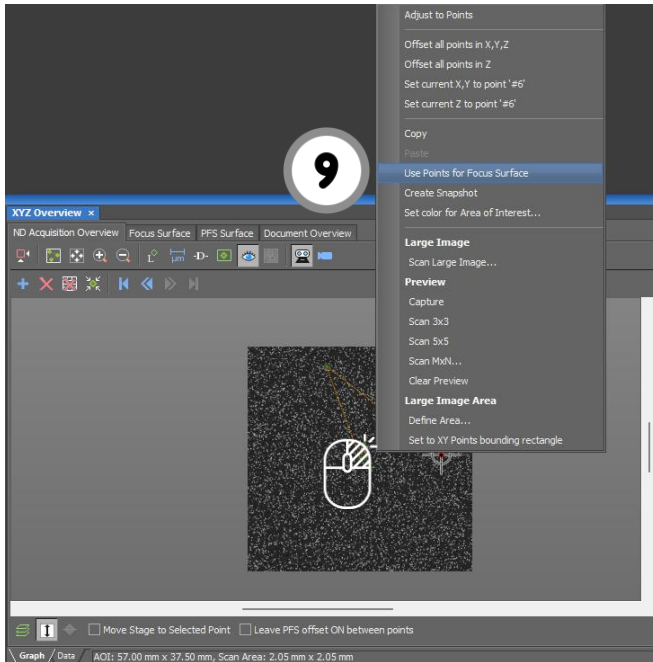
3. (Optional) Right-click on the imaging area, and select “Clear Preview” .
4. Click “Live” , and adjust the light intensity and exposure time if necessary.



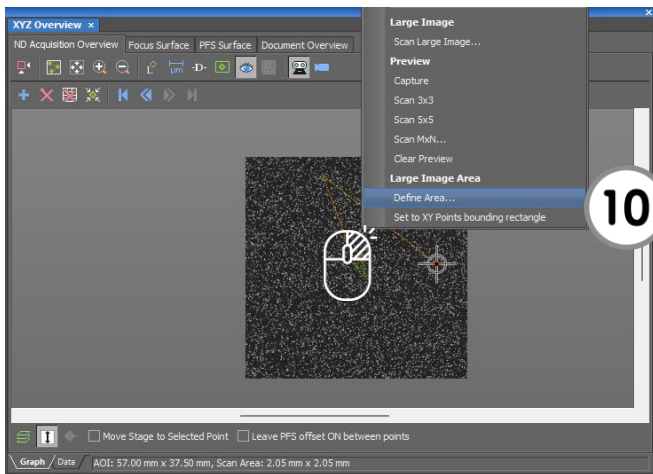
5. Right-click on the imaging area, and decide the preview area to scan a preview image.
6. Double-click on the image to move the stage to a specific point.



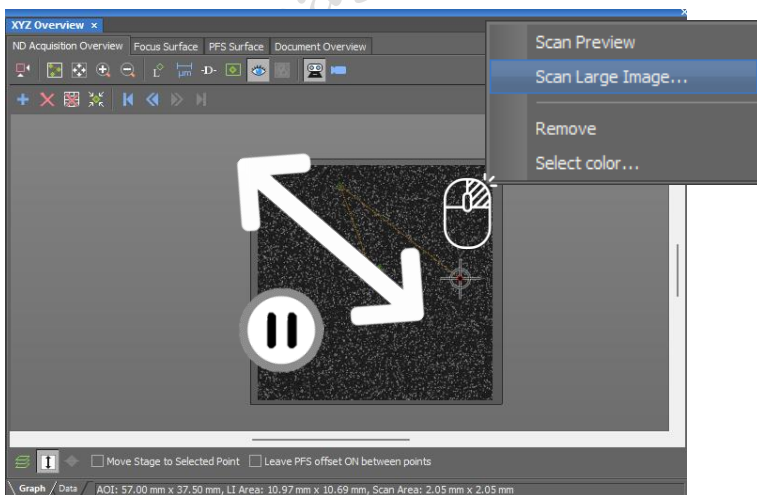
7. Adjust the focus and press  to add the points.
8. Repeat steps 8-9 until all points are added.
*points can be added at the edges of your sample and center (as shown in above picture).



