

# NIKON A1R CONFOCAL

## Basic Operation

1

System Basics

2

Software Overview

3

Sample Finding and Focus

4

Scanning and Capture

5

Channel Settings

6

Zooming and Resolution

7

High Speed Scanning

8

ND Acquisition

9

Advanced Routines

# System Basics

1

2

3

4

5

6

7

8

9

## Microscope

The A1R is mounted on an inverted stand, the Ti-E from Nikon.

- Motorized XY stage with encoders
- Motorized Z focus with encoders
  - Perfect Focus System 3
- Motorized turret of widefield cubes
  - DIC optics
- Tokai-Hit stage-top incubation chamber

## Optics

- Plan Fluor 4x/0.2
- Plan Apo 10x/0.5
- Plan Apo VC 20x/0.75
- Plan Apo Lambda 40x/0.95
- Plan Fluor 40x/1.3 (oil)
- Plan Apo Lambda 60x/1.4 (oil)

## Scanner

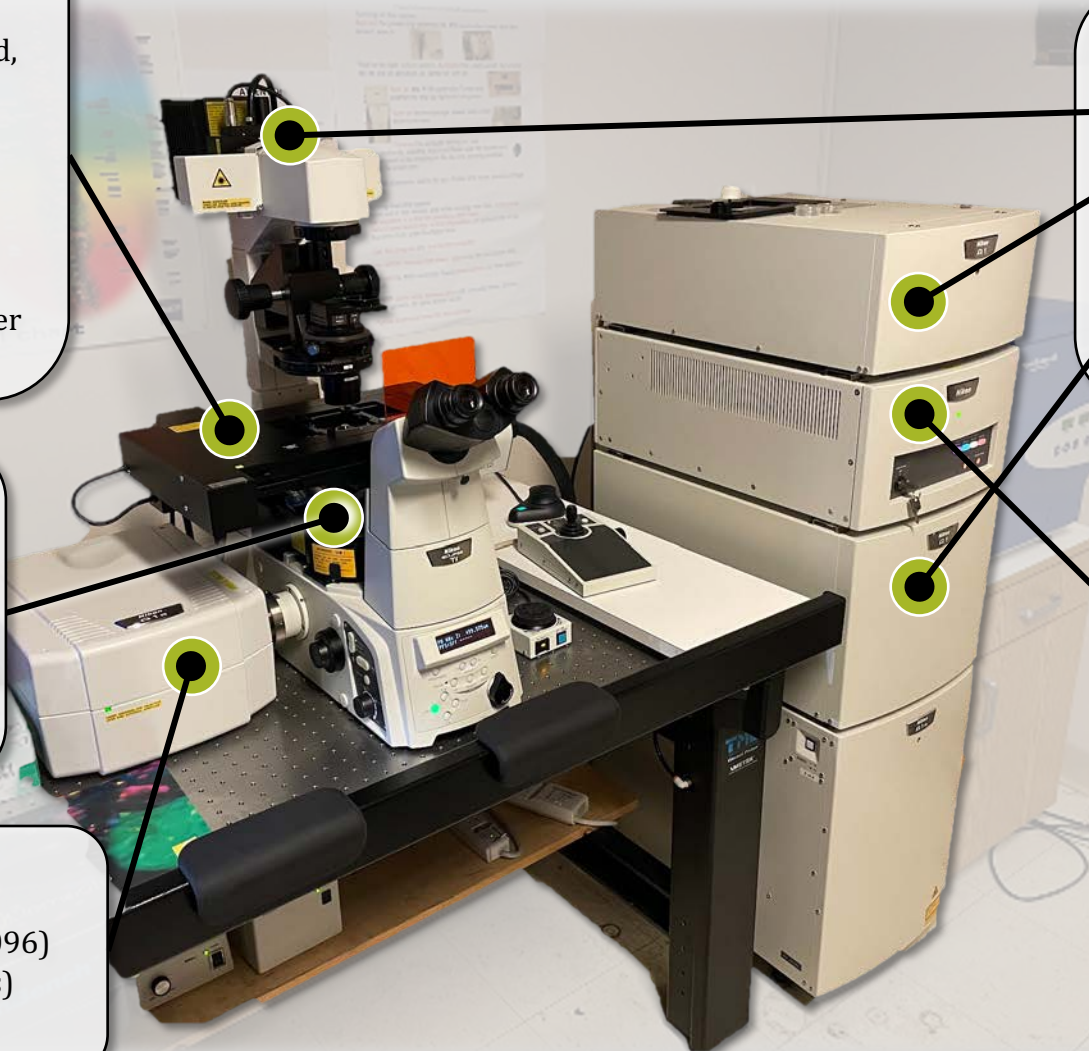
- Galvanometer Scanner (max 4096x4096)
- Resonant Scanner (512x512, 30fps)
  - Hybrid Scanner (405nm laser)

## Detectors

- DIC Transmitted Detector
- DUG: Four(4) filter-based detectors (2 high sensitivity MAL + 2 GaAsP)
- DUS: 32-channel spectral array (2.5nm, 6nm, or 10nm resolution)

## Lasers

- 405nm
- 488nm
- 561nm
- 640nm



# Software Overview

The A1R confocal runs in *Nikon's NIS-Elements software*. To help limit any user issues typically seen when several users overlap on a single system, the software is programmed to automatically load presets when it starts. These include a standardized hardware configuration and initial software layout. Users can adjust or change these settings during their imaging session without impacting future users.

## Default Configuration

- Galvanometer Scanning
- 512x512 Image window
  - 1x Scan Zoom
- Four (4) channel Acquisition  
DAPI, GFP, RFP, Cy5

The screenshot shows the Nikon NIS-Elements software interface. A yellow box highlights the 'A1plus Compact GUI' on the left, which includes controls for scan area, zoom, and channel selection. A callout points to the Nikon logo. Another yellow box highlights the 'A1 Scan Area' in the center, showing a 512x512 pixel window. A third yellow box highlights the 'LUT (docked behind)' area, which is currently docked behind the scan area. A fourth yellow box highlights the 'ND Acquisition' area on the right, showing a time schedule table with columns for Phase, Interval, Duration, and Loops. The table contains 10 phases, each with a 1-second interval and a 4-minute duration, and 61 loops per phase.

