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.

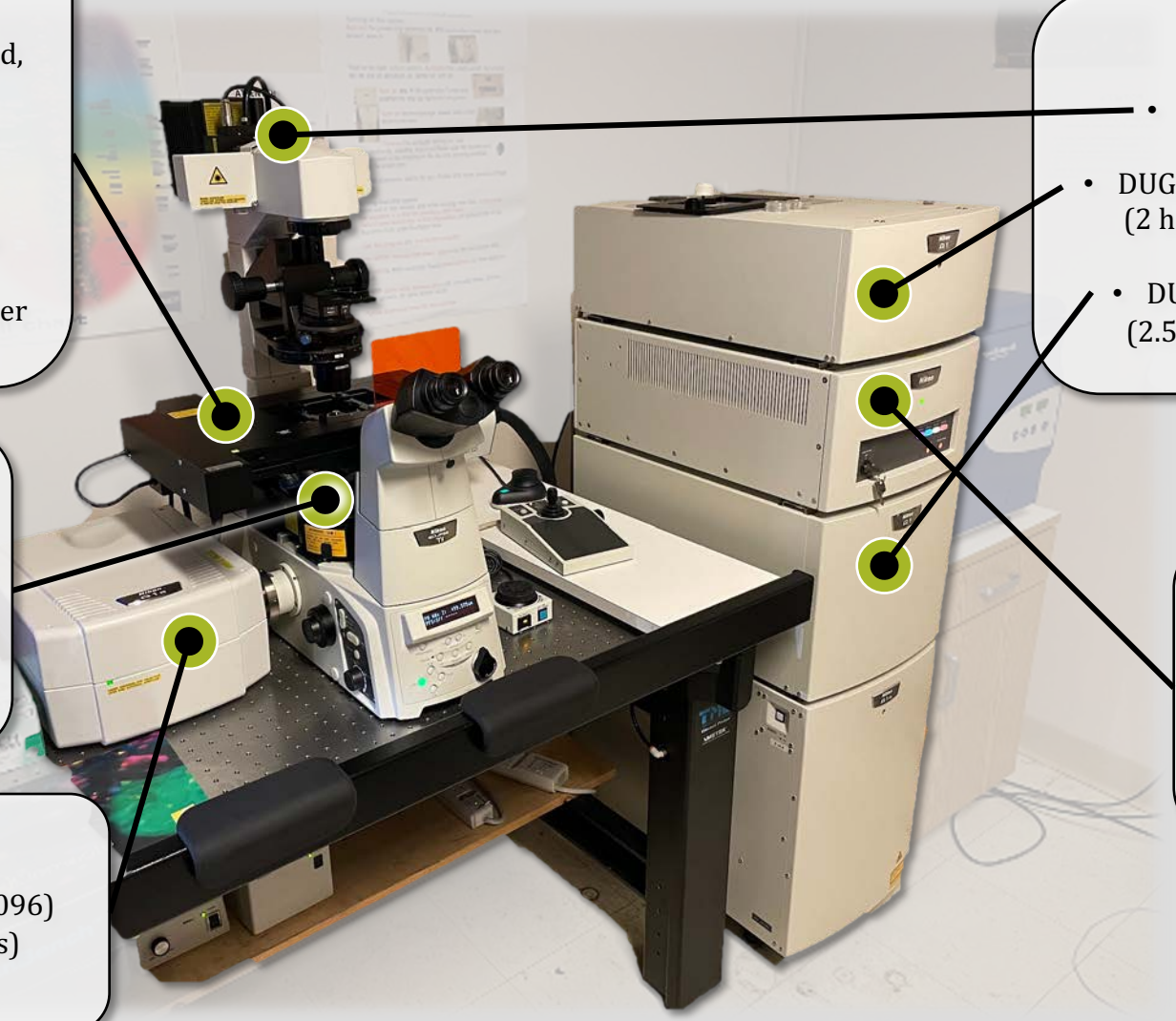
- Motorized XY stage with encoders
- Motorized Z focus with encoders
- Perfect Focus System 3
- Motorized turret of widefield cubes
- DIC optics
- Tokai-Hit stage-top incubation chamber

