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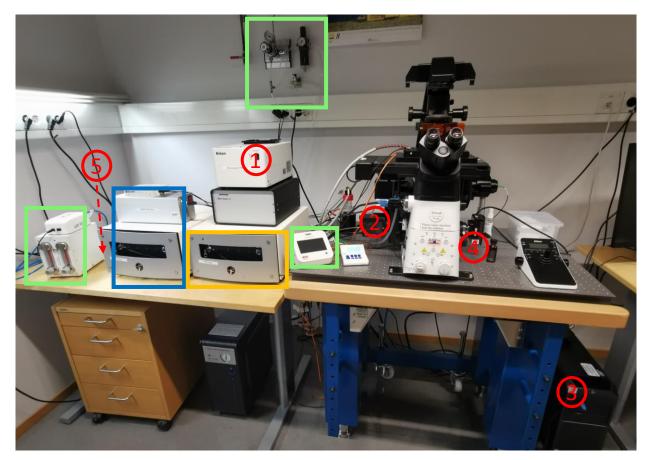
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- 1. Turn on the microscope ctrl box (1).
- 2. Turn on the TIS camera for fluorescence imaging (2) if needed.
- 3. Turn on the computer and log in using "user" and password " ".
- 4. Turn on the Fi3 color camera (4) if needed.
- 5. Turn on the CoolLed for fluorescence (switch on el. pad)

For live cell imaging, open the CO2 valve, turn on the OKO lab mixer and start the heating (green boxes). See page 7 for more information.

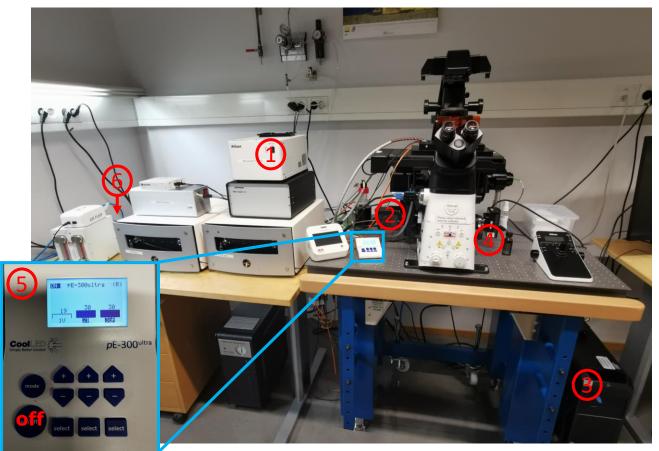
For FRAP imaging, remember to turn on the FRAP laser and the FRAP ctrl box (blue).

For TIRF, remember to turn on the TIRF lasers (yellow).



- 1. Transfer your data (page 22).
- 2. Close the software and start a computer shutdown.
- 3. Turn off the TIS camera (2) and the Fi3 camera (4) (if they were on).
- 4. Turn off the microscope control box (1).
- 5. Turn off the CoolLED (5).
- 6. Switch of the CoolLED on el. pad (6).
- 7. Remember to sign the logbook and report any issues to MIC personnel.





Starting the software?

- Log on to the computer with Nikon ٠ (password Nikon).
- Start "NIS-ELEMENTS

- Login using the "**user**" account (no password needed).
- · Select the camera you would like to use. If you do not want to acquire any images, select "passive mode". For more details about the camera options, see page 6.
- The objectives are the eyes of the system, so make sure you use them correctly. The 60x and 100x must be used with immersion oil. The other objectives are DRY! The two objectives in position 5 and 6 have large working distance. These can be used for cell culture plates and give nice phase contrast images.

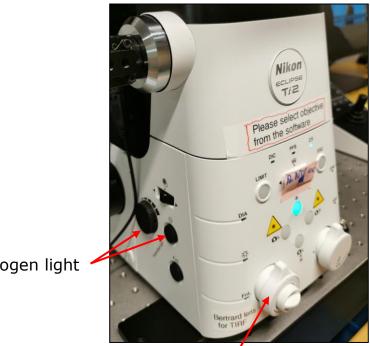


Login		×
User name:	user	•
Password:	 	
	Login	Close

NIS-Elements AR 5.30.05 (Build 1559) 64bit - Setup and Configuration
Prime BSI EPI only
TIS, 🎌 Prime BSI Express
Prime BSI EPI and TIRF
🔀 TIS, 🕋 Prime BSI Express
Fi3
Fi3
Passive Mode
1

DNLY FOX 60x, 1001	Ti2 Pad Nosepiec 20x 1 Zoom: 1x	40x 60x	100x	20x 10	×	
		Oil objective	es	DRY obje	ctives	
Immersion oil		with small		with large	<u>.</u>	
		working dist	ance	working		
		and high NA		distance.		

Viewing through the microscope



Bertrans lens

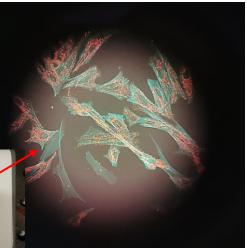
Fluorescence filter turret. Use the multiband filter. Press the button to open/close the shutter.