Phase	Interval	Duration	Loops
#1	1 sec	4 min	241
#2	1 sec	1 min	61
#3	1 sec	1 min	61
#4	1 sec	1 min	61
#5	1 sec	1 min	61
#6	1 sec	1 min	61
#7	1 sec	1 min	61
#8	1 sec	1 min	61
#9	1 sec	1 min	61
#10	1 sec	1 min	61

A1 Compact GUI

A1 Scan Area

LUT (docked behind)

ND Acquisition

1

2

3

4

5

6

7

8

9

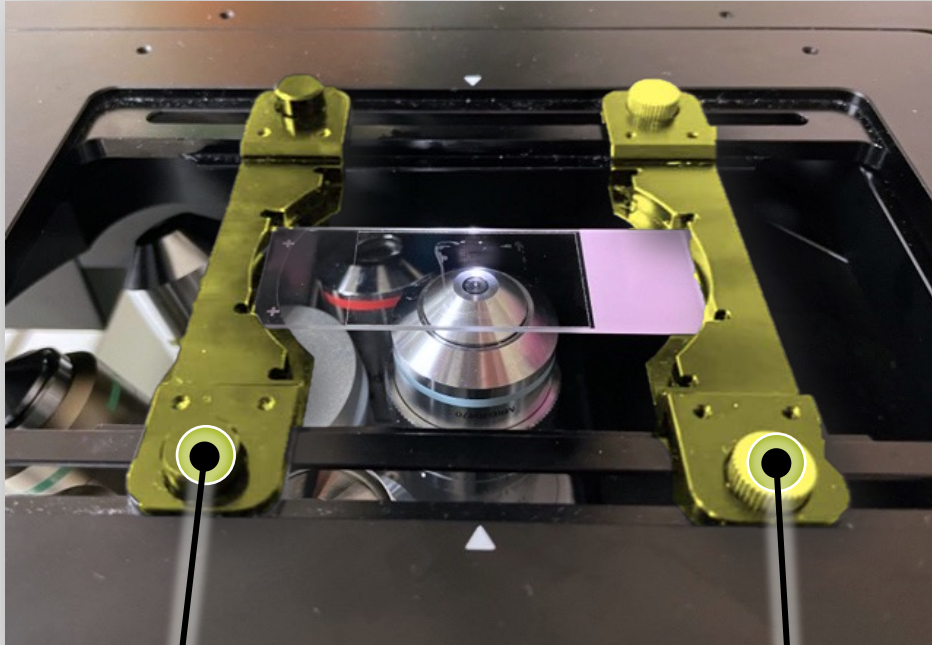


Sample Finding and Focus

A. Stage Placement

The Universal Stage Adapter can accommodate

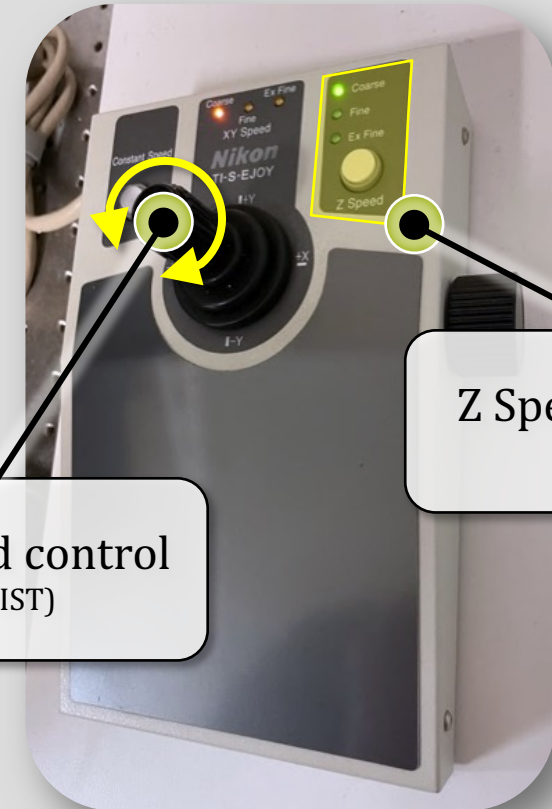
- Slides
- 35mm dishes
- Multi-well chambers



(L) Adjustable

(R) Fixed

Joystick can control Stage (XY) and Focus (Z)



XY Speed control  
(TWIST)

Z Speed control  
(PRESS)

1

2

3

4

5

6

7

8

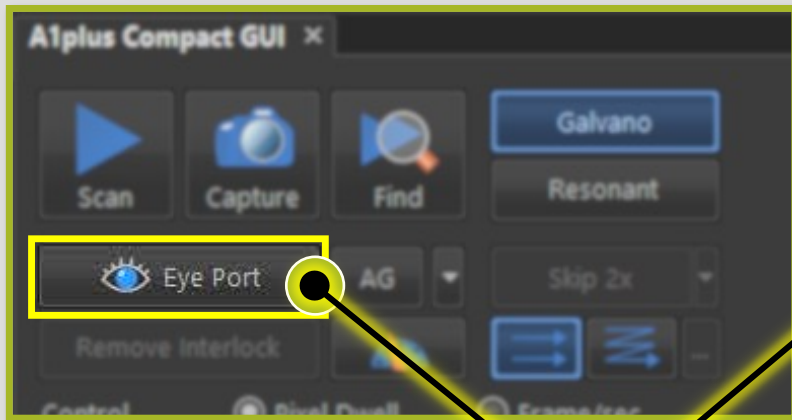
9

## Sample Finding and Focus

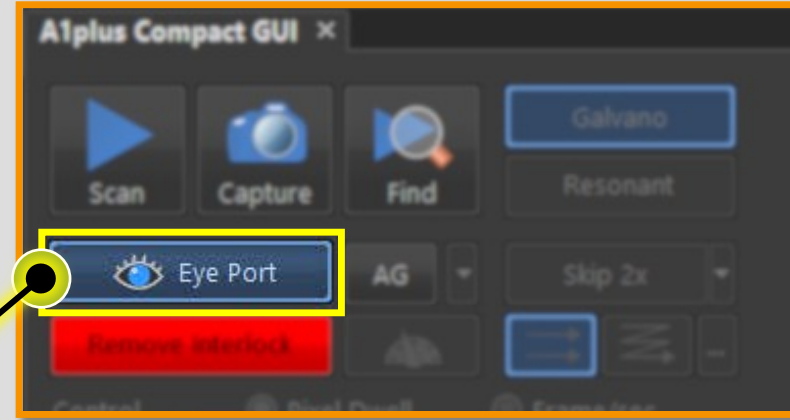
## B. Eyepoint Viewing

Once the sample is on, you must bring it into focus. This is usually done by eye using widefield fluorescence. There is a shortcut button in the software to switch from **CONFOCAL** to **EYEPPOINT** viewing.

## Confocal Mode



## Eyepoint Mode



PRESS TO SWITCH BETWEEN MODES



When viewing sample in **EYEPPOINT** Mode:  
Change colors with the *rocker* switch on  
the **RIGHT** side of the microscope

1

2

3

4

5

6

7

8

9

## Sample Finding and Focus

## C. Focusing and PFS

- The front of the microscope will show its current focus position
- Its LOWEST position is **~499um**.
- Typical slide work will be in the **1900-2300um** range\*\*

## Bottom of Range



## Working Position

**When changing samples or objectives:**

**ESCAPE** will lower the objective to the **bottom of its range**

**REFOCUS** will bring it back to the previous **working position**

**Protip!** The Perfect Focus System will light up when a sample interface is reached. This is a good clue that you're close.

