# NIKON A1R HD CONFOCAL

Basic Operation

BioImaging Shared Resource – 2026

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

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1
2
3
4
5
6
7
8
9

## Microscope

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

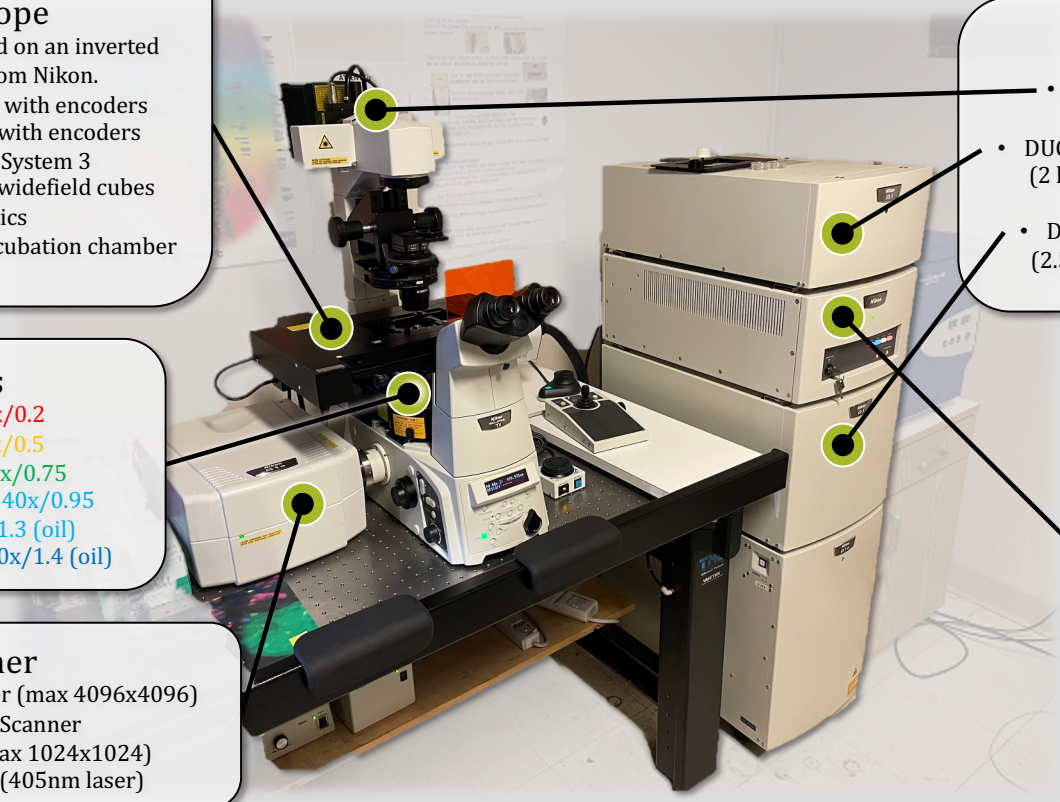
- Motorized XY stage with encoders
- Motorized Z focus with encoders
- Perfect Focus System 3
- Motorized turret of widefield cubes
- DIC optics
- Tokai-Hit stage-top incubation chamber

## 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; max 1024x1024)
- 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