Halogen light

Click on a fluo setting in the software in order to get the multiband filter in position.



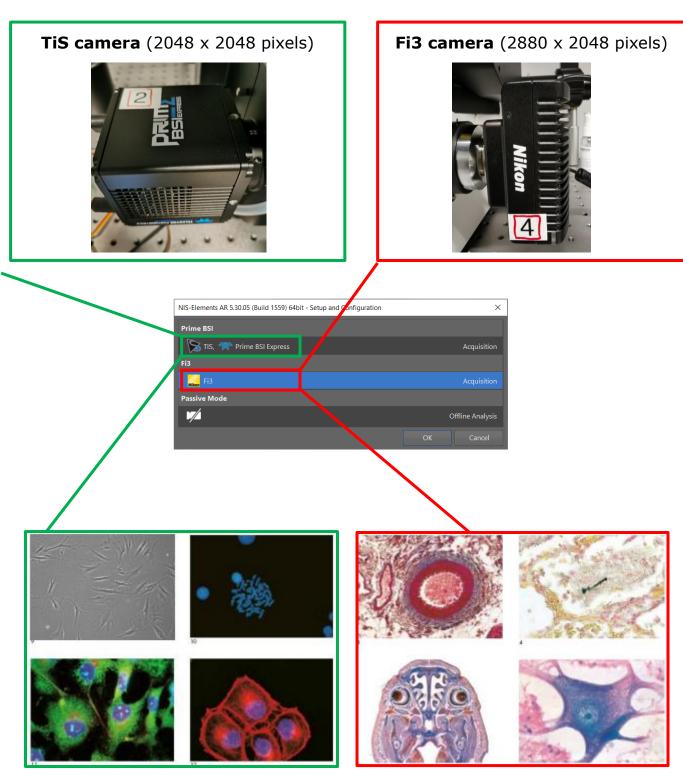


You can leave the 2nd and 3rd channel on to see both green and red! To change Z speed, press here



To change the XY speed, press here

CoolLed ctrl panel

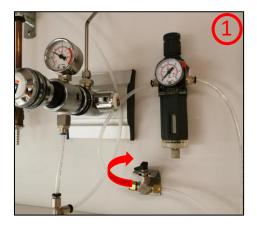


Use this camera for imaging of fluorescent samples.

Use this camera for imaging of brightfield, phase contrast and histological samples.

Live cell imaging setup

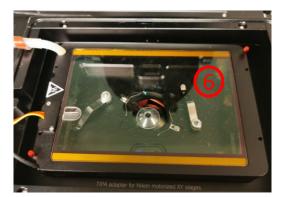
- Flip the small switch upwards to open for the CO2 gas (1). Do Not touch any of the other valves!
- Turn on the air pump (2). Do not touch any of the valves on the box underneath!
- Turn on the small OKO control box (3) to start the heating of the insert.
- Make sure you connect the correct heating cable (4) for the objective you will use (60x or 100x). The red heating element will start to warm up the objective. For good stability allow 0,5-1 h warm up time.
- Make sure there is enough distilled water in the humidifier (5).
- Use the live cell insert and heating lid (6).













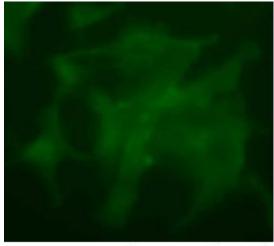


Objectives available on the system

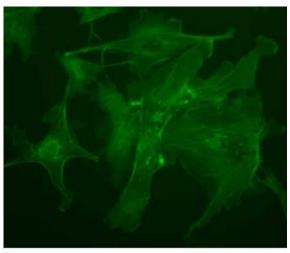
Magnification	Correction	NA	WD (mm)	Contrast method and application
10 x Air	Plan Fluor	0,30	15,2	Ph 1 / phase contrast
20 x Air	Plan Fluor (coverslip correction)	0,45	7,4	Ph 1 / phase contrast
20 x Air	Plan Apochromat lambda	1,20	1,0	fluorescence
40 x Air	Plan Apochromat lambda (coverslip correction)	0,95	0,21	fluorescence
60 x Oil	CFI Apo TIRF (coverslip and temperature correction)	1,49	0,12	TIRF / fluorescence
100 x Oil	CFI Plan Apochromat lambda	1,45	0,13	fluorescence

Some objectives have a correction collar (A) to adjust for "cover slip" thickness between 0 and 2 mm (B). In order to adjust:

- Look through the eyepiece at all times.
- Turn the correction collar slightly and refocus. Does it look sharper?
- Turn the correction collar again slightly and refocus.
- Plan Fluor WD 40 X/0.60 Ph2 ADL %/0-2 WD 3.7-2.7
- Repeat until you get a sharp, crisp image.



Correction collar not adjusted



Correction collar adjusted

Objectives available on the system continues...