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

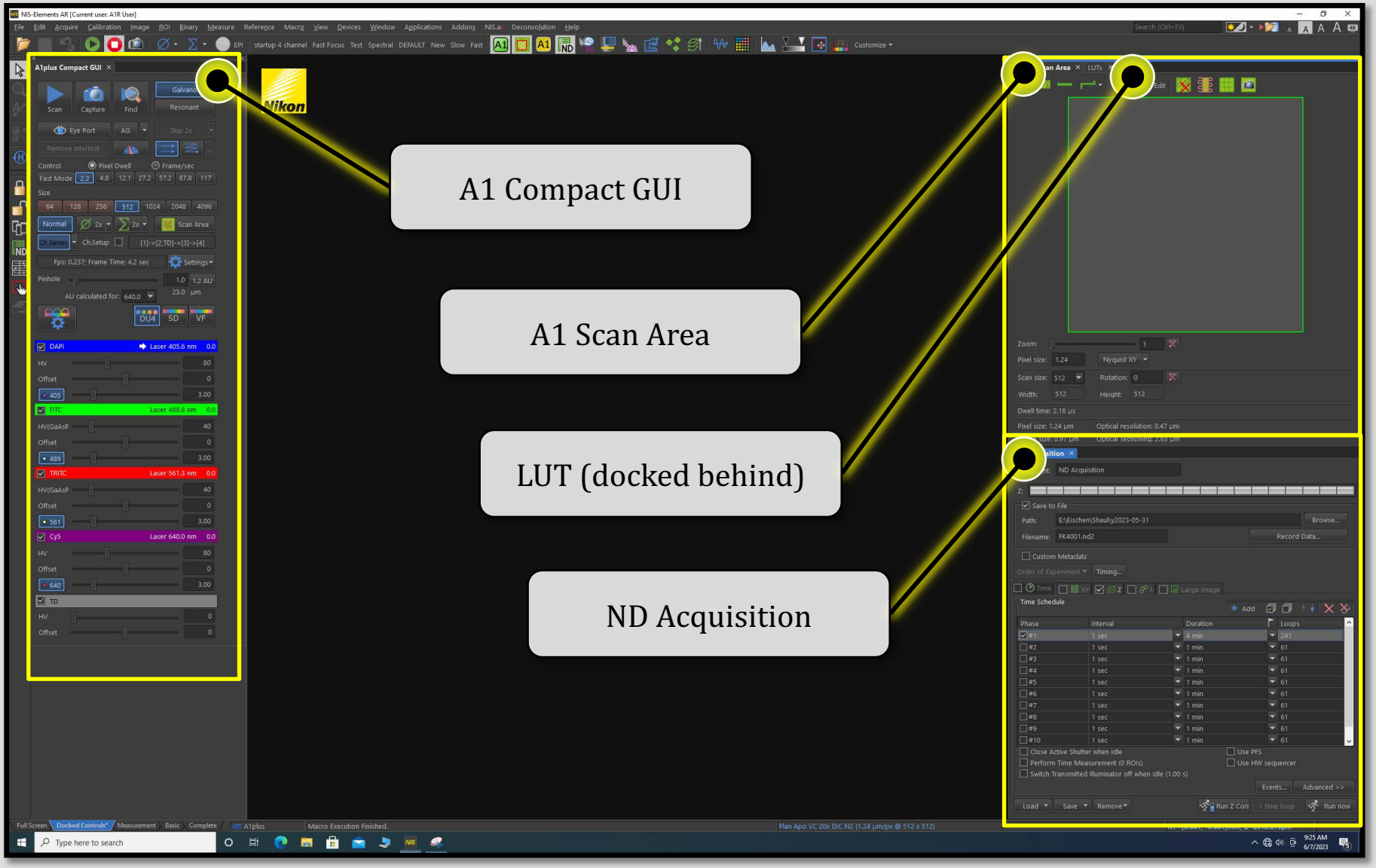
1 2 3 4 5 6 7 8 9

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

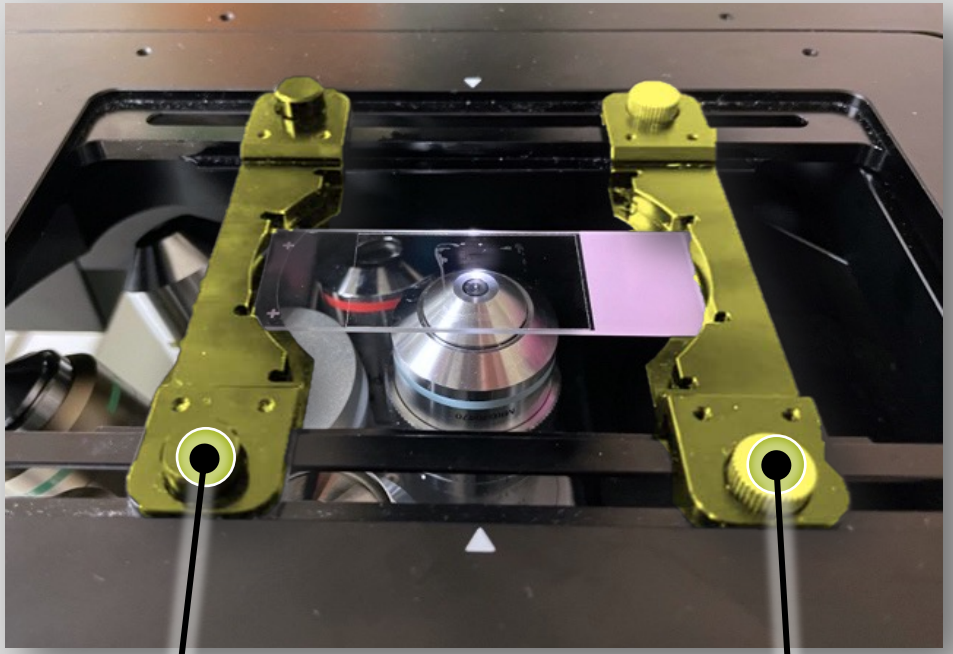


Sample Finding and Focus

A. Stage Placement

The Universal Stage Adapter can accommodate

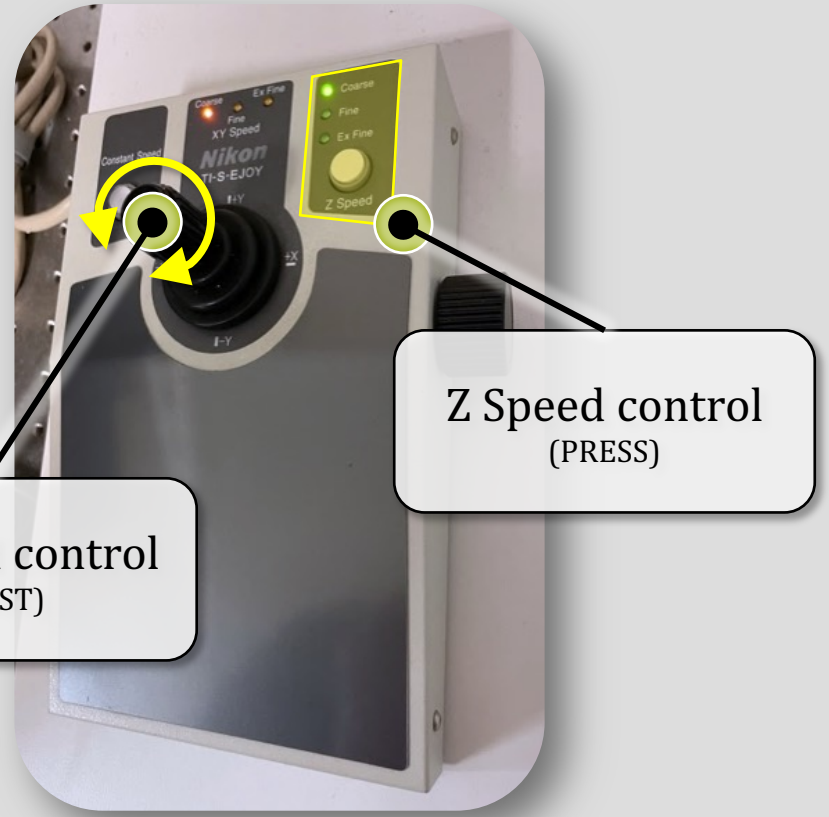
- Slides
- 35mm dishes
- Multi-well chambers



(L) Adjustable

(R) Fixed

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



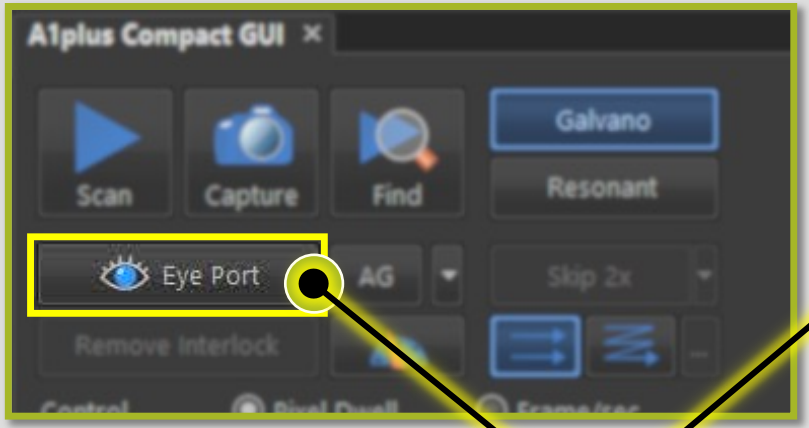
1 2 3 4 5 6 7 8 9

Sample Finding and Focus

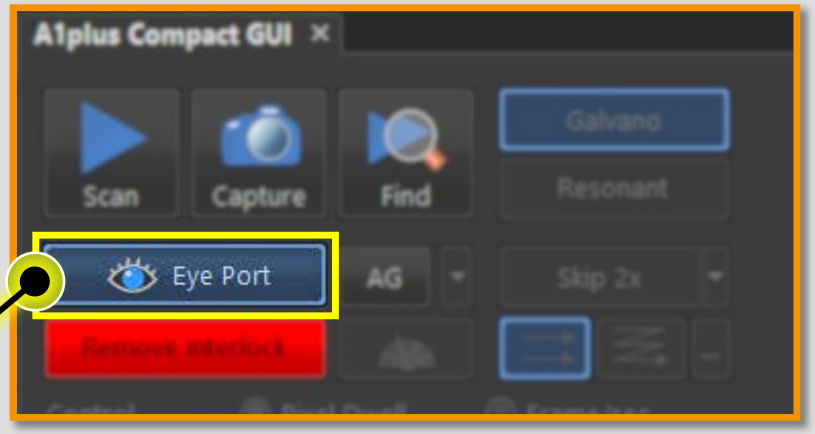
B. Eyeport Viewing

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 **EYEPORT** viewing.