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

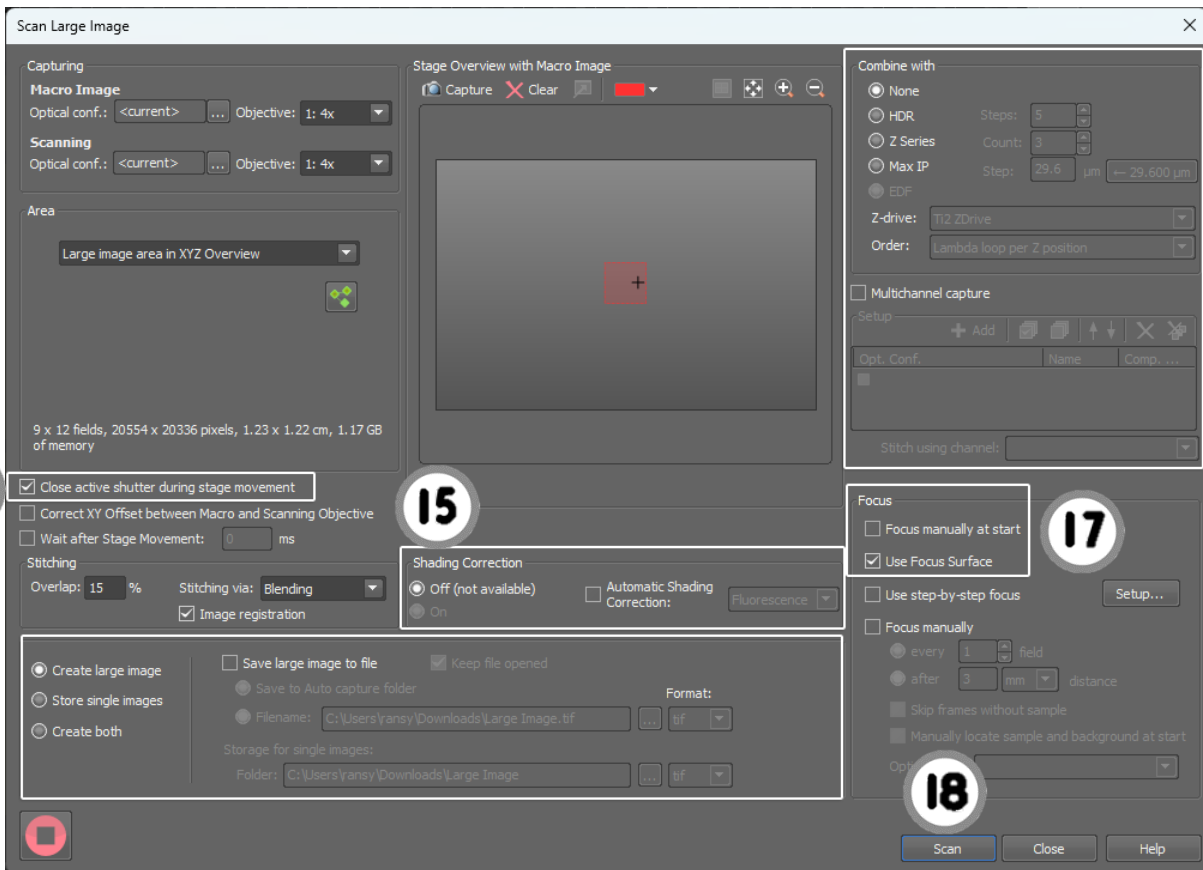


10. Right-click, and select “Define Area”



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.