100x 20x

5

6

4



Immersion oil only for 60x and 100x

Objectives suitable for coverslip (170 µm thick)

Nosepiece

20x

Zoom: 1x

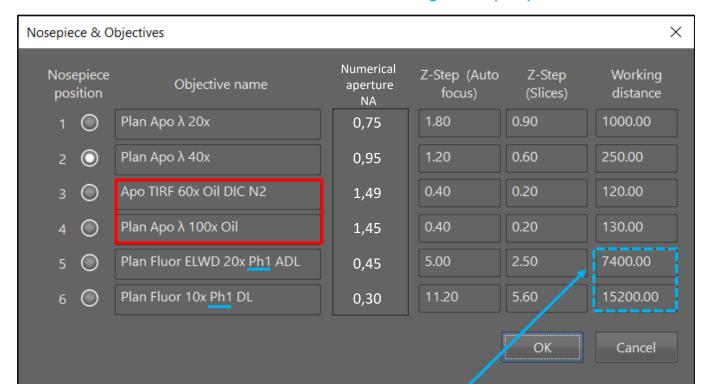
40x

2

3

DRY objectives suitable for coverslip as well as plastic bottom dish (cell culture, spheroids, larger samples)

N



Before using a high magnification objective, check confluency and fluorescence with 10/20x objective. Remember to adjust the correction collar (20x) to match the thickness of your bottom dish or coverslip.

Phase contrast acquisition

- It is important to adjust Köhler and phase contrast (see page 11) for the objective you want to use (10x, 20x). These two objectives are optimal for phase contrast. We also have a 40x objective (0,60 NA, Ph2) but this will only be placed on the system upon demand.
- Select the Fi3 camera at startup.
- Press "+" on the keyboard to get the live image or press the "Freeze" icon.
 Adjust exposure time and DIA lamp power to get the best result. You can also use the AE (auto exposure) or leave the continuous autofocus on.
- Move outside your sample to adjust for the white balance (**auto White**).
- Acquire the images by pressing "capture" at the top.

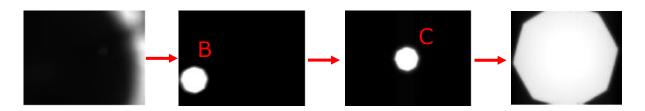
<u></u>	
Acquisition ×	
	ge Image Save Save As Open
Eyepiece - DIA Fi3 - DIA	🗲 Lightpath 📃 Add
Fi3 Pad	
Resolution <u>Fast (Focus)</u> 3x8bit 1440x1024	Nosepiece 20x 40x 60x 100x 20x 10x 1 2 3 4 5 6
Quality (Capture) 3x8bit 1440x1024	Zoom: 1x DIC Prism: Out
Exposure	PFS Glass Dichroic Offset
Analog Gain 🔹 🕨 1.0x	
AE Compensation -+0.0 EV - +	DIA
Live Acceleration: 1.0x	0.0 100.0 23.8
Size 💌	Filters Analyzer Slider: <mark>Out</mark>
Color	Configure
Auto White	
Scene Mode > <u>C</u> ommands ▼	

Köhler adjustment and fase contrast

Always adjust Köhler after changing objective or replacing sample.

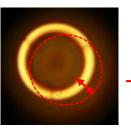
- Close the field stop diaphragm (A) in order to see the edges of the diaphragm.
- Move the condenser up and down (B) until you see a sharp image of the diaphragm.
- Center the diaphragm by using the allen key (C).
- Open the field stop diaphragm (A) until the edges lie just beyond the field of view.
- Increase depth/contrast of field by adjusting the aperture stop (arrow).

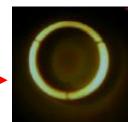


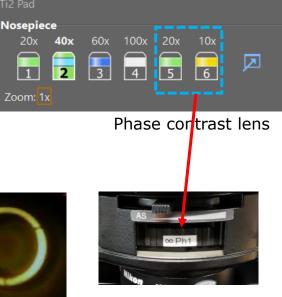


The system is equipped with a setup for phase contrast with 10x, 20x and 40x (on request). Select the correct phase matching the objectives inscription. Insert the Bertrans-lens (BI) and use the eyepieces to align the two rings using allen keys.









Automated ND acquisitions

Using the ND acquisition, you can set up experiments that include:

Timelapse, Multipoint, z-series, Multichannel, Large image

- Open the ND acquisition window (icon on the top menu).
- Check the module(s) you need (A) and define each as described on the following pages. Define the order of the experiment (B). Define also the save path (C), preferably Biomic.
- If you need to define autofocus, open "advanced" (D).