Confocal Mode



Eyeport Mode



PRESS TO SWITCH BETWEEN MODES

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

1 2 3 4 5 6 7 8 9

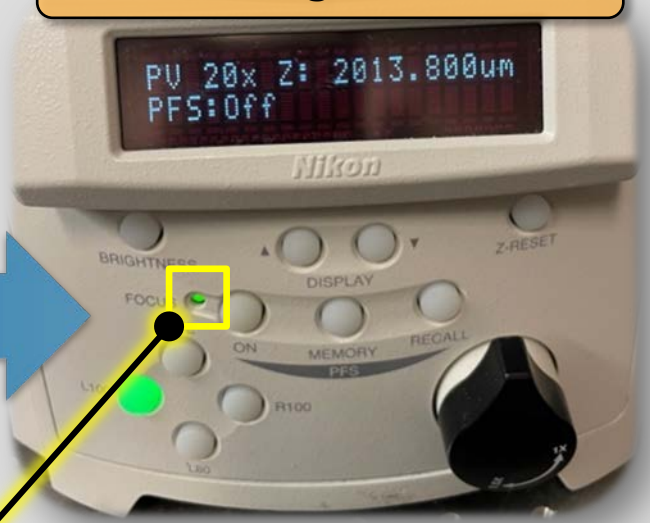
Sample Finding and Focus

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 Postiion



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

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

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

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

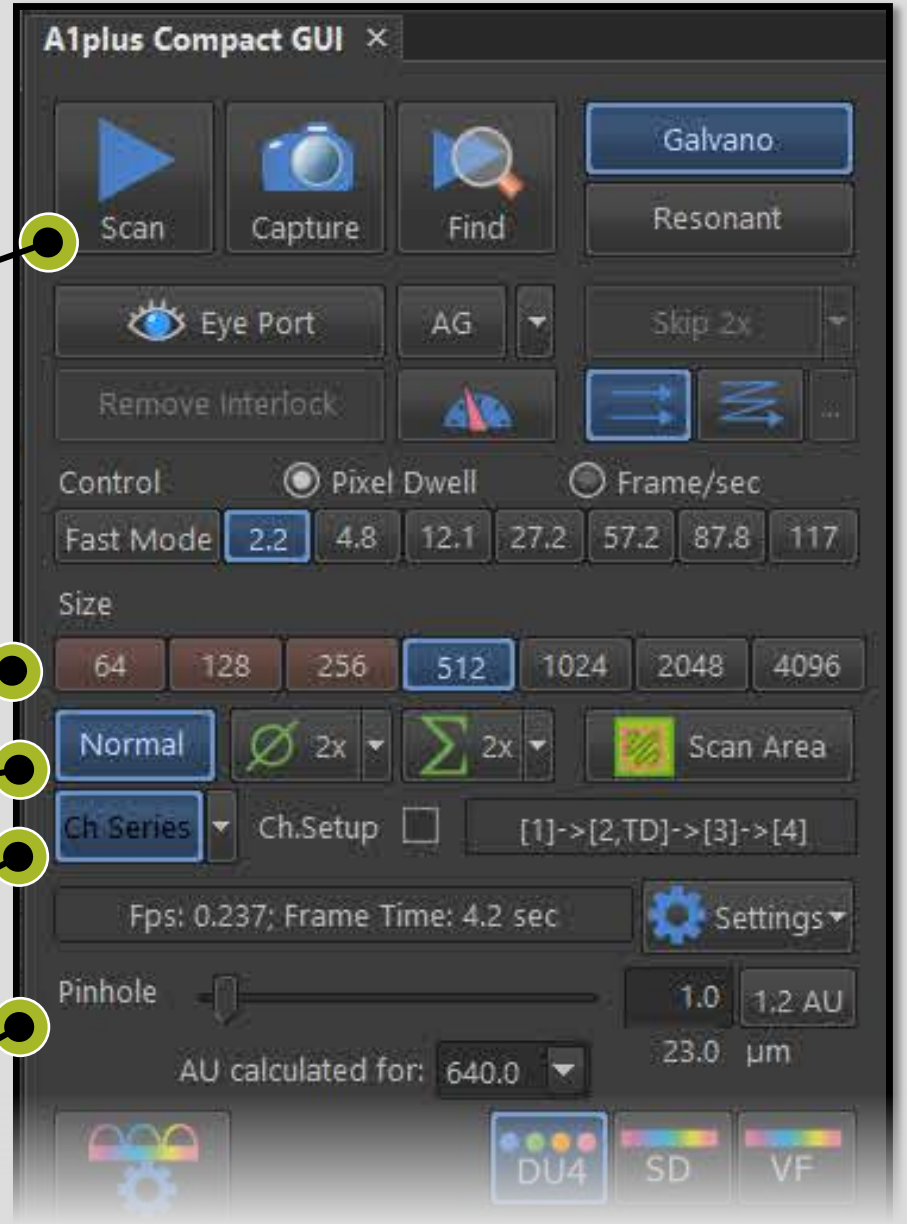
Collect image multiple time to improve signal to noise

Channel Series

Collect colors in separate scans to remove bleedthrough/crosstalk

Pinhole Size

Controls optical section thickness and removes out of focus light



Channel Settings

A. Controls

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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)

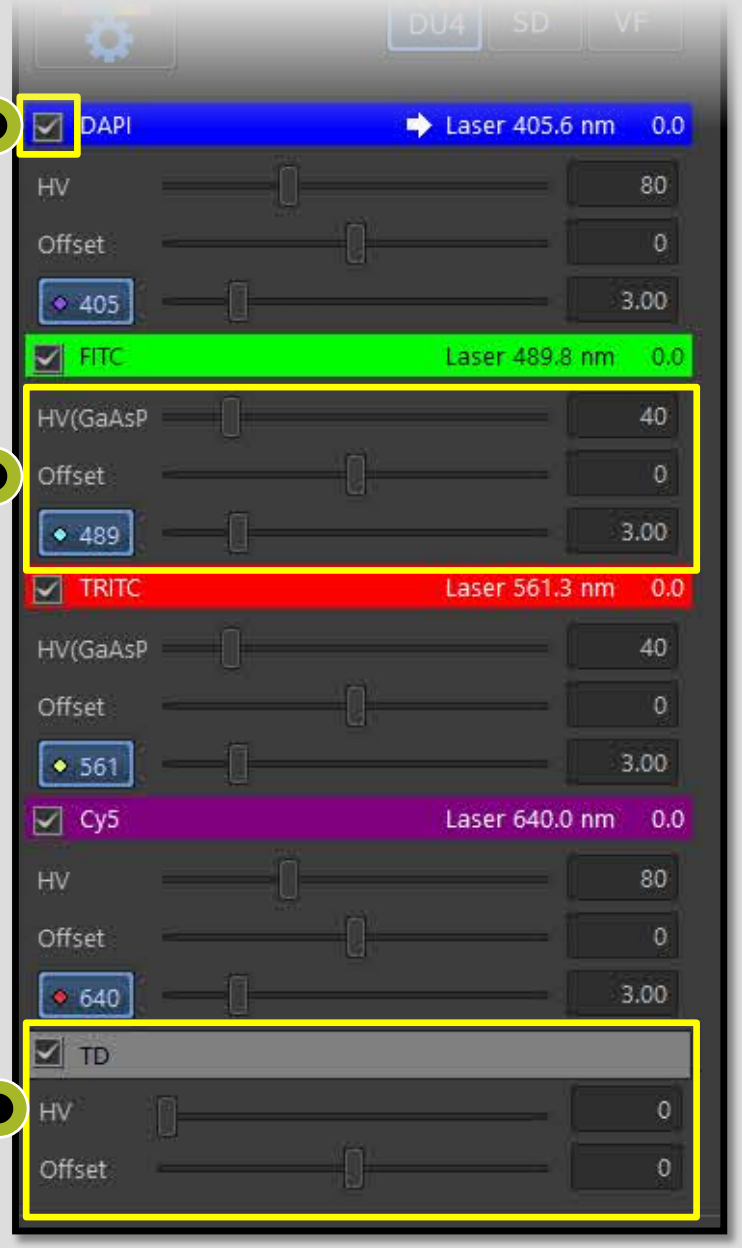
LASER: controls the amount of laser delivered to the sample