\*\*Live cell and Wellplate work will have different typical ranges

1

2

3

4

5

6

7

8

9



## Scanning and Capture

1

2

3

4

5

6

7

8

9

## Basic Scanning Controls

## Live/Capture/Find

LIVE: constant scanning for optimization and setup

CAPTURE: collects a single image with the current settings

FIND: constant scanning with reduced resolution for a faster frame rate

## Scan Array Size

Sizes shown in **red** are not possible at the current scan speed

## Averaging/Integration

Collect image multiple time to improve signal to noise

## Channel Series

Collect colors in separate scans to remove bleedthrough/crosstalk

## Pinhole Size

Controls optical section thickness and removes out of focus light

The screenshot shows the A1plus Compact GUI with the following controls visible:

- Scan Array Size:** The 'Size' section shows buttons for 64, 128, 256, 512, 1024, 2048, and 4096. The 512 button is highlighted in blue, while 64, 128, and 256 are in red.
- Averaging/Integration:** The 'Normal' button is highlighted in blue. There are also buttons for '2x' and '2x' with a summation symbol.
- Channel Series:** The 'Ch Series' dropdown menu is open, showing options like [1]->[2,TD]->[3]->[4].
- Pinhole Size:** The 'Pinhole' slider is set to 1.0 AU. The 'AU calculated for' dropdown is set to 640.0.

# Channel Settings

## A. Controls

### Channel Selection

Check channel to include it in the scanning settings

### GAIN/OFFSET/LASER

**GAIN:** controls the detector sensitivity (displayed as HV/HV GaAsP)

**OFFSET:** controls the detector baseline (**Typically not changed**)

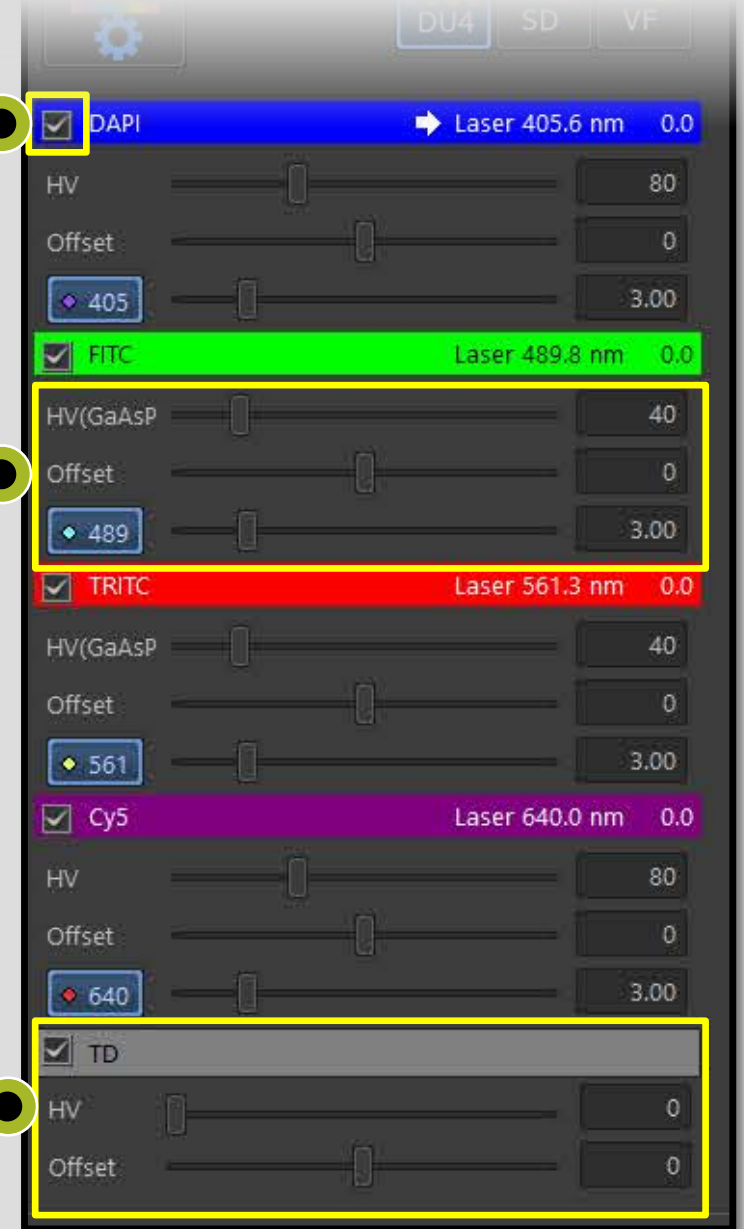
**LASER:** controls the amount of laser delivered to the sample

### Transmitted Detector

Uses the excitation laser that passes through the sample

Adjust Gain (HV) to control brightness

Requires DIC optics to produce DIC image



1

2

3

4

5

6

7

8

9

# Channel Settings

## B. Oversaturation

Oversaturation refers to signal that is beyond the quantifiable range of the detector.