Acquisitie

- When you are ready, start the experiment by clicking on "Run now".
- It is recommended to heat up the system 0,5-1 hour before starting timelapse.

in statement					
Experiment:	ND Acquisition				
T: λ: L:					
Save to I	File				
Path: C	F:\Users save here!\Endy				Browse
Filename:	nd063.nd2	ND2			Record Data
Custom	Metadata				
Order of Expe	rir <mark>B</mark> nt 🔻 Timing				
🗹 🕑 Time [_ ⅲ XY □ ☞ ℤ ☑ ℰ λ ☑	Large	Image		
Til. • Schedul				🕂 Adc	00++×*
Phase	In erve!		Duration		Loops
#1	o min	-	6 hour(s)		▼ 37
<u> </u>					
Close Acti	ive Shutter when idle			🗌 Use F	PFS
Perform T	ime Measurement (0 ROIs)				
Switch Tra	ansmitted Illuminator off when Idle (0.0	1 s)			
					Events Advanced < <



Multichannel acquisition

- Check the fluorescence on your sample using the oculars. Check each fluorescent channel in live view and set the exposure time and coolLED intensity for each channel. Changes with exposure time will automatically be saved. If you see the red exclamation mark "!" behind the name of the channel, it means that you have change the LED setting. To save this new intensity, right click on the "!" and "assign current microscope setting".
- To set up an automatic acquisition, see next page.

	» Acquisition ×							×
	Freeze	Capture NI	D Acquire	Large Im	age S	ave	Save As	Open
	Prime BSI Express	: - DIA 📜 Prir	ne BSI Expres	s - EPI			4	Lightpath
	📃 DAPI 📙	GFP! m0	Cherry 📃	CY5	Qdot	📃 FAST L	.ED	📕 + Add
	Prime BSI Express Pad				i2 Pad Nosepiece			
	Format	No Binning	-		20x 40x	60x 100»	x 20x 10x	_
Assign current camera setting	Bit Depth	12-bit		-	1 2	3 4	5 6	
Assign current microscope setting*	Auto Exposure	64 ms			Zoom: 1x PFS			
	ROI				Glass	Dichroic (Offset	
	Temperature 0.1 °C		3	¢	PFS		17346]
					Off			
	pE-300 ultra/pE-340 F			-	ilters			
	1: • 400 nm		19	[%]	Turret-Lo			
				100	EM Wheel1	\bigcirc	$\bigcirc \bigcirc \bigotimes \bigcirc$	\otimes
	2: • 460 nm		12	[%]				
	0 3: 💽 550 nm		16	100 ['] [%]		Confi	gure	
	0	Cor		100				
	(A) pE-300	Cor	nfigure			=		

- Open the ND acquisition window (icon on the top menu).
- Check the lambda window and tick the optical configurations you need.



								×
ſ	ND Acquisitio	on ×						
I	Experiment:	ND Acquisition						
I	λ:							
I	Save to	File						
I	Path:	F:\Users save here!\En	ıdy				Browse	
I	Filename:	nd093.nd2		ND2		Decord	Data	
I	Fliename.	10033.102		NDZ		Record	I Dala	
I	Custom	Metadata						
I	Order of Expe	riment 🔻 Timing						
	Time [_ ⅲ xy 🗆 ᢓ ℤ 🗹]					
ľ	Setup					44		~
I							↑ ↓ X	ď
I	Opt. Conf.		Name DAPI		Comp. Color		Focus Offset	X
	Prime BS		DAPI GFP				0	
	Prim: mC		mCherry				0	
I	Prime BS		CY5				0	
I	Prime BSI.	: BF					0	
I								
I								
I	Close Act	ive Shutter during Filter	Change		Use			
I	Use Ratio		enange					
I						_		
I	Wait for u	iser before changing to	next channel				Advanced <-	
l	Advanced for			 Apply to 	All			
I	Autofocus	None	Define					
	Execute	e Command before Cap	ture					
	Execute	e Command after Captu	re					
	Merge Ca							
	Wait for F	PFS after setting Optical	Configuration					
	Load 🔻	Save 🔻 Remove					Run r	now



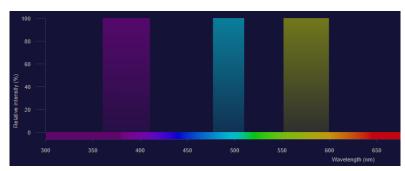
 Use the "M" in the left toolbar to start capturing with the option to focus manually. Click "continue" to record the image.



 Use the "A" to automatically capture all the channels. You might want to define "autofocus" in the ND acquisition.

Fluorescence filter setup

The system comes with two filter turrets and by combining them we get the following setup:



Multi exitation filter for coolLED is used for the following filters: dapi, GFP and mCherry.

Wa



Large image acquisition

- Before you start an automatic large image acquisition, make sure the exposure time and LED intensity is correct for all the channels you want to acquire (see page 13).
- Find "scan large image" under the "acquire" menu (this has more options than through the ND acquisition mode. If you need multichannel capture, define your channels (A).
- Define the size of the field (B) and placement of start position (C).
- Choose which objective you want to use (D).