1
2
3
4
5
6
7
8
9

# Software Overview

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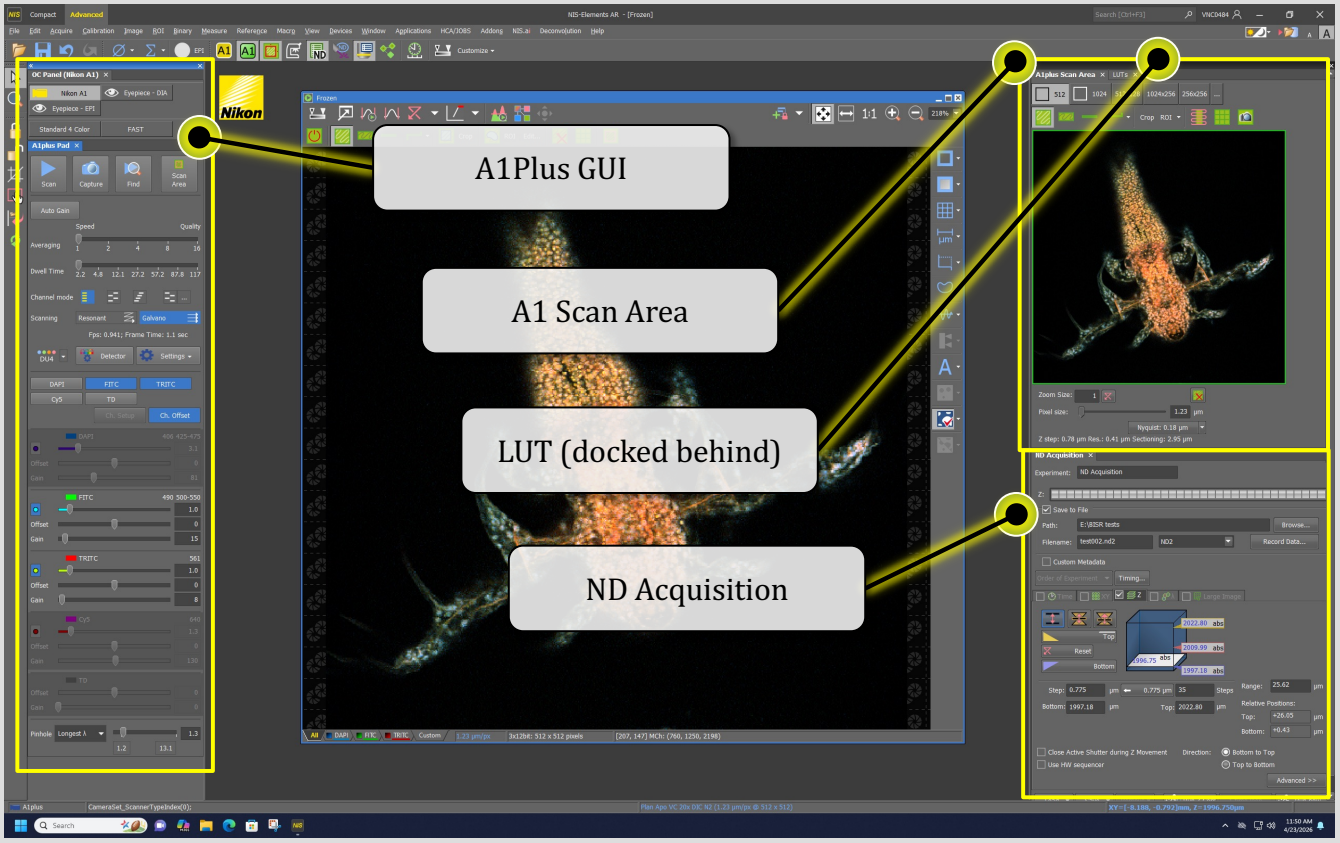
*The A1R confocal runs in Nikon's NIS-Elements software. To help limit any user issues typically seen when several users overlap on a single system, the software is programmed to automatically load presets when it starts. These include a standardized hardware configuration and initial software layout. 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



1
2
3
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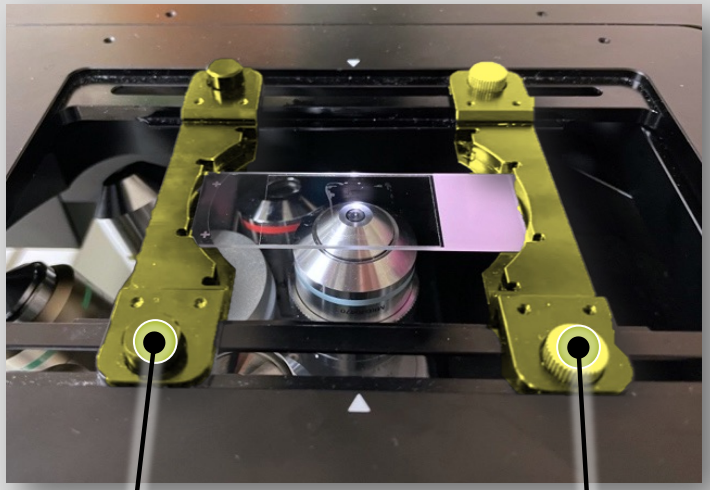
# Sample Finding and Focus