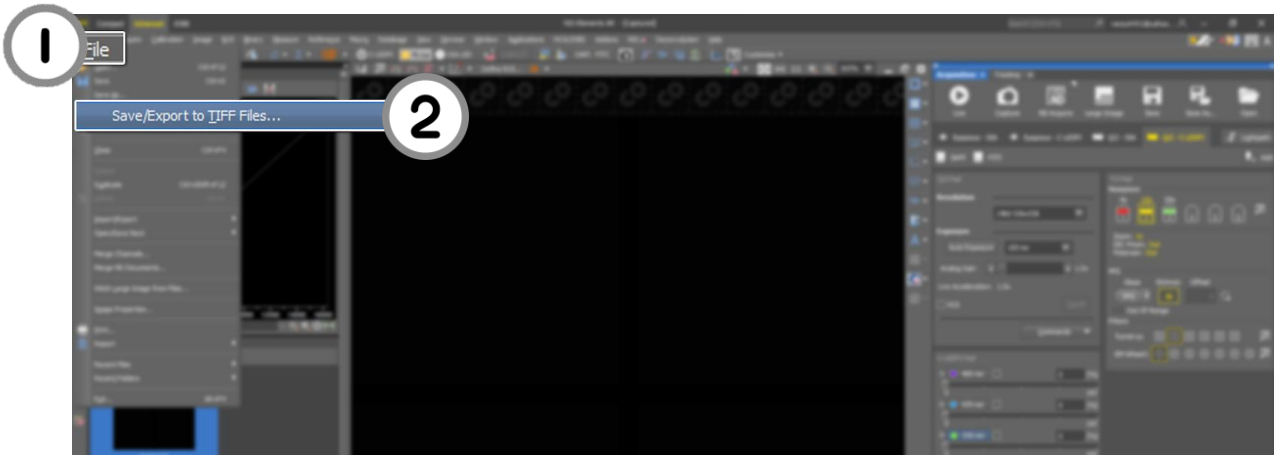
16. Select the file save format.

17. Ensure “Use Focus Surface” is ticked.

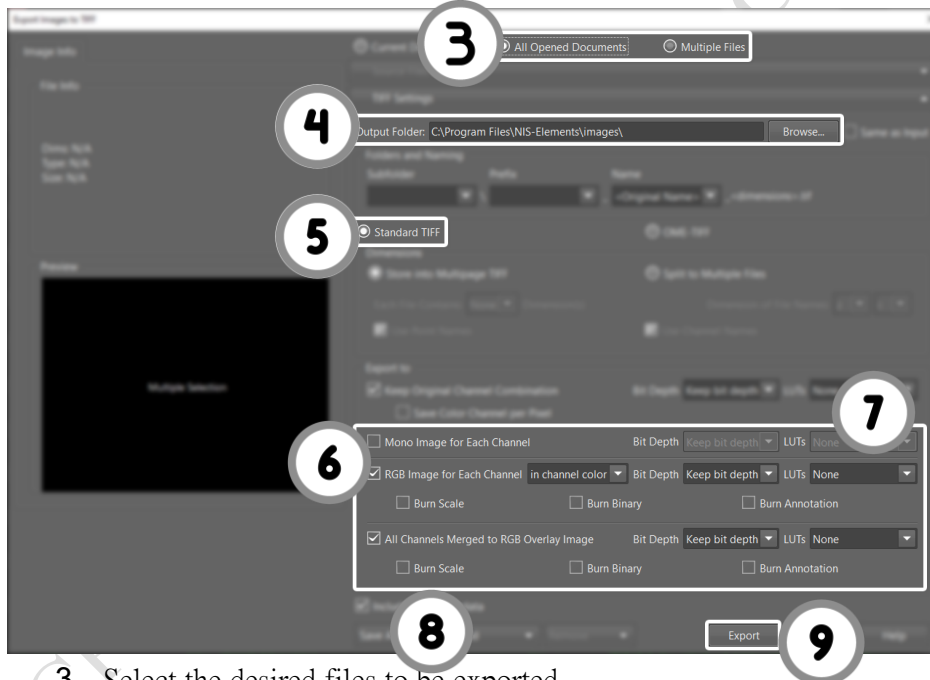
18. Click “Scan” .