Scan Large Image			×
Scan Large Image Capturing Macro Image Optical conf: <urrent> Scanning Optical conf: <urrent> Fields: 4 * Fields: 4 * Vumber of fields in X and Y Fields: 4 * Value * 4 Fields: 4 * Value * 4 Correct XY Offset between Macro and Scanning Correct XY Offset between Macro</urrent></urrent></urrent></urrent></urrent></urrent></urrent>	6: 10x		Combine with
Overlap: 15 % Stitching via: Blendi	ng 🔽 🔘 Off (not available)	Automatic Shading Correction	Use step-by-step focus Setup
Create large image Save large Save to Save to	image to file Keep file opened Auto capture folder e: FAUsers save here!\Endy\Large Image.tif ngle images:	Format	Focus manually every 2 field after 3 Manually distance Manually distance Manually other Optical conf. Prime BSI Express - EPI: GFP
Тwo ра	Range Continuous in Range sses □ Step 112 [µm] ← 11.200µm Rang Criterion: Brightfield		Scan Close Help
Offset Z a	ffter AF 0 μm 2 Active Shutter during Stage Movement	Use Opt. Conf: Conf: Conf: New	autofocus with fluo
	Jest	Save and <u>C</u> lose Cancel	16

• Define how you want to focus (E).

Timelapse acquisition

- Open the ND acquisition window (icon on the top menu).
- Check the "Time" flag (A) and define "intervals" at which the images should be taken. Define the "duration" (B) of the experiment.
- For long term experiments, use **PFA** (perfect focus system) or **autofocus** (remember to start up the heating at least 0,5-1 hour before imaging, se page 7).
- If you want to image multichannels or/and multipositions, remember to check the flag and set up these too (D).
- You can define where the data is going to be saved (E), preferably Biomic.

ND Acquisition ×	
Experiment: ND Acquisition	ND
T:	
λ:	
Sav To File	
Path: F:\Users save here!\Endy Browse	
Filename: nd093.nd2 ND2 Record Data	
Custom Metadata	
Rrder of Experiment	
Time 🗍 🇱 XY 🗌 🛃 Μ 🖗 λ 🔲 🕼 Large Image	
Time Schedule + Add 🗇 🗇 🛉 😾 ╆	
Phase Interval Duration Loops	
✓#1 15 sec Continuous B 🔽 ???	
Close Active Shutter when idle	
Perform Time Measurement (0 ROIs)	
Switch Transmitted Illuminator off when Idle (0.01 s)	
Events Advanced <<	
Autofocus None I At the Beginning Define	
Execute before each Time	
Execute after each Time	
Advanced fc Time Phase 1	
Execute before Time phase	
Execute after Time phase	
Keep object in view Settings	
Load 🔻 Save 🔻 Remove 🔻 1 time loop 🚀 Run now	17

Multi-point acquisition

- Open the ND acquisition window (icon on the top menu).
- Check the "multipoint" flag under ND acquisition (A).
- Define your multipoint positions in the XY window with a tick into an empty square (arrow). Remember to check "include Z" (B) if your positions are far apart.
- Define your channels (C) and interval (D) of your timelapse.
- For long term experiments, use **PFA** (perfect focus system) or **autofocus**.
- You can define where the data is going to be saved (E), preferably Biomic.
- Remember to start the heating 0,5-1 hour before imaging (see page 7).

ID Acquisition ×				X
periment: ND Acquis	ition			
	_			
Save to File				
Path: F:\Users sa	ave here!\Endy			Browse
	<u>`</u>		-	
Filename: nd093.nd2	2	ND2		Record Data
Custom Metadata				
Order of Experiment	Timing			
] 🗹 Time 🗹 🏭 XY [<u>□ \$</u> Z \ 7 } \ □			
Points	Stage to Selected Point		🕂 Add 🗇	♬ ♦ ↓ 🗙 १०
			· · ·	
Point Name	X [mm]	Y [mm]	Z [µm]	
✓ #1 ✓ #2	-14.365	20.088	2958.180	
▼#2 ▼#3	2.597	24.281 24.902	2357.900 2358.520 <-	Offset All X,Y,Z
	-> 2.900	24.902	2536.320	Offset All A, Y,Z
_ <u>B</u>				
🗹 Include Z 🛛 🗌 Rela	ative XY	Optimi	ze Load Sav	/e Custom
Close Active Shutter o	during Stage Movement			Use PFS
				Advanced <<
Redefine Reference Z	after Auto Focus			
Autofocus N	lone	-		
Execute Command be				
Execute Command after	er Capture			
Split Multipoints				
Load 🔻 Save 🔻			1 time	loop 🔗 Run now

How to use autofocus (PFS)

- Open ND acquisition.
- Check "Use PFS". You should now hear a different noise when changing the z position.
- If you are registering multipoint, make sure the offset comes up in the table.

Order of Experiment \checkmark Timing $\square $ Time $\square \blacksquare XY \square \boxminus Z \blacksquare & \lambda \square \blacksquare$ Large Image					
Points 🗌 Move Stage to Selected Point + Add 🗇 🗇 🛉 🕇 🗶 ╆					
Point Name	X [mm]	Y [mm]	Z [µm]		
/ #1	-14.365	20.088	2958.180		
/ #2	2.597	24.281	2357.900		
∀ #3 ->	2.966	24.902	2358.520	<- Offset All X,Y,Z	
☐ Include Z ☐ Relative XY Optimize Load Save Custom					
Close Active Shutter during Stage Movement					

Z-acquisition

This is not frequently used as it requires an additional module in NIS-Element to handle these images.