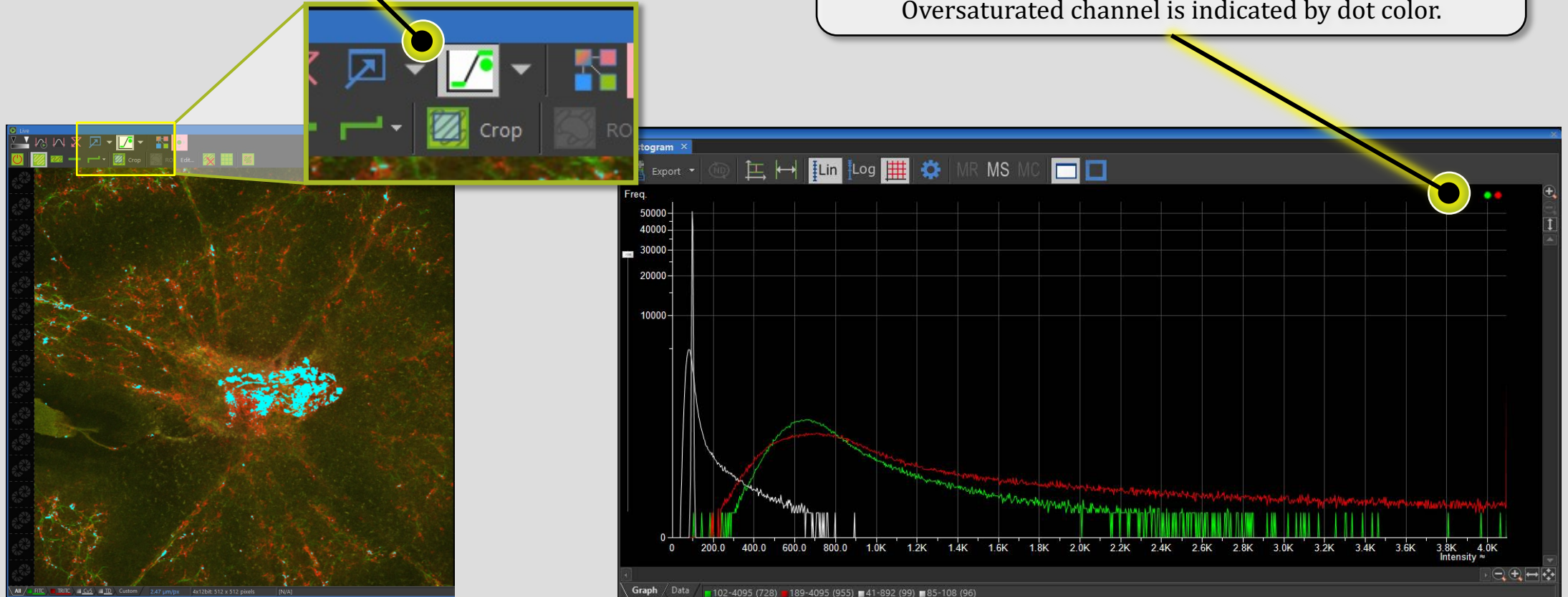
If intensity-based measurements are needed, **avoid oversaturation**

### Oversaturation Indicator

Saturation color is complimentary to each channel

### Histogram Saturation

Oversaturation is visualize by a dot in the top/right corner.  
Oversaturated channel is indicated by dot color.

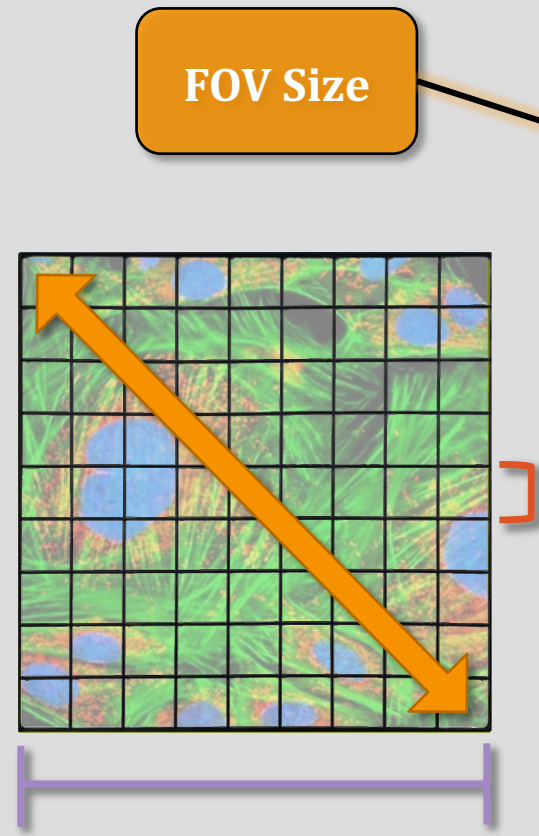


- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9



# Zooming and Resolution

- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9



FOV Size

Pixel Size

Array Size

A screenshot of the A1plus Scan Area software interface. The 'Crop' button is highlighted in yellow. A yellow box highlights the 'Zoom' slider and the 'Pixel size' field. Another yellow box highlights the 'Pixel size' and 'Optical resolution' fields in the bottom status bar.

**CROP**  
Reduce Field  
Reduce Array  
Maintain Pixel  
Increase Speed

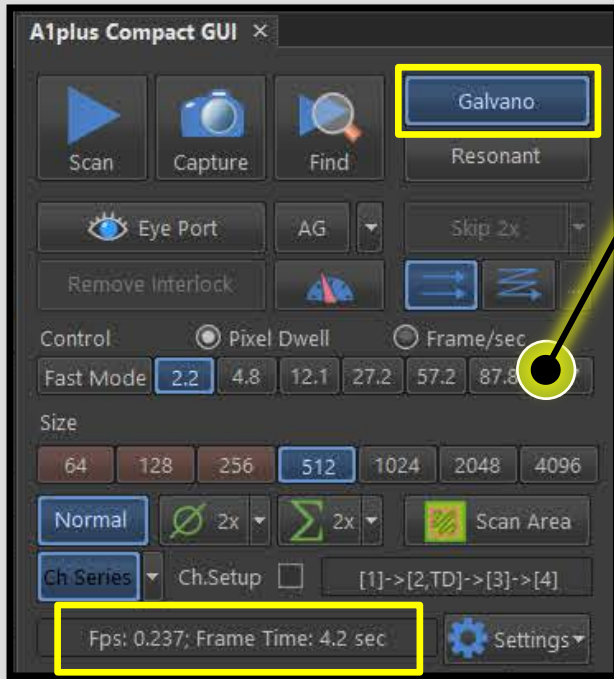
**ZOOM**  
Reduce Field  
Reduce Pixel  
Maintain Array  
Increase Resolution

**System Resolution**  
Current settings and theoretical limit

# High Speed Scanning

The Nikon A1R confocal can also be run in **RESONANT MODE**  
 The faster frame rate has several application **benefits**, but users may face limiting **tradeoffs**.

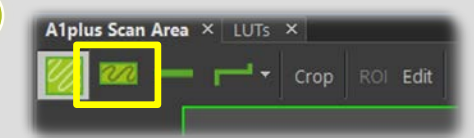
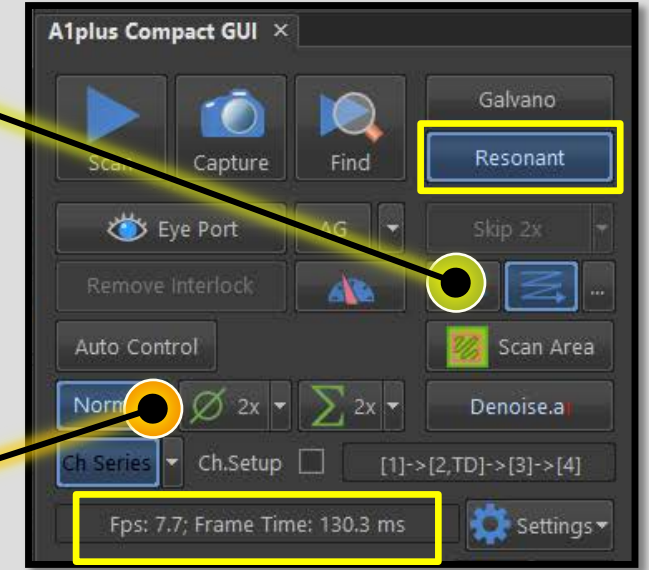
- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9



**Frame Rate**  
 1fps → 30fps  
 No speed choices  
 Bidirectional Scanning  
*Fast Acquisition, Live cells, High-speed dynamics*

**Signal:Noise**  
 Higher perceivable noise  
 Averaging likely needed  
 Increased signal helps  
*Denoise.ai* post-processing helps

**Resolution**  
 Array is fixed at 512x512  
 Resolution increase by Zooming ONLY  
 "Band Scanning" provides additional speed



## ND Acquisition

NIS-Elements runs MOST of its multi-dimensional experiments through **ND ACQUISITION**.