CPOS Imaging and Flow Cytometry Core

Batch Export of ND2 Images into Tiff

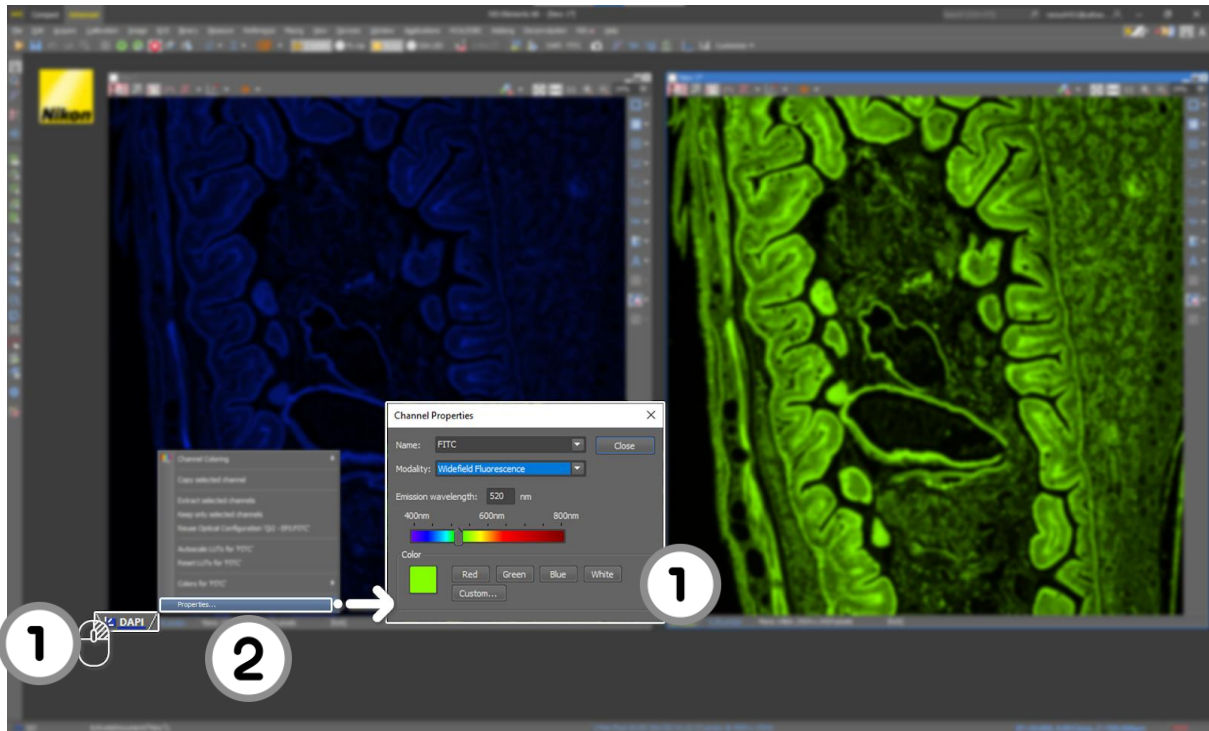


1. Click “File” .
2. Select “Save/Export to TIFF Files...” .



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. Select the desired color to be exported.
7. Select “Apply Saved LUTs” if you adjusted the LUTs(Do not recommend if for intensity quantification).
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.

CPOS Imaging and

Turn off system

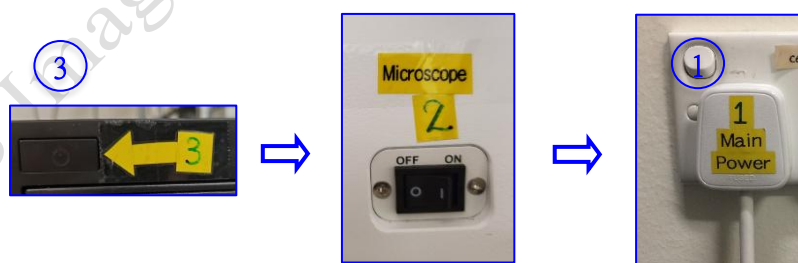
Please check if the equipment will be used by other users. Please switch off system if no one books equipment over two sessions (1h) after you.

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

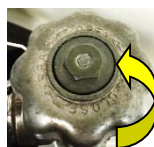
10. Switch off the Power of Tokai Hit incubation system controller.
11. Turn off CO₂ tank by turning the main switch clockwise
12. Turn off CO₂ regulator by turning regulator clockwise to the end
13. Take off objective heater on objective
14. Release the valve and remove the water from the chamber by plugging a 50ml syringe (located in the tool box) to the tube.



Incubation System controller



Main Switch



Regulator