- Open the ND acquisition window (icon on the top menu).
- Check the "Z" flag under ND acquisition (A).
- Move through your sample in z and define the "top" and "bottom".
- Define the step size (C) or leave it as default (according to Nyquist sampling).
- You can define where the data is going to be saved (D), preferably Biomic.

ND Acquisition ×			×
Experiment: ND Acquisition			
M:			
Save to File			
Path: F:\Users save here!\Endy		Browse	ND
Filename: nd093.nd2	ND2	Record Data	
Custom Metadata			
Order of Experiment 🔻 Timing			
□ ([®] Time			
	2350.72 abs		
Top 7347.58 abs	N/A		
Reset	2347.81 abs		
Bottom	2344.90 abs Move 1		
Step: 0.2 🜔 µm ← 0.6 µm 31	Steps Range: 5	.82 μm	
Bottom: 2344.90 µm Top: 2350			
		-3.14 µm 2.68 µm	
	Bottom: -	2.68 µm	
Close Active Shutter during Z Movement Dir	rection: O Bottom to Top		
	Top to Bottom		
		Advanced <-	
Execute Command before Capture			
Execute Command after Capture			
Load - Save - Remove -		1 time loop 🛷 Run n	ow 20

Adding a scale bar

- The images you acquire on the system will have information about the pixel size incorporated into the metadata due to the objective calibration. You can therefore add a scale bar later and in any other software.
- If you need a scale bar burned (it's the only way) into your image, click on the scale bar icon on the left tool bar menu.
- A new window will open where you can customize the design.

Properties: Scale	\times
Scale Font	
Orientation Type Size	
Line color:	
Background:	
 Automatically adjust size Keep in view Show Text 	
Burn Scale Method:	
Original Fit to Screen 1:1	
OK Cancel Apply	

A

~

How do I save my data

Nd2 format are the raw format from Nikon. You can open these in most software, but you will not be able to visualize these in power point, word etc.

JPG format will remember the changes you did in the LUT, but JPG images have reduced size because the image has gone through a lossy compression (some data from the original image file is eliminated). The human eye will not see the difference. Choose lowest compression.

ND2 Image File Format (*.nd2)

Tagged Image Format (*.tif;*.tiff) JPEG2000 (*.jp2;*.j2k) LIM Image File Format (*.lim) JPEG - JFIF Format (*.jpg;*.jpeg) EZ-C1 ICS/IDS (*.ics) Windows Bitmaps (*.bmp) Portable Network Graphics (*.png) CompuServe Graphics Interchange (*.gif) OME-TIFF (*.ome.tif;*.ome.tiff;*.ome.tf8;*.ome.btf)

Image File:	Multichannel						
Save as type:	e: JPEG - JFIF Format (*.jpg;*.jpeg)						
	Compression:	Lowest	~				
	Save Color Image	Burn Binary In	nage	Burn Annotations	Read Only		

Tif/tiff format is a standard in printing and publishing industry.

Tif format will have a bit depth and will use lossless

compression. To save each channel separately, check '	"RGB
---	------

e File: Multichannel					
type: Tagged Image Fo	rmat (*.tif;*.tiff)				
Compression:	None	Less Options			
Save Color Im	age 📃 Save Binary Image	Save Annotations	Read Only		
Color Image Opt	ions				
Keep Original	Channel Combination	Bit Depth Keep bit de	pth LUTs	None	Y
Save Colo	r Channel Data per Pixel				
Mono Image	for Each Channel	Bit Depth Keep bit de	pth LUTs	None	
RGB Image fo	r Each Channel In channel color	 Bit Depth Keep bit de 	pth ~ LUTs	None	~
Burn Scale	e 🗌 Burn Binary 🗌 Burn A	nnotations			
All Channels I	Merged to RGB Overlay Image	Bit Depth Keep bit de	epth V LUTs	None	~
Burn Scale	e Burn Binary Burn A	nnotations			

We prefer that you **do not** use USB keys and external hard drive on the system. The pc is on the net, so there are a few options.

 Save onto Biomic. (<u>\klient.uib.no\felles\mofa\biomic</u>). Ask MIC personnel if you would like access.

	Windows Security X
	Enter network credentials
	Enter your credentials to connect to: klient.uib.no
	uib\hts076 ×
	•••••
· · · · · · · · · · · · · · · · · · ·	Remember my credentials
😍 Windows (C:)	
ACQUISITION1 (D:)	More choices
ACQUISITION2 (E:)	م dag017@uib.no
STORAGE (F:)	
✓ biomic (\\klient.uib.no\felles\mofa) (Z:)	A Use a different account
Network	
-	OK Cancel

 Save on to your cloud/one drive. Go to any browser and search for "office 365" and sign in using your email. In the left menu you will see the cloud icon. Here you have access to 1 TB.

