Nikon Eclipse Ti2-E Widefield Microscope Standard Operation Protocol

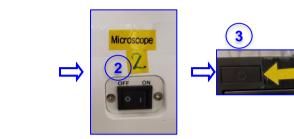
(Optional) Set the temperature and CO2 contro	
Sample locating and focusing	
Eyepiece Observation	
Bright-Field Imaging	
Fluorescent Imaging	
Save Images	
Capture and Store	
Save Separately	
ND Acquisition	
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Define by top and bottom	
Symmetric mode defined by range	
Time Lapse	
Stitching / Scan Large Image	
Batch Export of ND2 Images into Tiff	
Change the Channel Color	
Turn off system	

Turn on system

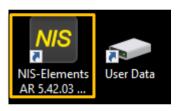
1 Main Power

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"

NIS-Elements AR 5.42.03 (Build 1812) 64bit - Setup and C	onfiguration	×		
Nikon Ti2				
T Prime 95B	Acquisition			
Passive Mode				
M/A	(Offline Analysis		
Select automatically on startup	ок	Cancel		

(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 CO_2 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 \rightarrow 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

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- 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.

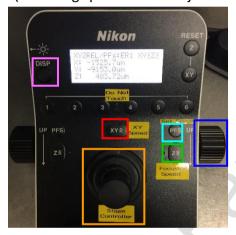
Brightfield – light intensity is adjusted using the knob on the side of the microscope.



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

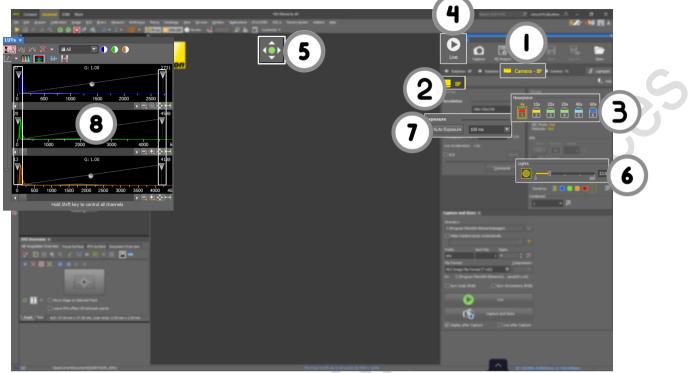
	43	[%] 100
3: О WHT		
0 2: O 380 nm	37	100 ['] [%]
pE-300 ultra/pE-340 Pad 1: O 340 nm	74	[%]

- 5. Press to adjust XY position by moving mouse.
- 6. OR Move the Stage Controller to adjust XY position (XY speed can be adjusted: $\frac{2}{3} > 2 > 3$)
- 7. Focus the sample with the focusing knob \rightarrow Clockwise_Down; Anti-clockwise_Up



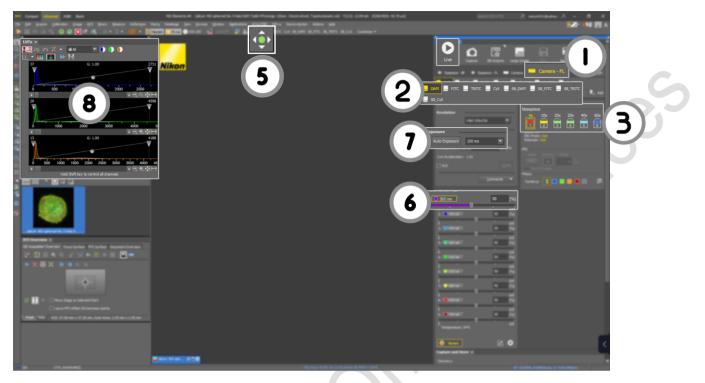
(Focusing speed can be adjusted: $\frac{1}{2} > \frac{1}{2} > \frac{1}{2}$

Bright-Field Imaging



- 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

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 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

Save Images

Capture and Store

Explore and Nove 1.	
Directory:	
C:\Program Files\VIS-Elements\images\	
	- - 7
Parlies Institute Dayles	
File Format:	1141
2 ND2 Image File Format (*.nd2)	
Cherchae 2020 Cherchevel	
0 -	
Capture and Sto	re
2 Junio Ale Same	Capture

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

2. Select the file format.

10

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

Save Separately

- 1. Click to capture the image.
- Click Stock 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.

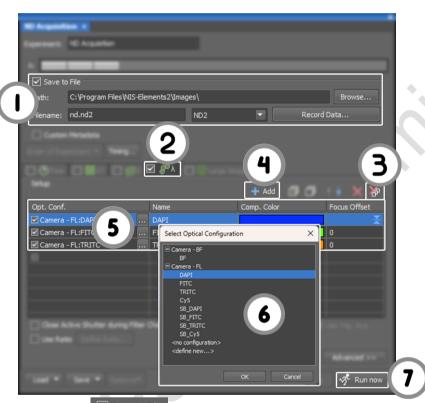
File format	Features
.nd2	 Recommended Largest file size can save all the information including the camera and device settings of your image Cannot be opened by Windows
.tiff	Can be opened by Windows

