1. Turn the key on the Nikon LUN-V Laser Launch.



2. Press the button the left side of the A1Rsi Controller unit.





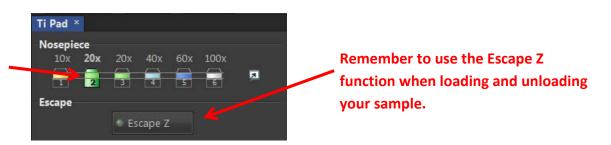
3. Turn on the power strip underneath the microscope.



- 4. Turn on the H.P. workstation (P.C. can be turned on first).
- 5. Open NIS-Elements.

Nikon A1RSi Confocal Workflow

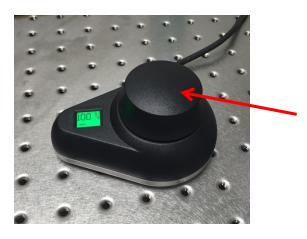
1. Start by selecting your desired objective and then place your sample in the sample holder on the stage.



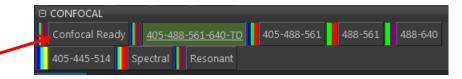
2. In the EYES optical configuration (OC) panel click brightfield/fluorescence color of interest. This will allow you to focus on your sample prior to using confocal.



3. Press the X-Cite controller to turn on the fluorescence light.



- 4. Once you have your sample in focus turn off the fluorescence light by pressing the controller again.
- 5. Next you will click on the Confocal Ready button in the CONFOCAL optical configuration menu.



6. The Confocal Ready button will allow the scanning of samples for all four channels plus transmitted light (TD). If you don't need all the laser lines you can select one of the other OC buttons for your fluorescent probe of interest.



7. Press the scan button and the live image window will appear with your sample. Press the scan button again to stop scanning.

8. Adjust the laser power and gain (HV) settings to your desired level for visualizing your sample.





- 9. Once you have set your laser power and gain, press the ^{Capture} button to obtain a 2D image.
- 10. To improve the resolution you can change the Pixel dimensions. Default is 512x512.

ize						
64	128	256	512	1024	2048	4096

11. For sequential scanning to prevent bleed-through or crosstalk between fluorescence channels, press the

button.

- **12**. Be aware of photobleaching when scanning for long periods of time on one particular region of your sample.
- 13. Utilize the Scan Zoom feature if your structure of interest is much smaller than the scanning window.

A1plus Scan Area ×
🧭 🚾 — ᢇ • Crop ROL Edit 🔀 📑 🎬 🚺
Zoom: 1
Pixel size: 1.24 Nyquist XY 💌
Scan size: 512 💌 Rotation: 0
Width: 512 Height: 512
Dwell time: 2.2 µs
Pixel size: 1.24 µm Optical resolution: 0.47 µm Z step size: 0.97 µm Optical sectioning: 2.83 µm

- 14. Once you have captured an image we recommend you save the image as an ND2 file which is the Elements preferred file format. Once you have saved as an ND2 file then you can export to a Tiff file.
- 15. Following your imaging session please clean any objectives used if you used oil or water immersion.
- 16. Leave the system on and the Imaging Center Director will turn off the equipment at the end of the day.
 - a. Please Note If it is afterhours please turn off the system following usage.
 - i. Close Elements
 - ii. Turn off powerstrip
 - iii. Turn off controller
 - iv. Turn off laser unit

Nikon Confocal Training Document

Ti Pad

- Nosepiece use to change objective
- Zoom should be at 1.00x unless using the manual 1.50x mag changer on the TiE

OCs – Optical Configurations

- Eyepiece OCs for finding sample through eyepieces
- Confocal OCs for imaging with the A1 confocal

Confocal GUI

• Scan (starts and stops live image); Capture (take picture); Fast (for faster preview live scan)

NOTE: If you are LIVE but do not see an image, make sure the red Interlock is turned off!

- Galvono slower scan mirrors
- <u>Resonant</u> faster scan mirrors (used mainly for live cell imaging)
- Eyeport changes to last used eyepiece setting; click again to return to confocal mode
- Autogain cycles through some gain settings and finds a good starting point for HV
- Single vs. Bidirectional Scan
 - Single need more resolution, don't need speed
 - Bi ONLY for fast speed, live-cell lose flexibility
- Pixel Dwell/Frame per Second scan speed (how long laser scanning over each pixel)

Start at 1 Frame/sec

Size – Scan size in XY; pixel size (resolution = how many pixels we put in the field)
 Start at 512; if want pretty picture with no z in a fixed sample, use 1024

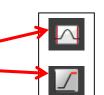
- Average cleans up image by decreasing noise Mainly used when Imaging with Resonant Scanner
 Use 2x to start and can go to 16x keep in mind the equivalent decrease in scan speed
- Ch Series = Channel Series excites and collects in sequentially rather than simultaneously
- Settings you don't need to use
- Laser Settings do not open
 - Pinhole click square 1.2 AU button to use recommended pinhole size
- Laser Lines

.