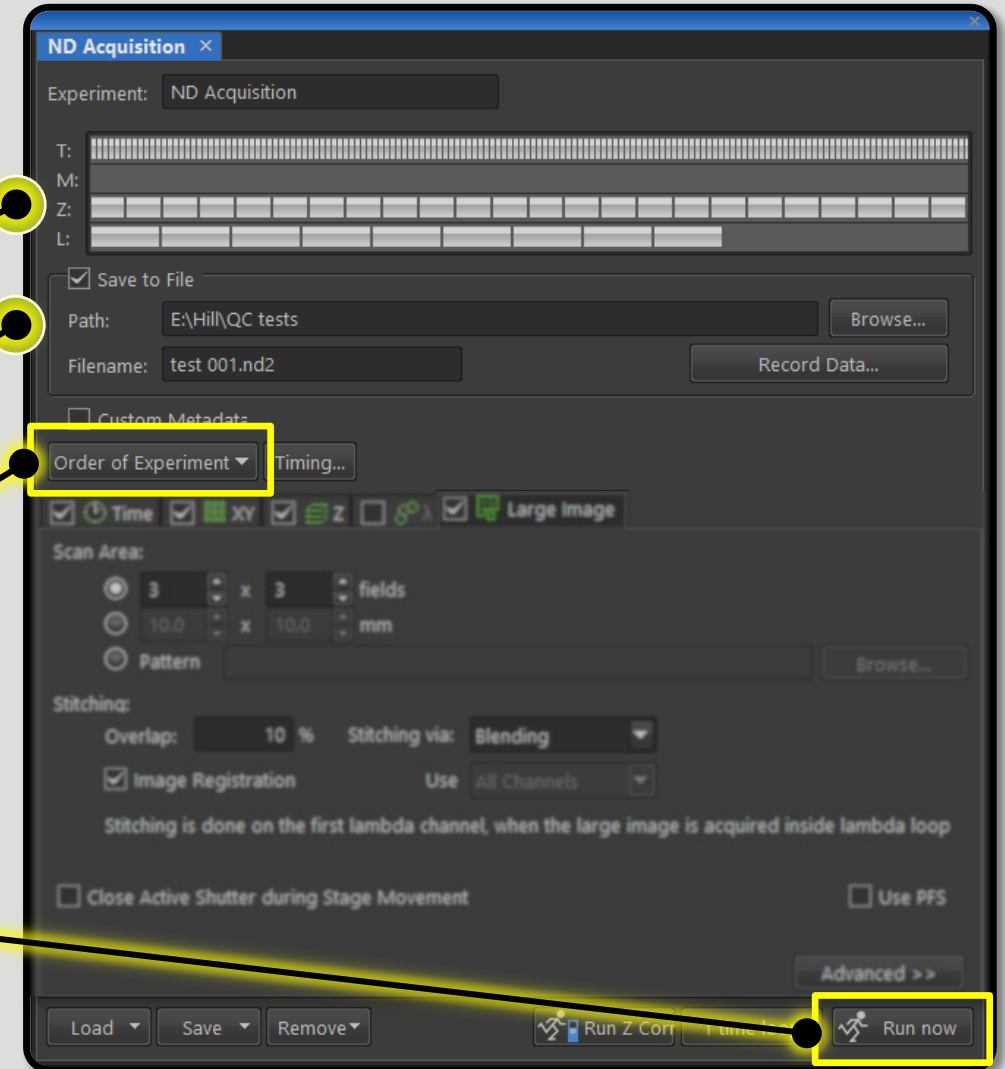
This dialogue allows you to set parameters for each dimension and control basic experimental logic

Selected Dimensions

Auto-Save Path

Experiment Order

Run Experiment



1

2

3

4

5

6

7

8

9



1

## ND Acquisition

## B. Multi XY

2

## XY

How OFTEN do you want to image  
"No Delay" runs as as possible

3

4

5

6

7

8

9

## Z

Can be saved along with XY position  
Serves as anchor when used along with PFS

## Perfect Focus System (PFS)

Remembers a PFS offset along with XY  
(and Z if selected)  
*Check box BEFORE marking positions*

The screenshot shows the ND Acquisition software interface. At the top, there are checkboxes for Time, XY, Z, and Large Image. Below this is the 'Points' section with a table of coordinates. The table has columns for Point Name, X [mm], Y [mm], and Z [μm]. Two points are listed: #1 and #2. Point #1 has X=1.919, Y=2.926, and Z=2036.325. Point #2 has X=-0.157, Y=6.422, and Z=2036.300. There are also buttons for 'Add', 'Move Stage to Selected Point', and 'Offset All X,Y,Z'. At the bottom, there are checkboxes for 'Include Z', 'Relative XY', 'Close Active Shutter during Stage Movement', and 'Use PFS'. There are also buttons for 'Optimize', 'Load...', 'Save...', 'Custom...', and 'Advanced >>'.

Point Name	X [mm]	Y [mm]	Z [μm]
<input checked="" type="checkbox"/> #1	1.919	2.926	2036.325
<input checked="" type="checkbox"/> #2	-0.157	6.422	2036.300
<input type="checkbox"/>			



## ND Acquisition

## C. Z-Stack

## Stack Type

**Absolute:** Top and Bottom defined for SINGLE POSITION

**Relative:** Range around middle defined for MULTIPLE POSITIONS

## Stack Parameters

**Step Size:** Recommended step based on *current optical settings*




**Stack Range:** Distance between defined TOP and BOTTOM

**Step Number:** How many slices of the defined size to achieve defined Range?

**Limits:** Top and Bottom of Range

The screenshot shows the Z-stack acquisition control panel. At the top, there are checkboxes for Time, XY, Z (checked), λ, and Large Image. Below these are icons for Top, Bottom, and Relative stack types. A 3D box represents the stack range with absolute positions: 2069.70 abs (left), 2088.83 abs (right), 2050.20 abs (front), and 2011.58 abs (back). The parameter fields are: Step: 0.2 μm, Range: 77.25 μm, Bottom: 2011.58 μm, Top: 2088.83 μm, and Relative Positions: Top: +19.13 μm, Bottom: -58.13 μm. There are also checkboxes for 'Close Active Shutter during Z Movement' and 'Use HW sequencer', and radio buttons for 'Direction: Bottom to Top' (selected) and 'Top to Bottom'. An 'Advanced >>' button is at the bottom right.