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

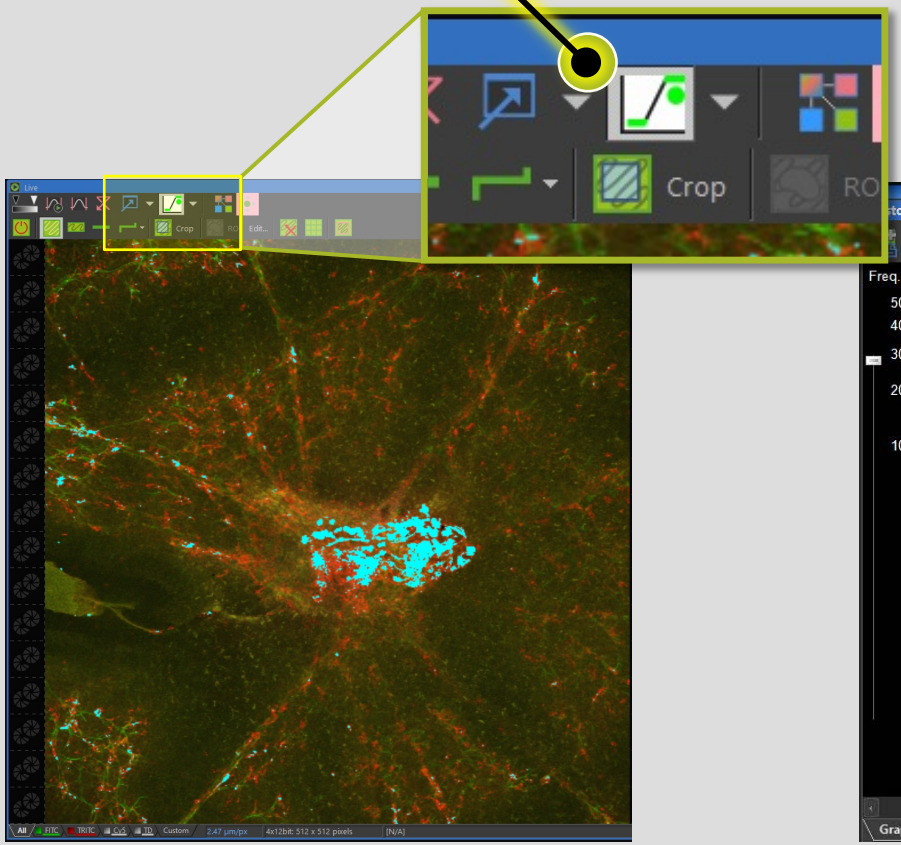
Channel Settings

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



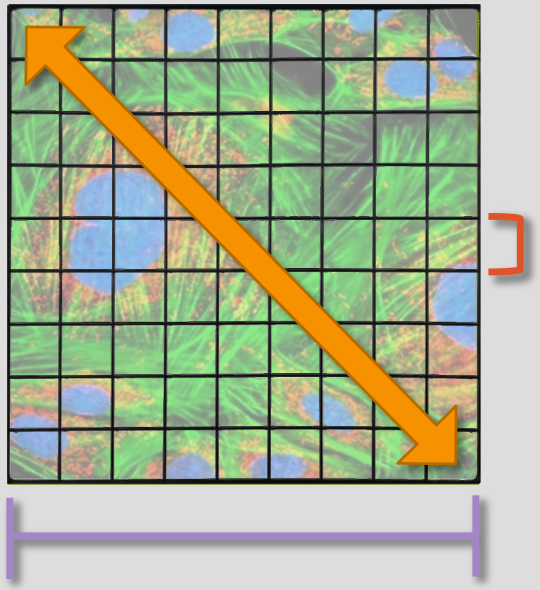
Histogram Saturation

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

Zooming and Resolution

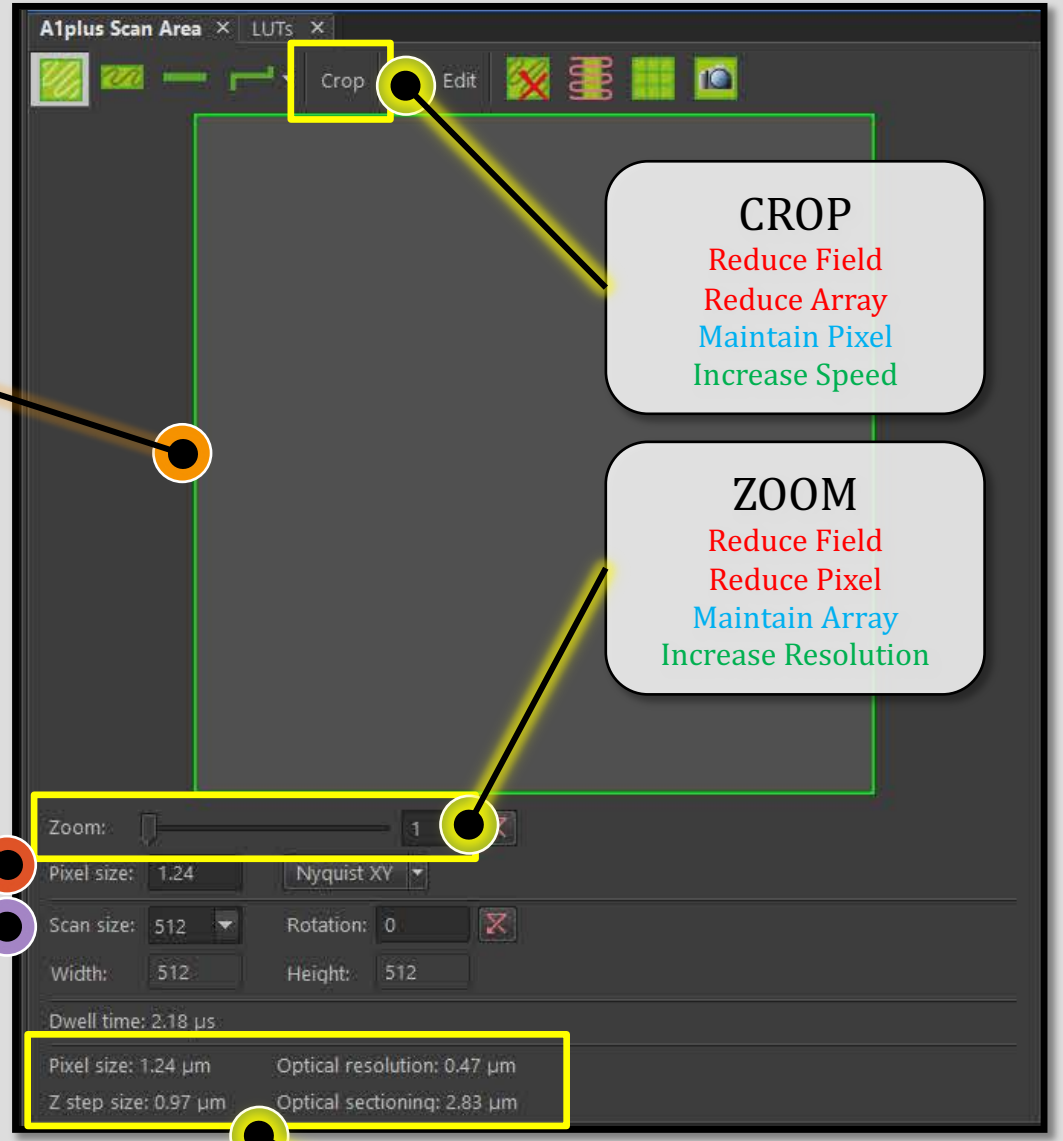
1 2 3 4 5 6 7 8 9

FOV Size



Pixel Size

Array Size



CROP
Reduce Field
Reduce Array
Maintain Pixel
Increase Speed

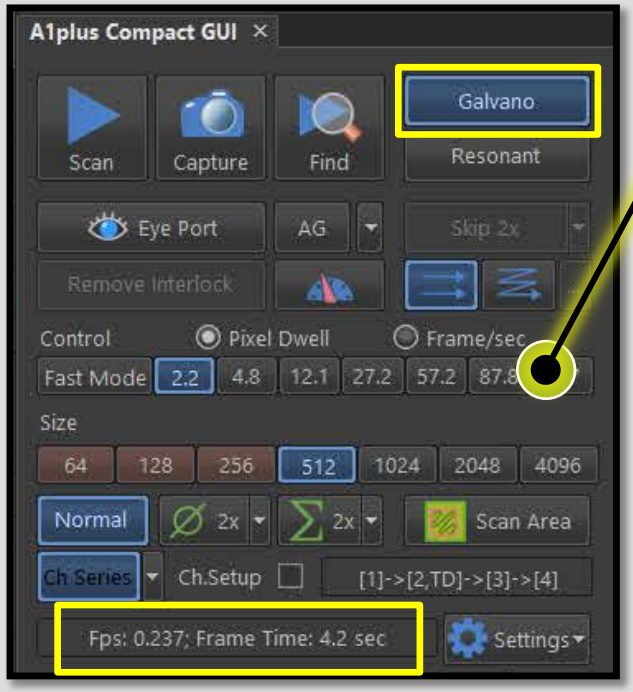
ZOOM
Reduce Field
Reduce Pixel
Maintain Array
Increase Resolution

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.



Frame Rate

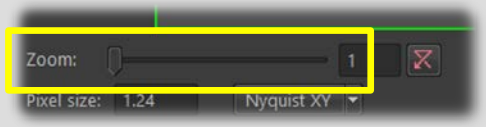
1fps ⟶ **30fps**
No speed choices
Bidirectional Scanning
Fast Acquisition, Live cells, High-speed dynamics