ND Acquisition

Multichannel



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 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

Opened Images x Z perimon Crime C Z perimon Reset Bottom Step: 1 µm ← 1µm 4 Range: 14.96 µm			
Bottom: 1911.62 µm Top: 19 Relative Positions: Top: +8.18 µm Bottom: 6.78 µm Close Active Shutter during Z Movement Direction: Bottom to Top Top to Bottom	Save to File	5	

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

Symmetric mode defined by range

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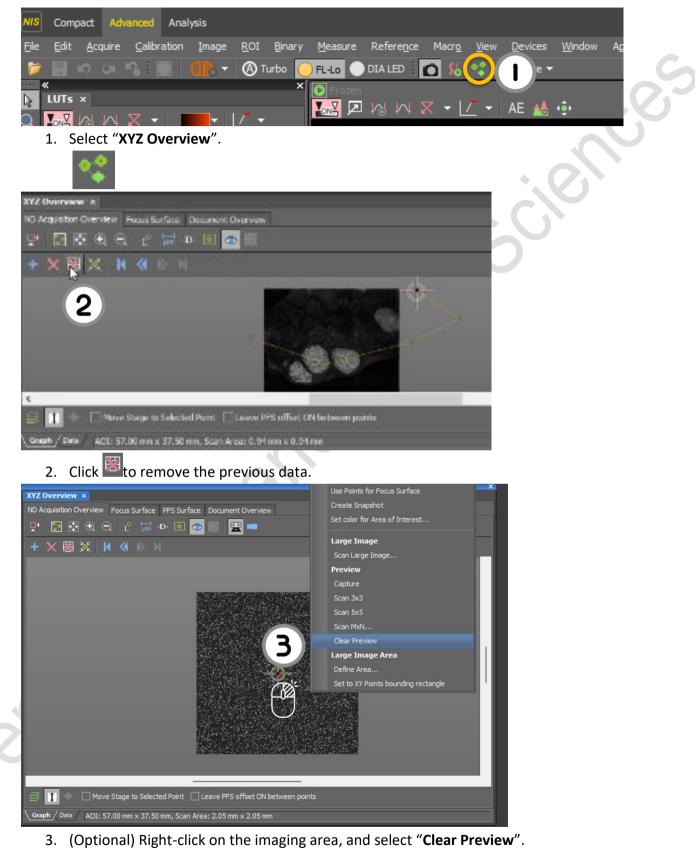
- Click Z Z for z stack imaging, and ensure unwanted such as time, λ is not ticked.
- 2. Select 🔀
- 3. Adjust the focus and click Home
- 4. Click to use the recommended steps or type the desired steps.
- 5. Click 📌 Run now

Time Lapse

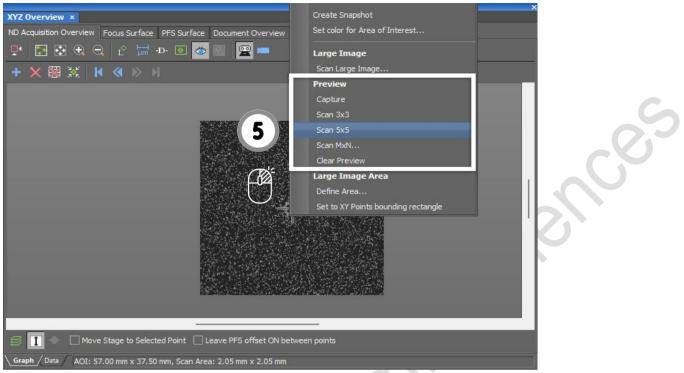
	Acquisition	× Capture	ND Acquire	Large Image	Save	Save As	Open	C
1. (Click "ND Ac	quire".						
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	Phase #1	6 Interval 1 min msec sec min hour(s) No Delay No Acqu	,	Duration IO min IO min IO IO		Loops 11		
	Perform	ctive Shutter when n Time Measuremen Transmitted Illumina Save 🔻 Rem	t (0 ROIs)	(0.01s)	Use PFS		lvanced >> Run now	

- 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 + Add to add time series.
- 6. Set the time-lapse detail.
- 7. Click 📌 Run now

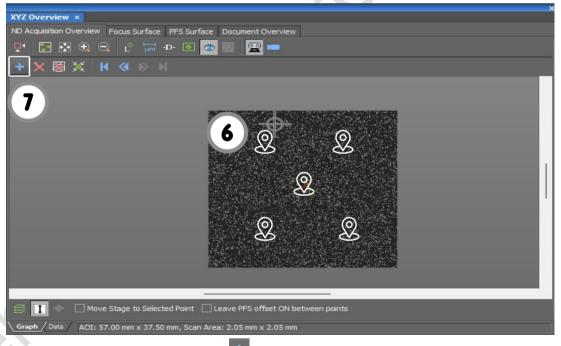
Stitching / Scan Large Image



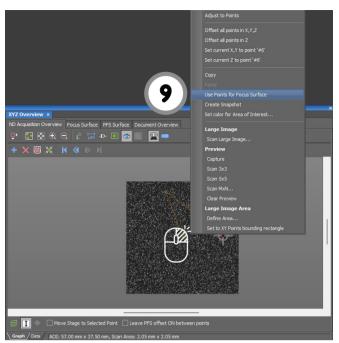
4. Click "Live", and adjust the light intensity and exposure time if necessary.