Microsoft				
Sign in				
Email, phone, or Skype				
No account? Create one!			•	
Can't access your account?				
Ba	ack	Next		

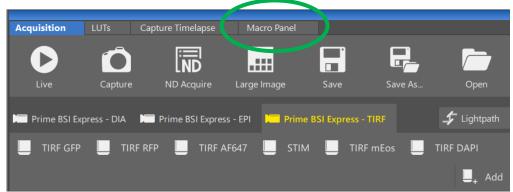
• Save to "users save here" on the hard disk for a limited time. This data must be transferred and deleted asap.

- 1. Open NIS elements in TIRF mode.
- 2. Choose the 60x TIRF objective (oil).
- 3. Find focus in your sample.
- 4. Add the black lid on top of the stage (laser safety protection). (the box on top of the FRAP-ctrl box should have a green light when software is opened).
- 5. On the microscope; turn from eye to camera (A) and turn 90⁰ left to Bertrand lens (B).

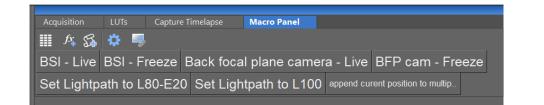




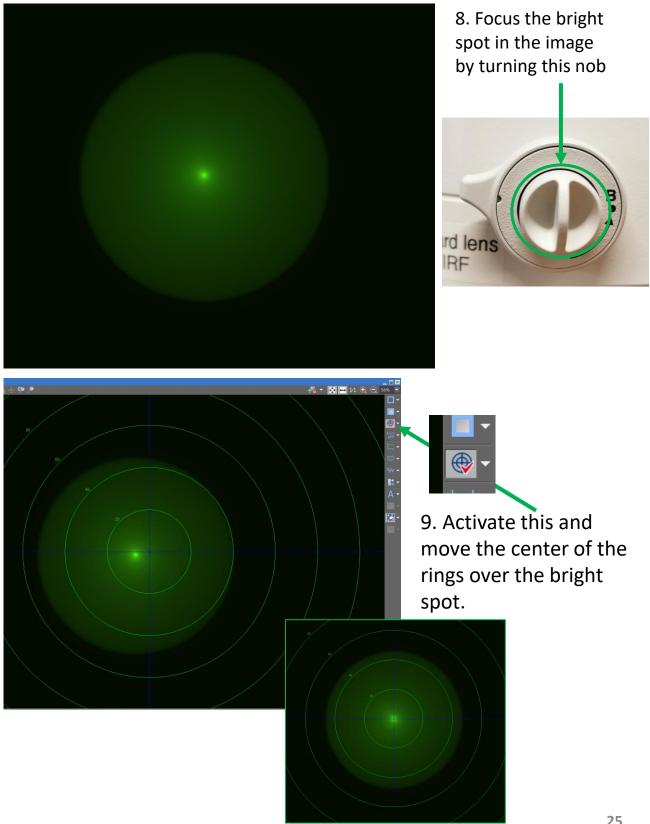
6. Open the Macro Panel.



7. Click on Set Lightpath to and L80-E20 and Back focal plane camera – live.

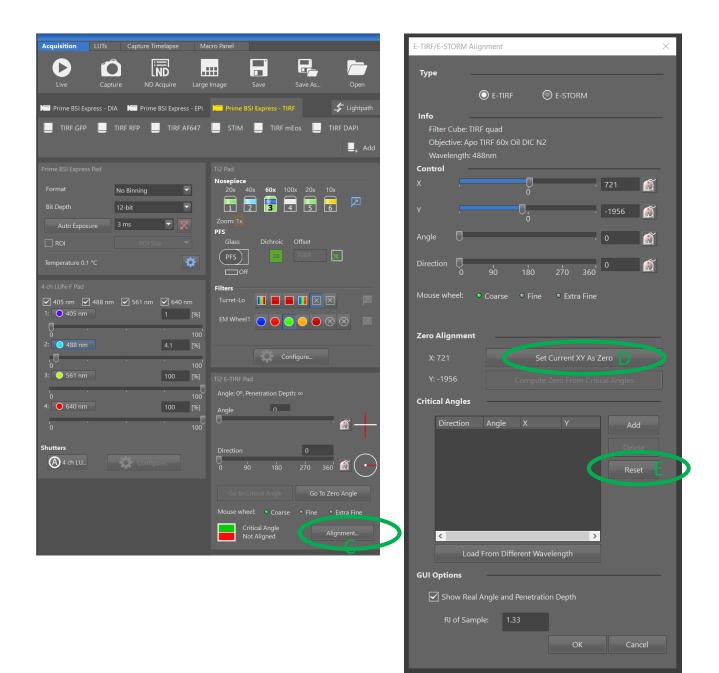


The image in the screen appears something like this. (1 % laser power is most likely enough)



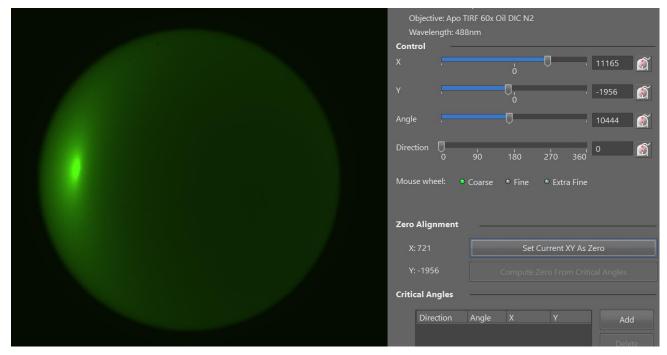
10. Click Alignment (C) and Set current XY to Zero (D).