**To set stack parameters:**

1. Focus to top; click 
2. Focus to bottom; click 
3. Confirm step size and adjust as needed
4. If acquiring a **RELATIVE** stack, click the middle stack type 



1

2

3

4

5

6

7

8

9



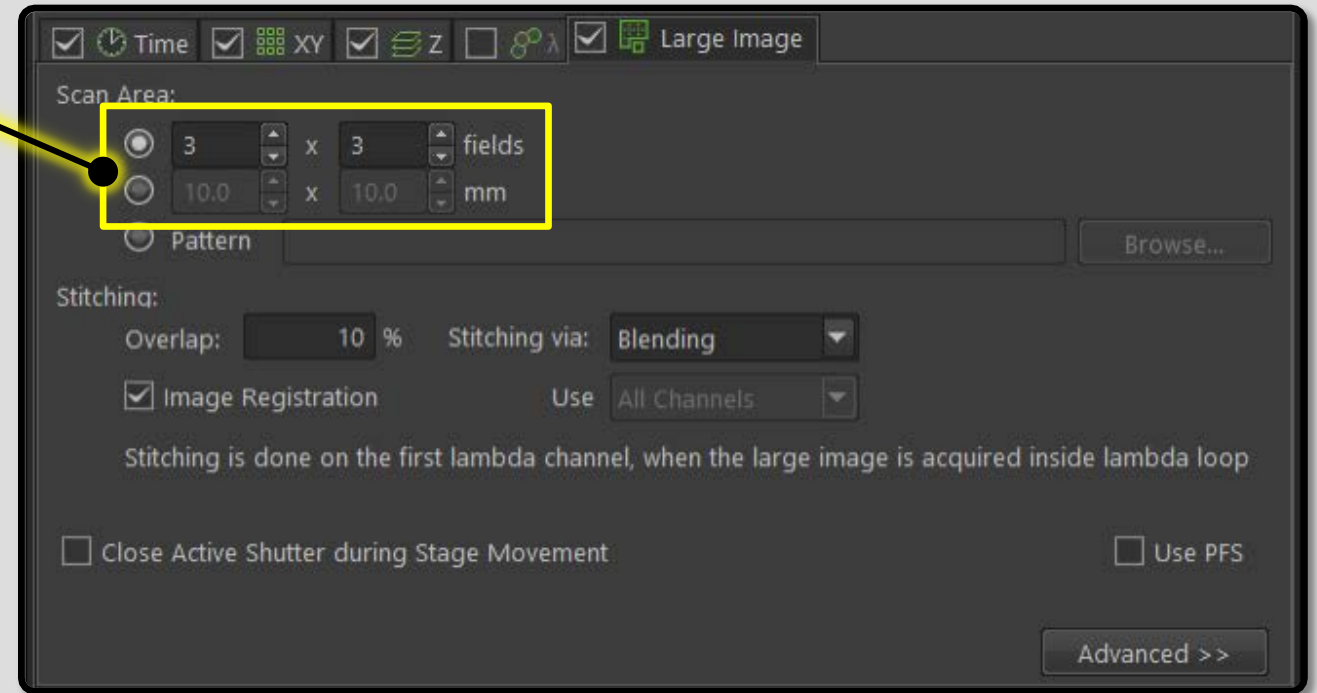
## ND Acquisition

## D. Large Image

### Stitched Field Dimensions

Dimensions listed as X by Y

- # Fields
- Total distance covered



1

2

3

4

5

6

7

8

9

## ND Acquisition

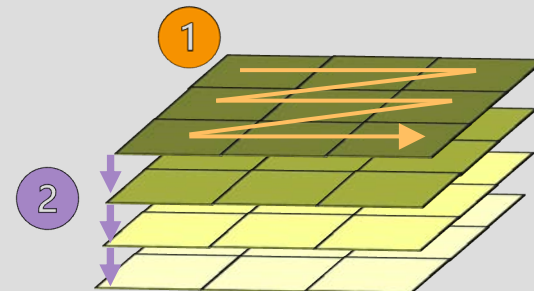
## E. Tips and Tricks

Advanced &gt;&gt;

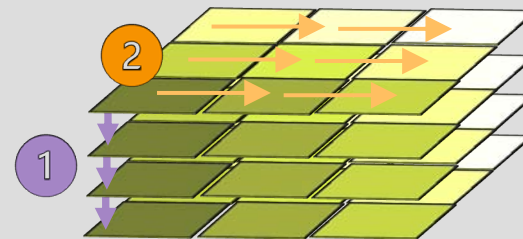
 Use PFS

Order of Experiment ▼

Large Image then Z



Z then Large Image



Each dimension in ND ACQUISITION has **ADVANCED OPTIONS**.

Two common ones are **Splitting Multipoints** and **Leave PFS On** (for use between points on a single coverslip)

When collecting data that returns to a set location in Z, consider **USE PFS** to maintain a log of focus positions. This can be helpful when stitching over a large area, running a live-cell timelapse, or setting up multiple or recurrent Z-stacks.

To optimize your experiment for speed or image registration, consider altering the **ORDER OF EXPERIMENT**. This option allows you to shift how the dimensions are collected. An example would be collecting a Z-stack of 3x3 images.