- 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.

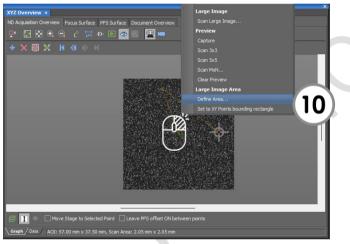


- 7. Adjust the focus and press **b**to add the points.
- Repeat steps 8-9 until all points are added.
 *points can be added at the edges of your sample and center (as shown in above picture).

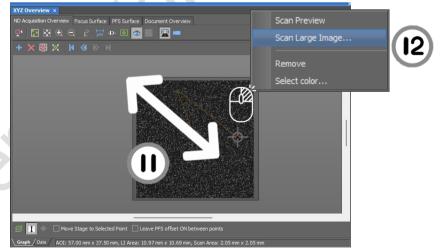


9. Right-click, and select "Use Points for Focus Surface".

, ces



10. Right-click, and select "Define Area

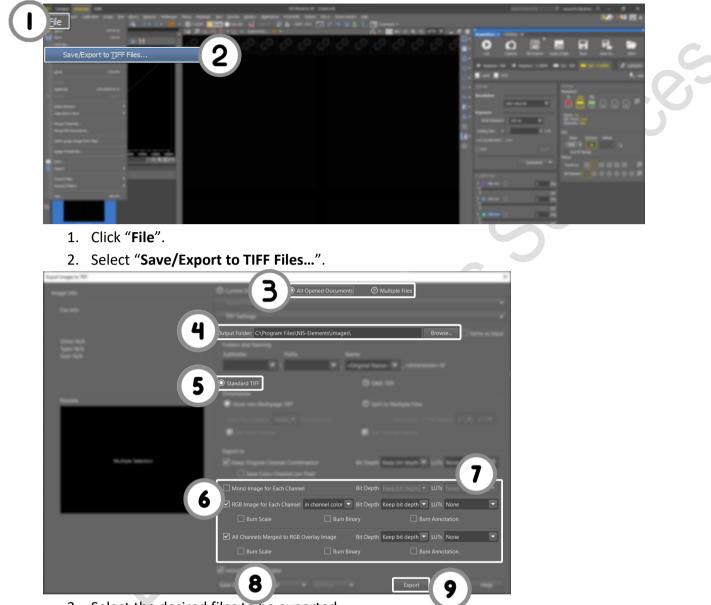


- 11. Drag to set the scan area. Adjust the scan area by dragging the boundary if necessary.
- 12. Right-click, and select "Scan Large Image...".

Scan Large Image				×
Capturing Macro Image Optical conf.: <current> Scanning Optical conf.: <current> Area Large image area in X 9 x 12 fields, 20554 x 2033 of memory</current></current>	Objective: 1: 4x Objective: 1: 4x	erview with Macro Image sture 🗙 Clear 🔎 🛑 🕶	eDF Z-drive: 112 2Drive Order: Lambda loop per Z p Multichannel capture Setup Add 2	© ↓ ↓ × ₩ Name Comp
Wait after Stage Moveme Stitching Overlap: 15 % S	n Macro and Scanning Objective nt: ms Shading C Shading C Shading C Off (n Image registration	Correction ot available) Automatic Shading Correction:	Focus Focus manually at start Focus Surface Use step-by-step focus Focus manually every focus manually focus ma	Setup
Create large image Store single images Create both	Save large image to file k ke Save to Auto capture folder Filename: C:\Users\ransy\Download Storage for single images: Folder: C:\Users\ransy\Downloads\Lar		after 3 mm Skip frames without sam Manually locate sample : Opt	distance

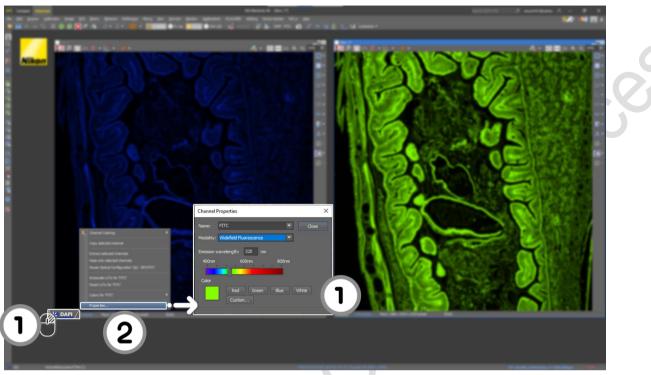
- 13. Combine with other acquisitions if necessary.
- 14. Tick "Close active shutter during stage movement" if you want to reduce the bleaching.
- 15. Tick "Automatic Shading Correction" and select the imaging method if necessary.
- 16. Select the file save format.
- 17. Ensure "Use Focus Surface" is ticked.
- 18. Click "Scan".

Batch Export of ND2 Images into Tiff



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

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.