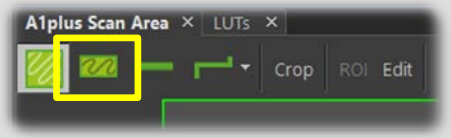
Signal:Noise

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





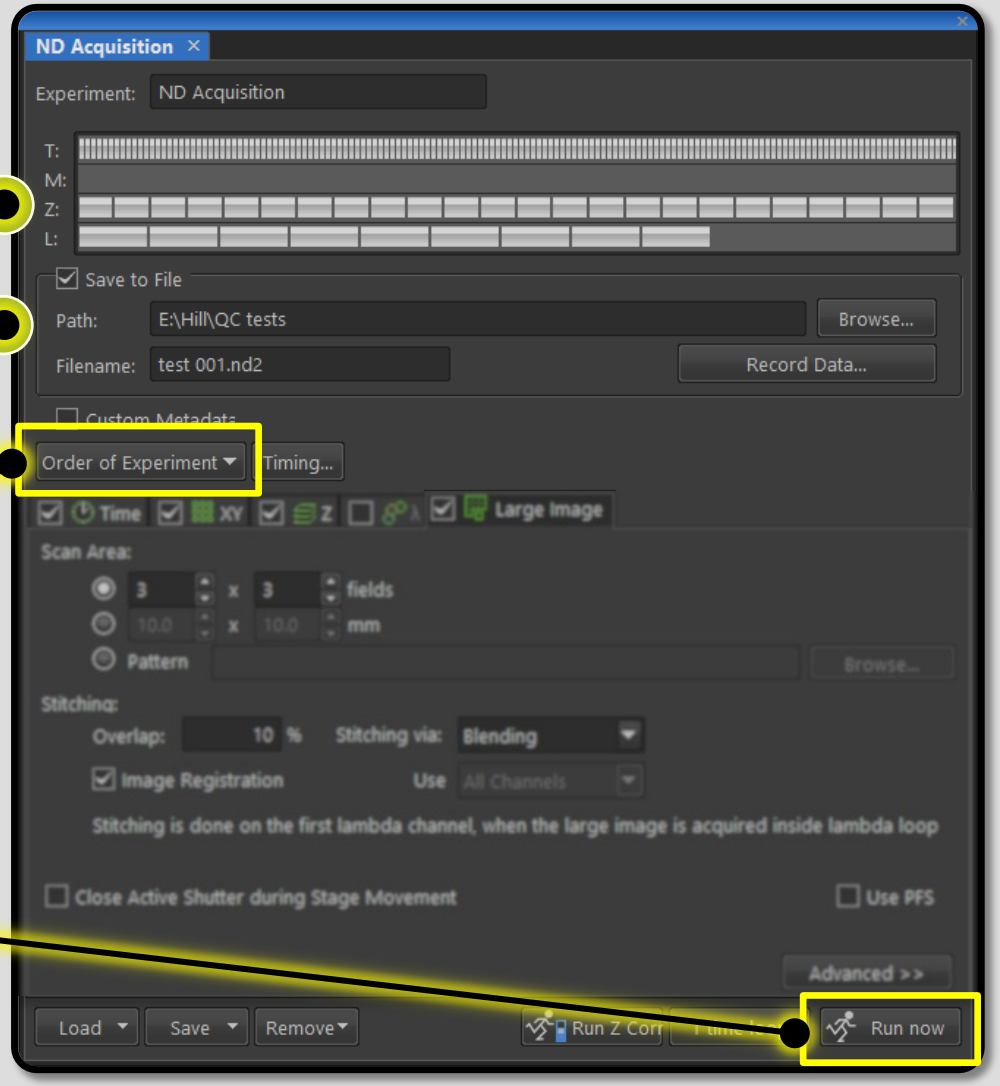
ND Acquisition

1 2 3 4 5 6 7 8 9

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

ND Acquisition

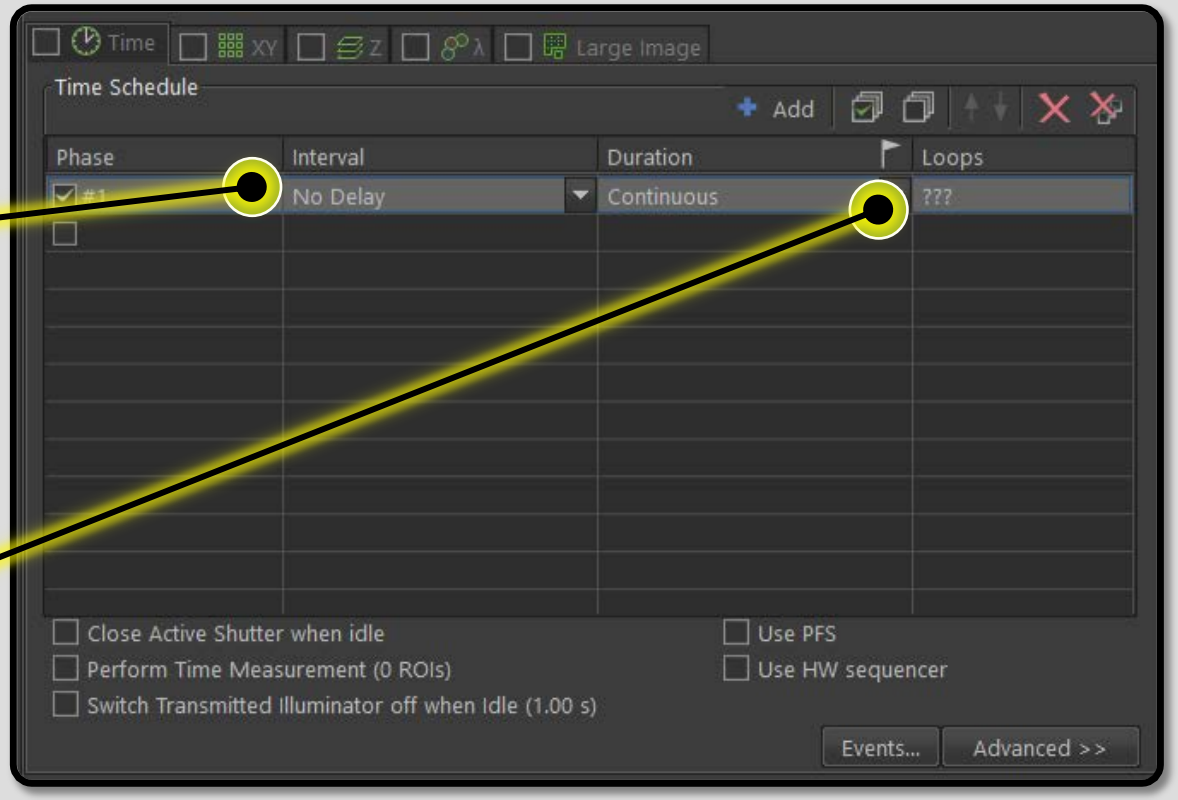
A. Time Lapse

Interval

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

Duration/Loops

How long do you want to image in total
Duration = Time
Loops = number of images
FLAG ICON shows Duration/Loops priority
"Continuous" goes until manually stopped



ND Acquisition

B. Multi XY

1 2 3 4 5 6 7 8 9

XY

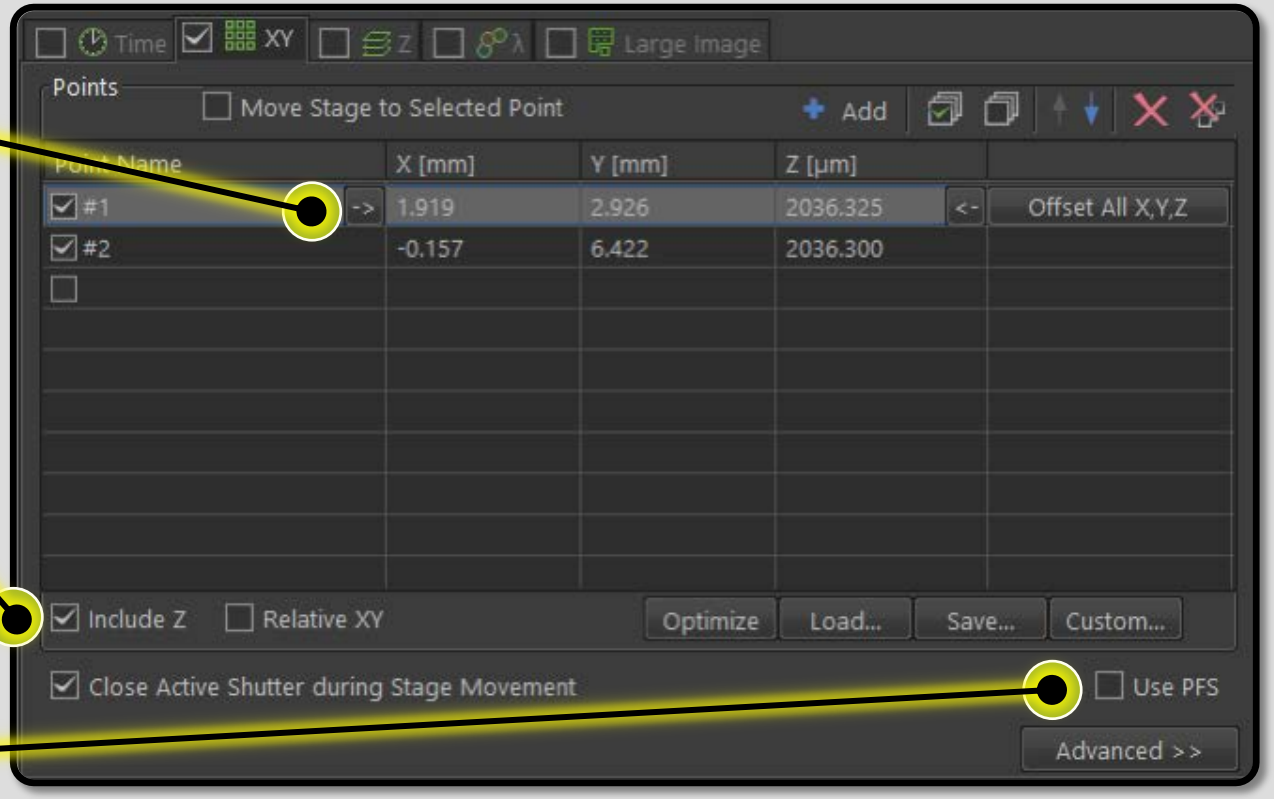
How OFTEN do you want to image
"No Delay" runs as as possible