## Advanced Routines

## A. Sample Overview



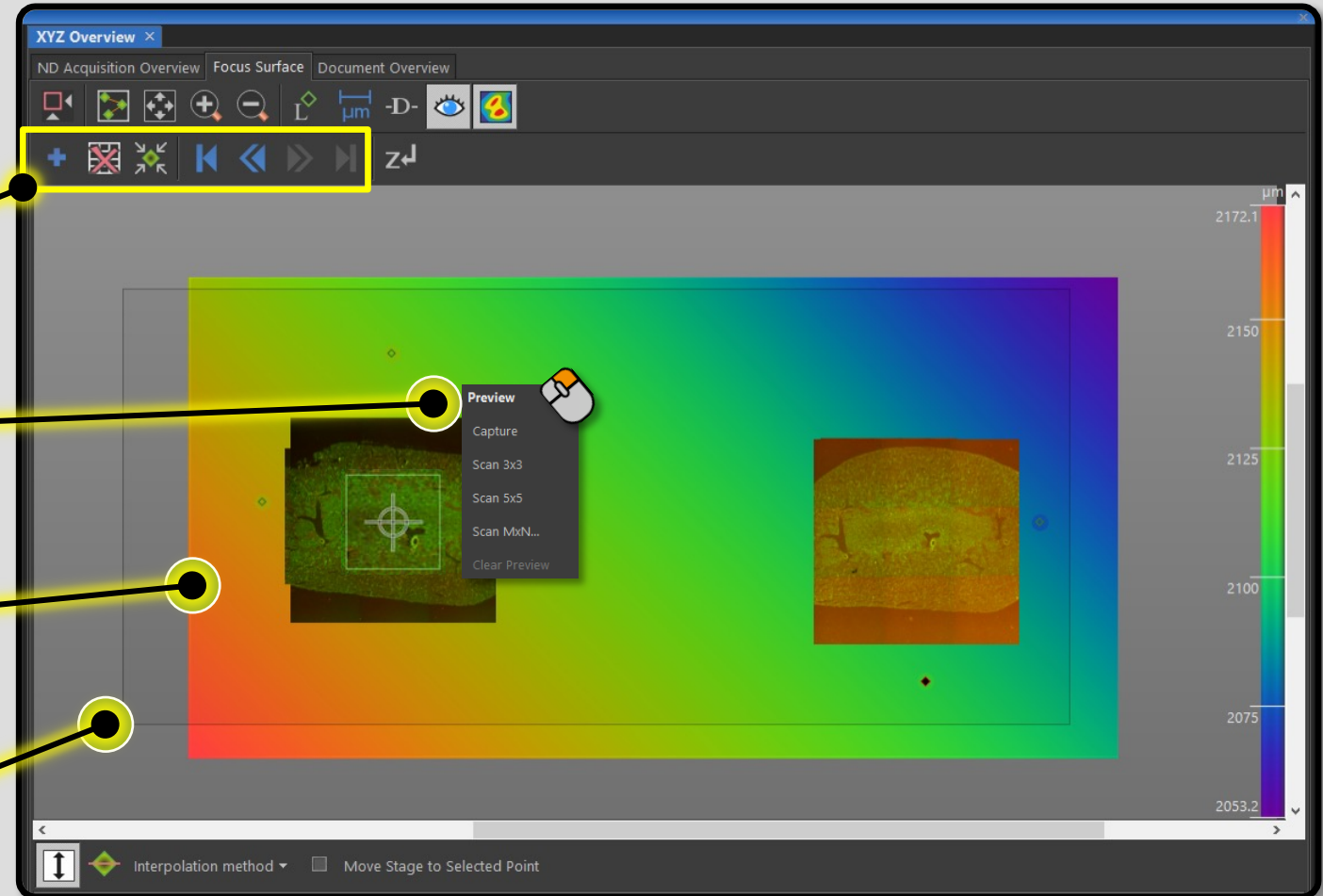
**XYZ OVERVIEW** is a control window that assists in sample navigation. It contains functions for XY point navigation, preview stitching, and can be used to create a virtual plane of focus for complex stitching routines

XY Location Navigation

Quick Preview Stitching

Interpolated Focus Surface

User-defined Sample Area



1

2

3

4

5

6

7

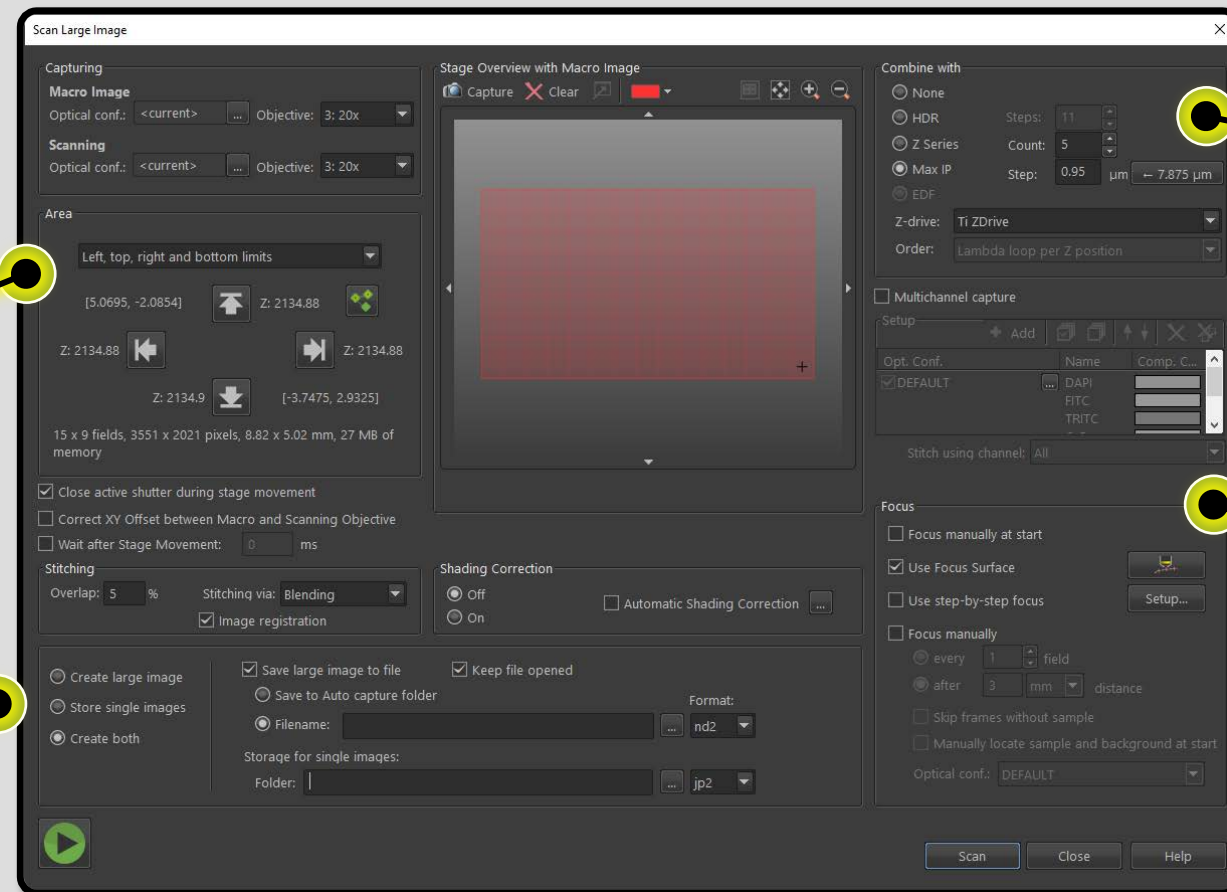
8

9

## Advanced Routines

## B. Large Image Stitching

**SCAN LARGE IMAGE** is a control window dedicated to stitching multiple fields together into a single image. Stitched fields can be defined as # of fields (X by Y) or by the boundaries (TOP, LEFT, BOTTOM, RIGHT). This dialogue also allows for the collection of a Z-stack at each position, but unlike any other dialogue, offers the option to only save a compression of that stack.