11. If there are any pre-stored coordinates under Critical Angels, press Reset (E).



12.

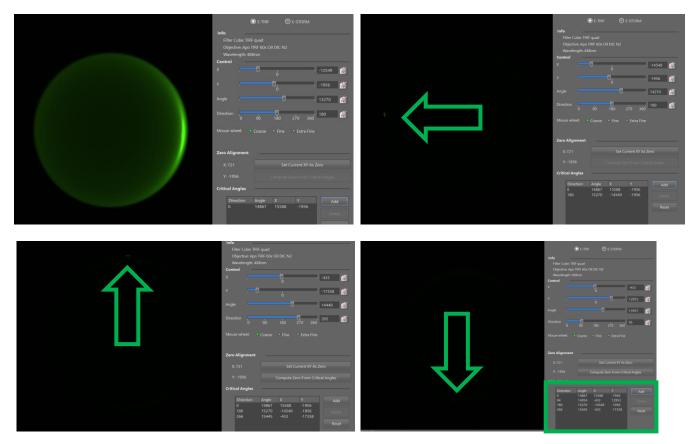
- Start adjusting by moving the X-slider in one direction.
- The bright spot moves accordingly.
- Move it until the bright spot is at the edge and just disappears (put the mouse wheel to "fine" for more precision at the edge).
- At the same time a tiny signal appears on the opposite side of the circle (F).
- Then you stop moving the slider and ADD the coordinates under Critical Angels.



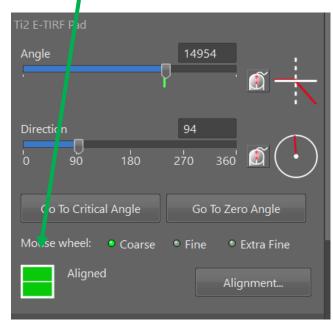
Live - Fast: TIS - Active	E-TIRF/E-STORM Alignment
Ive - Fast: TIS - Active	Type
	Y: -1956 Compute Zero From Critical Angles Critical Angles Direction Angle X Y Add_7 0 14867 15588 -1956 Create Reset

13. Repeat this in the other three directions (opposite X, Y up and down), and ADD coordinates for each direction.

14. Click OK at the bottom of the Alignment window and the Critical Angels are saved.



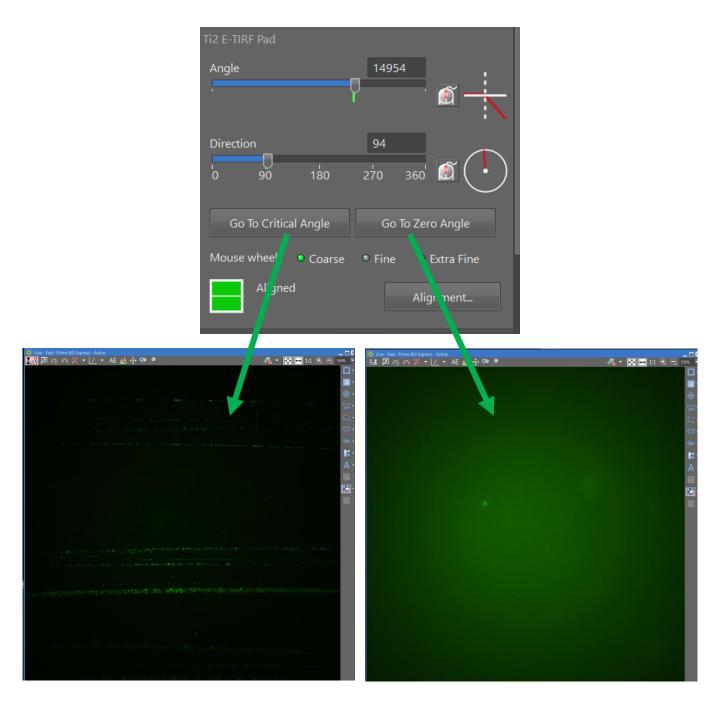
The panel now lights up in green and shows that the TIRF is aligned.



15. On the microscope; turn back the knobs to the starting positions (eye & 90⁰ upwards).

16. In the software; in Macro Panel deactivate Back Focal Camera and set Light path to L100.

You're ready to TIRF



No TIRF

TIRF

REMEMBER:

Imaging parameters (laser, exposure time, adjust display parameters etc.) must be optimized as you do for regular fluorescence imaging.