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



ND Acquisition

C. Z-Stack

1 2 3 4 5 6 7 8 9

Stack Type

Absolute: Top and Bottom defined for SINGLE POSITION

Relative: Range around middle defined for MULTIPLE POSITIONS

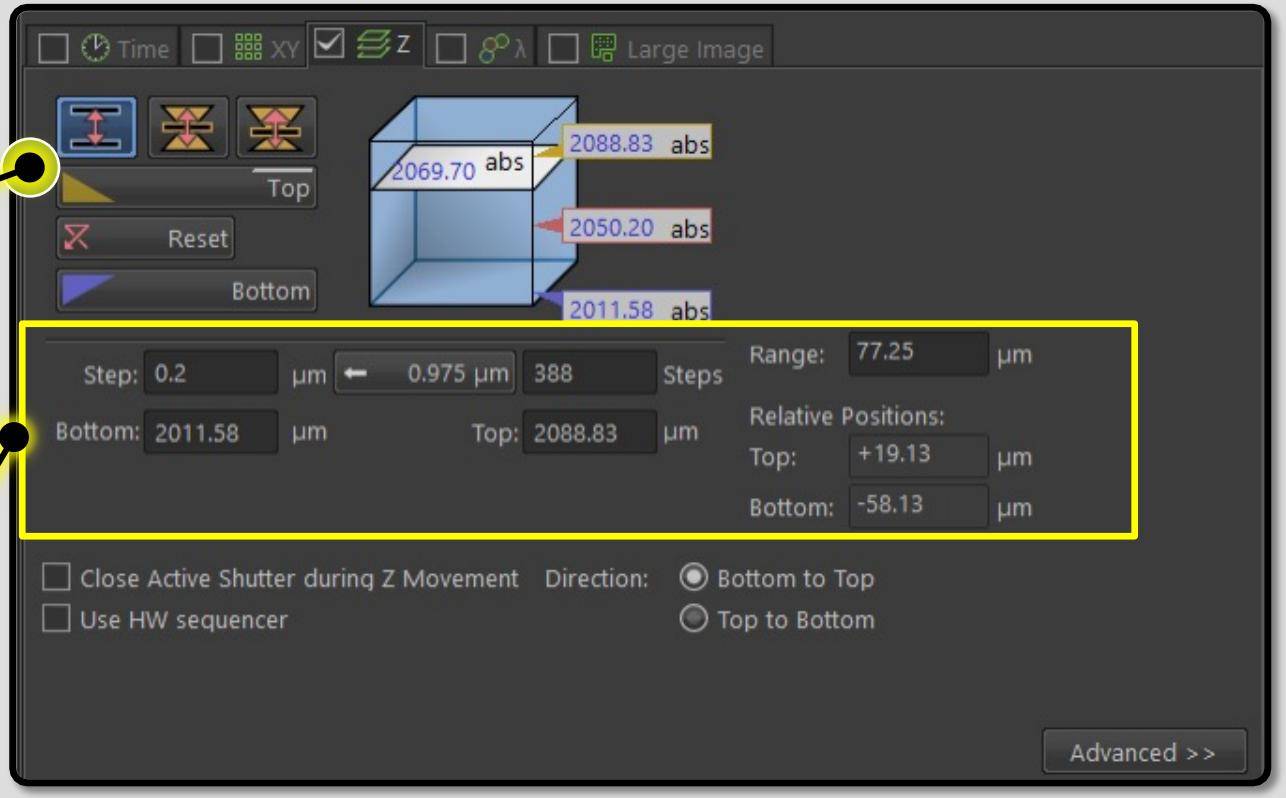
Stack Parameters

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

Stack Range: Distance between defined TOP and BOTTOM

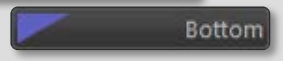
Step Number: How many slices of the defined size to achieve defined Range?

Limits: Top and Bottom of Range



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 

ND Acquisition

D. Large Image

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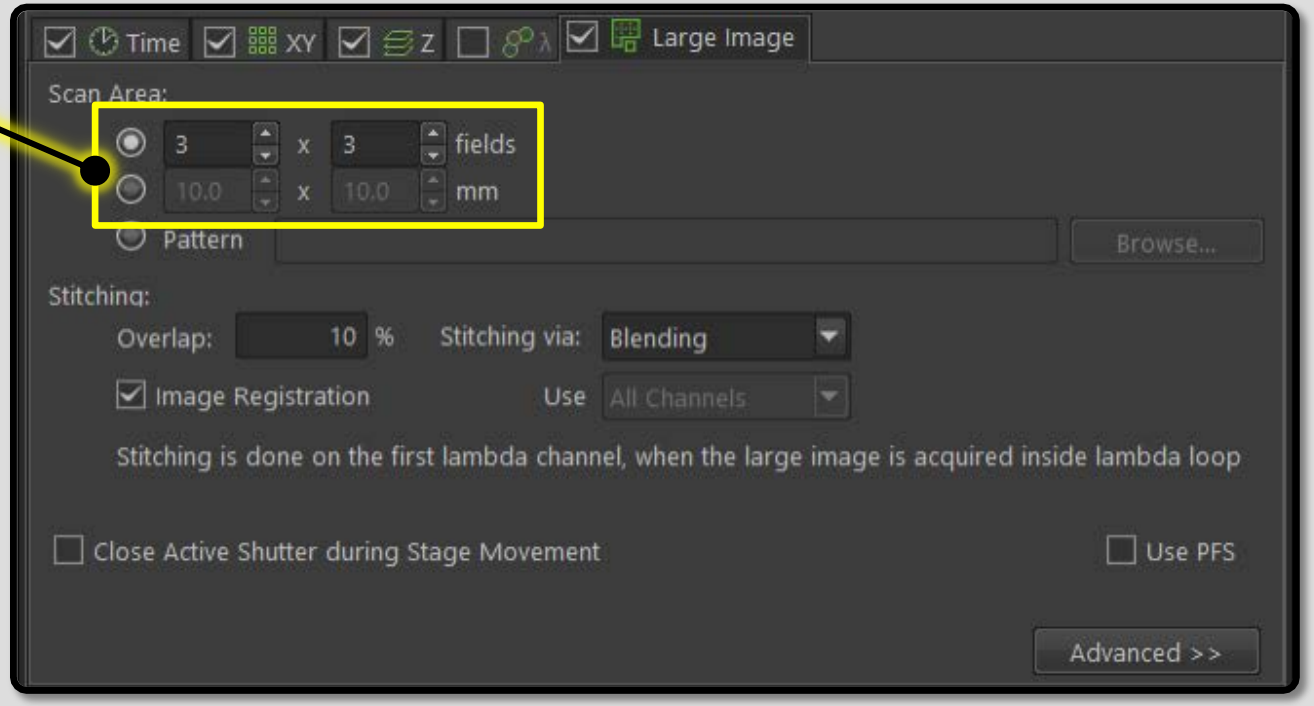
Stitched Field Dimensions

Dimensions listed as X by Y

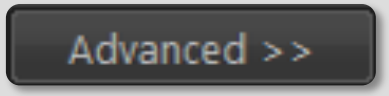
- # Fields
- Total distance covered



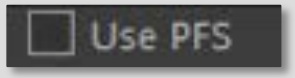
ND Acquisition

E. Tips and Tricks

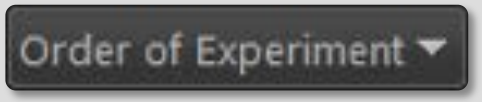
1 2 3 4 5 6 7 8 9



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

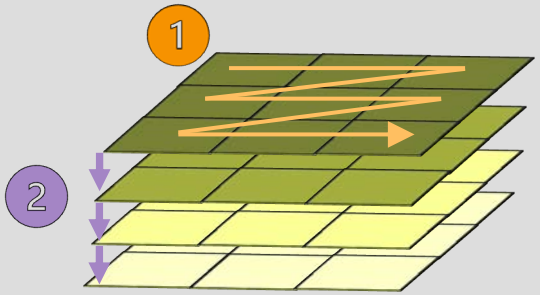


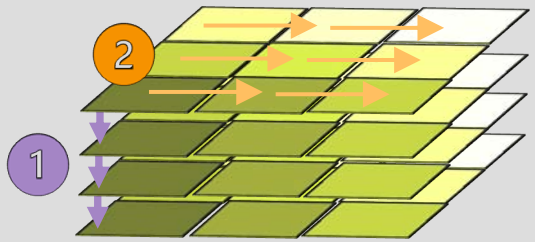
When collecting data that returns to a set location in Z, consider **USE PFS** to maintain a log of focus positions. This can be helpful when stitching over a large area, running a live-cell timelapse, or setting up multiple or recurrent Z-stacks.