Field Boundaries

Z-Stack Parameters

Save Options

Focus Method

1

2

3

4

5

6

7

8

9

## Advanced Routines

## C. Stimulation/Bleaching: Method Choice

1

2

3

4

5

6

7

8

9

For a **SEQUENTIAL STIMULATION**, use

Galvano

This method defines the conditions for

1) Prestimulation, 2) Stimulation, 3) Poststimulation.

For a **SIMULTANEOUS STIMULATION**, use

Resonant

This method defines the conditions for

1) Total Experiment Duration, 2) Stimulation Timing

Time schedule (A1plus Galvano / A1plus Device)

Phase	Group	Acq/Stim	ROIs	Interval	Duration	Loops
#1		Acquisiti...		132 sec	132 sec	2
#2		Stimulati...	S1	No Delay	2 sec	2
#3		Acquisiti...		132 sec	20 min	10
<input type="checkbox"/>						

Perform Time Measurement (0 ROIs, 1 stim./bleaching ROIs)  
 Close Active Shutter when idle  Use HW sequencer

Apply Stimulation Settings

Time schedule (A1plus Resonant / A1plus Galvano)

Acquisition:

Interval	Duration	Loops
No Delay	5 min	2301

Stimulation/Bleaching:

Wait	Duration	Loops	ROIs
5 sec	2.11 sec	2	S1

Perform Time Measurement (0 ROIs, 1 stim./bleaching ROIs)  
 Galvano Shutter

Apply Stimulation Settings Enable lasers for acquisition



Advanced Routines

C. Stimulation/Bleaching: Region Definition

1

2

3

4

5

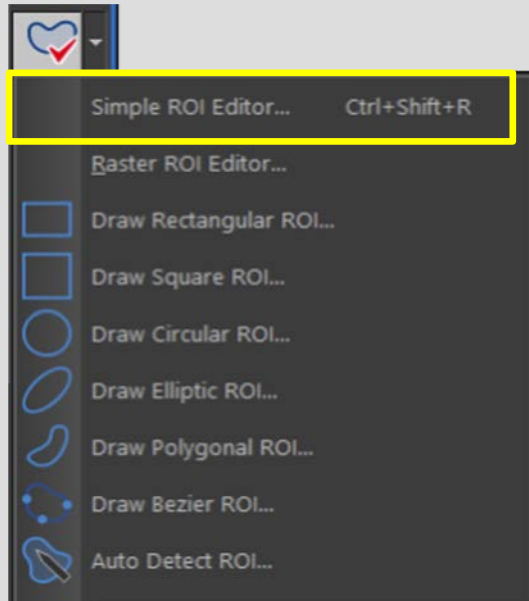
6

7

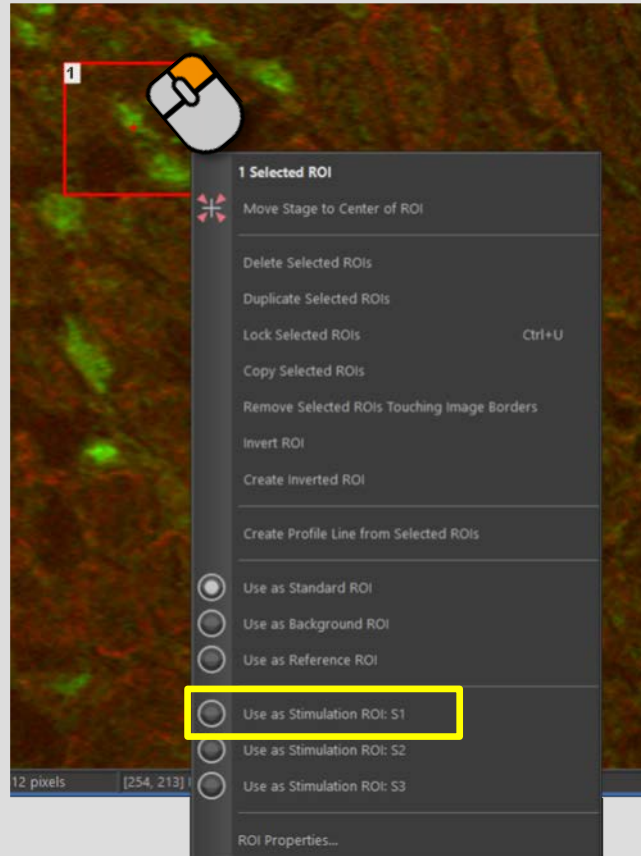
8

9

**1 Create Region of Interest**  
Use the ROI editor  
OR  
Select a pre-defined shape



**2 Define ROI as STIMULATION Region**



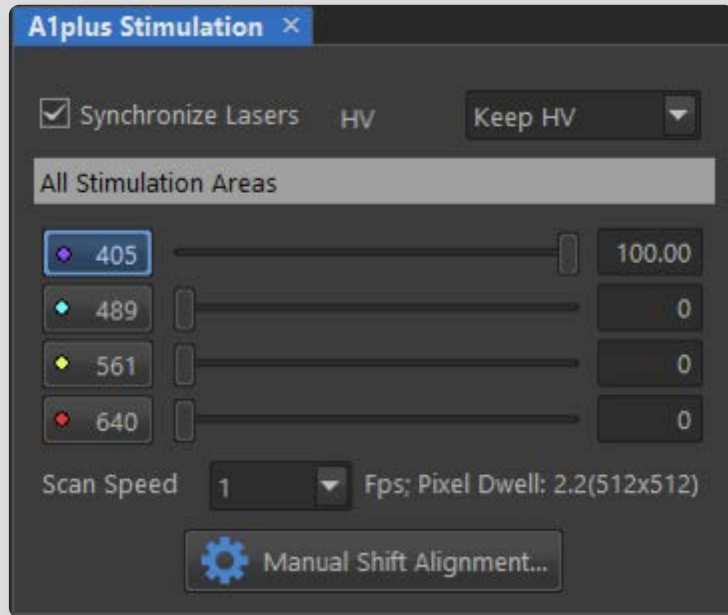
**3 Apply Settings**  
Any time you change location or definitions, you will need to CLICK *Apply Stimulation Settings*

Apply Stimulation Settings



## Advanced Routines

## C. Stimulation/Bleaching: Stimulation Laser



Once a Stimulation Region is defined and the Stimulation Experiment parameters are set:

Define:  
**Stimulation Laser Line**  
**Power**  
**Scan Speed**

**NOTE: ONLY the 405nm LASER IS AVAILABLE FOR SIMULTANEOUS STIMULATION EXPERIMENTS**

1

2

3

4

5

6

7

8

9