- Checked = collecting that channel
- Depressed = laser on _____
- o HV (Gain)
 - ***Start at 80-110 for 405 and 647 lasers* ***Start at 20% for 488 and 561 lasers***
- o Offset
 - ***keep at 0***
- Laser Power
 Start at 5% for 405 and 647 lasers
 Start at 1% for 488 and 561 lasers



- Increasing Laser Power Increases signal w/o increasing noise, but photobleachs sample faster
- Increasing HV (Gain) doesn't bleach more, but increases noise
- LUTs Look Up Tables
 - Autoscale doesn't change data just scales visual image –
 - o Oversaturation Indicator







Timelapse

	ND Acquisition ×		×
	Experiment: ND Acquisition		
	T: [
	Save to File		
	Path: C:\Program Files\	IIS-Elements\Images\	Browse
	Filename: nd.nd2		Record Data
	Order of Experiment 🕶 Timing		
		⁷ Ζ 🔲 🖉 λ 🔲 🖷 Lai	ge Image
	Time schedule	+	00 + + × >
Click to add	Phase Interval	Duration	Loops 🕨
a new Time		^	· •
Phase			
	Close Active Shutter when I	dle Perfo	rm Time Measurement (0 ROIs)
	Switch Transmitted Il uminat		
			Events Advanced >>
	Load	ve•	1 time loop
	Interval – How often	7	Loops – How many
	you want to image (ie.		images will be
	every 3 sec, every 10		acquired during this
	min, no delay = image)		duration
	continuously)		
		Interval – How long you want the	
		timelapse experime	nt
		to run	
		L	

Multipoint

ND Acquisition x			×	
Experiment: ND Acquisition	ו ו			
T:				
M:				
	iles\NIS-Elements\Images\	Bro	wse	
Filename: nd.nd2		Record D	ata	
Order of Experiment -	iming			
Time 🗹 🎬 XY		arge Image		
Points	age to Selected Point 👍		x x	
Point Name	X [mm] Y [mm]		- °' FS	
				Click this box to add a point. Pressing the spacebar will also add points.
Include Z Relativ		Save Cus	tom	Once a point is added, XYZ can be updated by clicking the arrow next to those values.
		Advanc		
load ▼ Save ▼ R	Remove	1 time loop	Run now	

Check this box to have Z remembered, too. Your set focal plane will be remembered when this point is visited.

Large Image Stitching – Tile Scanning

	- 1	ND Acquisition ×			
		Experiment: ND Acquisition			
		Save to File			
		Path: C:\Program Files\NIS-Elements\Images\ Browse			
		Filename: nd.nd2 Record Data			
You must add an XY		Order of Experiment Timing			
point! This		🗖 🕒 Time 🚬 🏥 XY 🔲 🖅 Ζ 🔲 🔗 λ 🗹 🛱 Large Image			
will serve					
as your		Scan Area:	Choose size		
center		• 2 • x 2 • fields	of stitch		
point to		○ 6.0 × 6.0 × mm			
stitch		O Pattern Browse			
around.		Stitching:			
		Stitch Use All Channels for Stitching			
		O Do Not Stitch Stitching is done on the first lambda channel, when the large image is acquired inside lambda loop			
		Overlap: 15 %			
		Close active Shutter during Stage Movement			
		Load Save Remove Remove Remove Run now			
		Use 10-15%			
		Overlap			

Z Stack

	ND Acquisition ×			× `
	Experiment: ND Acqu	isition		
	Z: [
	Save to File			
	Path: C:\Progr	am Files WIS-Element	ts\Images\	Browse
	Filename: nd.nd2]		Record Data
	Order of Experiment 🔻	Timing		
	🗖 🕑 Time 🗖 🏭 X	γ 🗹 🛃 Ζ 🔤 🖉	🤊 λ 🔲 🔚 Large Ima	ige
Set Top and Bottom of Stack			N/A	
Scroll to top and click to set	Reset	0.00	bs N/A	
Scroll to bottom and click to	Botto	m	N/A	
set	Step: 8.725	µm ⇐ 15.825µm	N/A Steps R	ange: N/A µm
	Bottom: N/A	µm Top:		elative Positions:
	Z Device: Ti ZDrive		-	op: N/A µm ottom: N/A µm
			В	ottom: N/A µm
	Close active Shutte	r during Z Movement	:	
				Advanced >>
	Load ▼ Save ▼	Remove	1 time	loop 🔗 Run now
	Set step size here		Set number of	
			steps here	
]
		Use		
		recommended		
		step size		

	r
	ND Acquisition ×
	Experiment: ND Acquisition
	T:
	Z:
	Save to File
	Path: C:\Program Files\VIS-Elements\Images\ Browse
	Filename: nd.nd2 Record Data
	Order of Experiment Timing
	🗹 🕑 Time 🔲 🏭 XY 🗹 😂 Ζ 🔄 🖉 λ 🔲 🖷 Large Image
Set Z Stack by Defining a Range	
Choose Relative if combining z stack with multipoint XY	Top 0.00 abs Relative Home Bottom N/A
	Step: 8.725 µm (= 15.825µm N/A Steps Range: N/A µm
Choose Home to set the	Range: N/A µm <n a="" a,="" n=""> Relative Positions:</n>
focal plane you would like	Z Device: Ti ZDrive
to take a stack around	Bottom: N/A µm
	Close active Shutter during Z Movement
	Advanced >>
	Advanced >>
	Load Save Remove Remove I time loop
	Set the range of the stack
	(ie.range of 10 = 5 above
	and 5 below)

	ND Acquisition x
	Experiment: ND Acquisition
Don't forget you can click here to	T: Z: Save to File
automatically save	Path: C:\Program Files\VIS-Elements\Images\ Browse
your ND experiments as they are acquired!	Filename: nd.nd2 Record Data
	Order of Experiment Timing
	$\fboxlarge Image$
Set Z Stack by Defining an Asymmetrical Range	Top Relative Home Bottom
	Step: 8.725 µm 15.825µm N/A Steps Range: N/A µm Below: N/A µm Above: N/A µm Relative Positions:
	Z Device: Ti ZDrive Top: N/A µm Bottom: N/A µm
	Close active Shutter during Z Movement
	Advanced >> Load ▼ Save ▼ Remove▼ 1 time loop I time loop