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.

Large Image then Z





Z then Large Image

Advanced Routines

A. Sample Overview

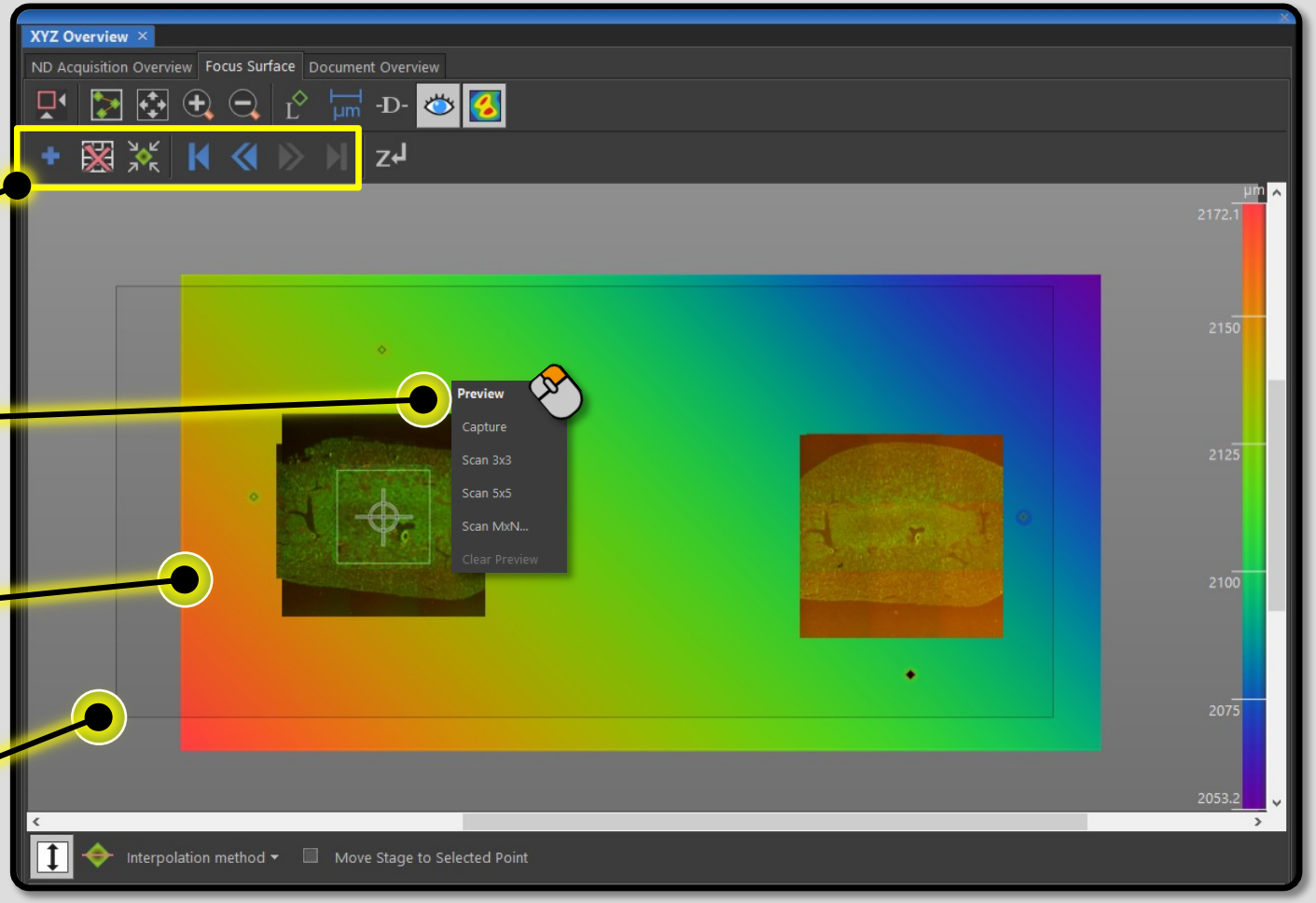
XYZ OVERVIEW is a control window that assists in sample navigation. It contains functions for XY point navigation, preview stitching, and can be used to create a virtual plane of focus for complex stitching routines

XY Location Navigation

Quick Preview Stitching

Interpolated Focus Surface

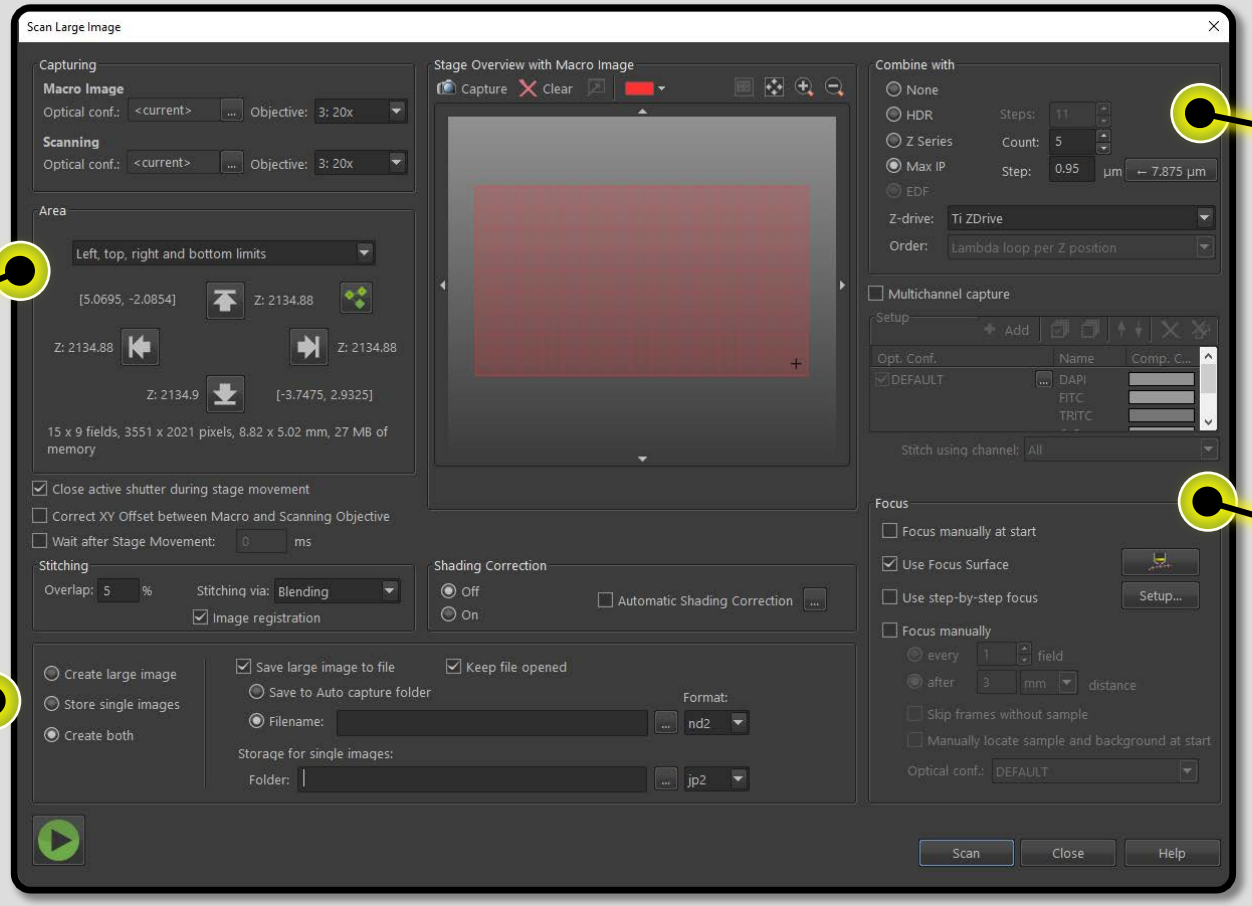
User-defined Sample Area



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

Save Options

Z-Stack Parameters

Focus Method

Advanced Routines

C. Stimulation/Bleaching: Method Choice

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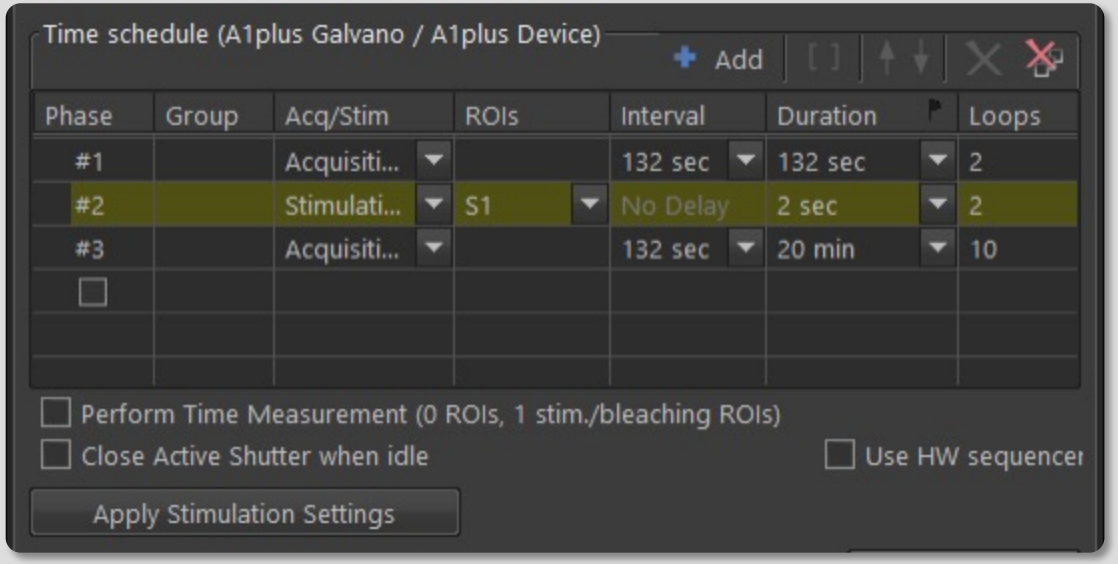
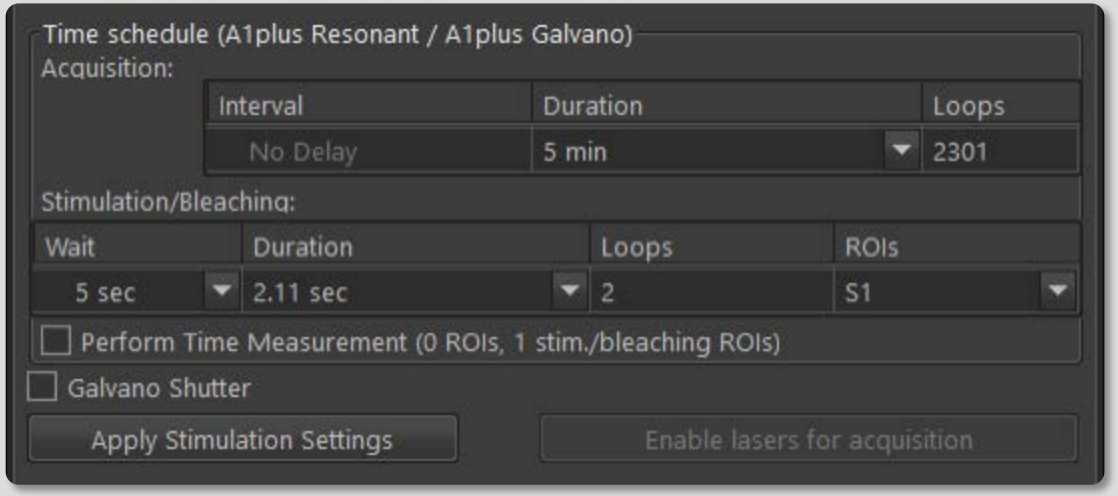
For a **SEQUENTIAL STIMULATION**, use Galvano

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



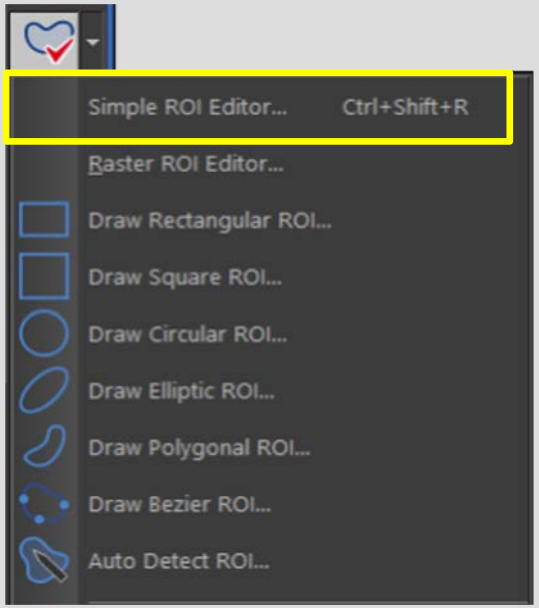
Advanced Routines

C. Stimulation/Bleaching: Region Definition

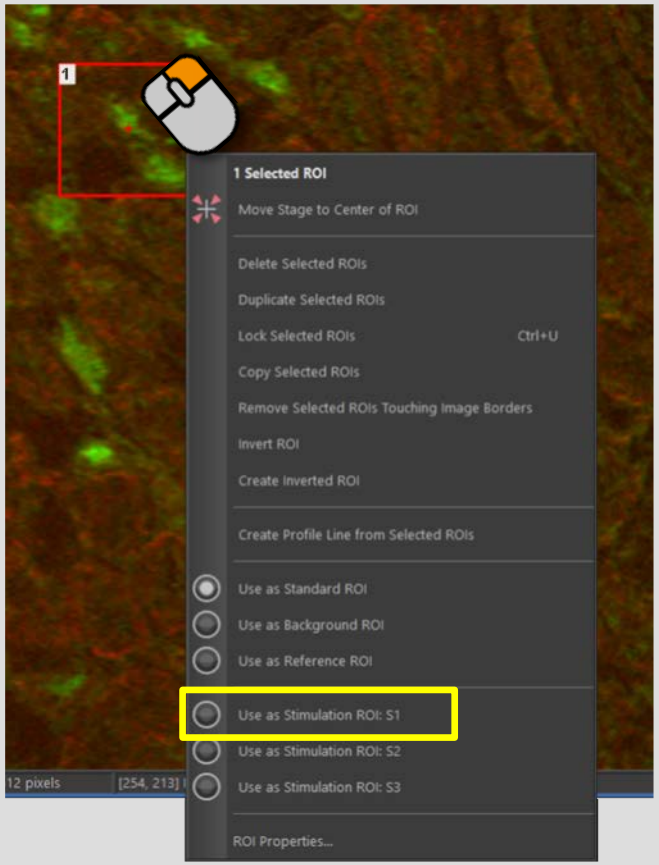
1 2 3 4 5 6 7 8 9

1 Create Region of Interest

Use the ROI editor
OR
Select a pre-defined shape

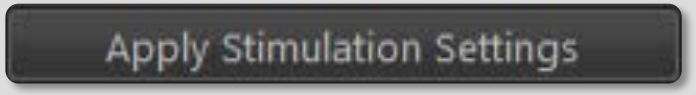


2 Define ROI as STIMULATION Region



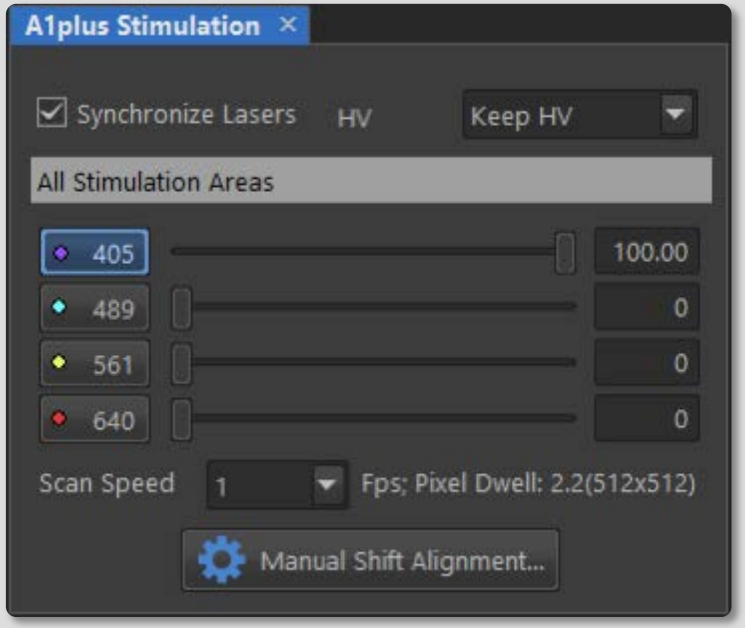
3 Apply Settings

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



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