## A. Stage Placement

The Universal Stage Adapter can accommodate

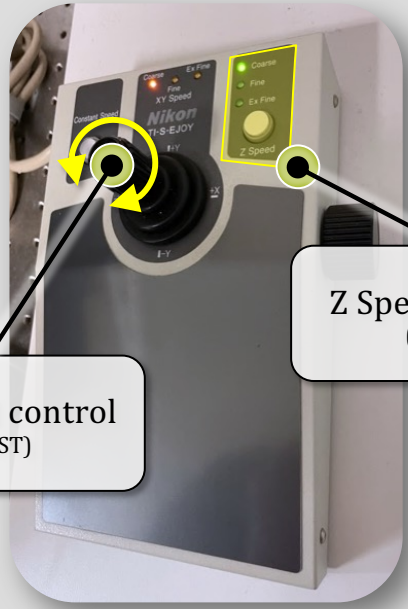
- Slides
- 35mm dishes
- Multi-well chambers



(L) Adjustable

(R) Fixed

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




XY Speed control
(TWIST)

Z Speed control
(PRESS)

1
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8
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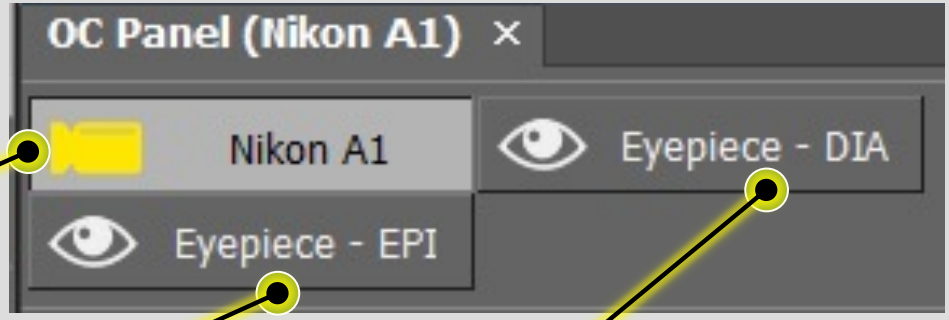
# Sample Finding and Focus

## 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

Eyepiece Fluorescence

Eyepiece Brightfield


When viewing sample in EYEPORT Mode:
Change fluorescence 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

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- 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

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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

## Averaging

Collect multiple images to improve signal to noise at the cost of speed

## Scanning Speed

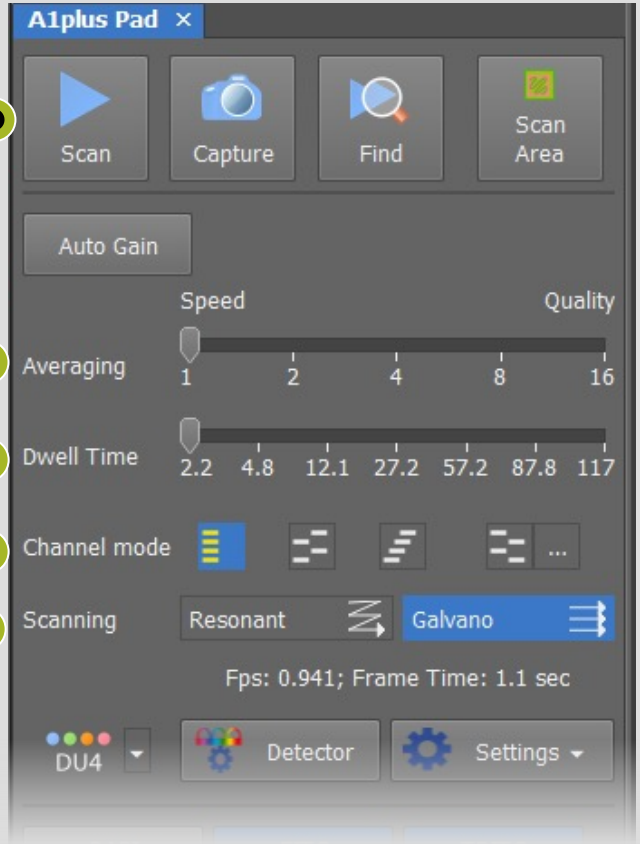
Collect images slower to improve signal to noise at the cost of speed

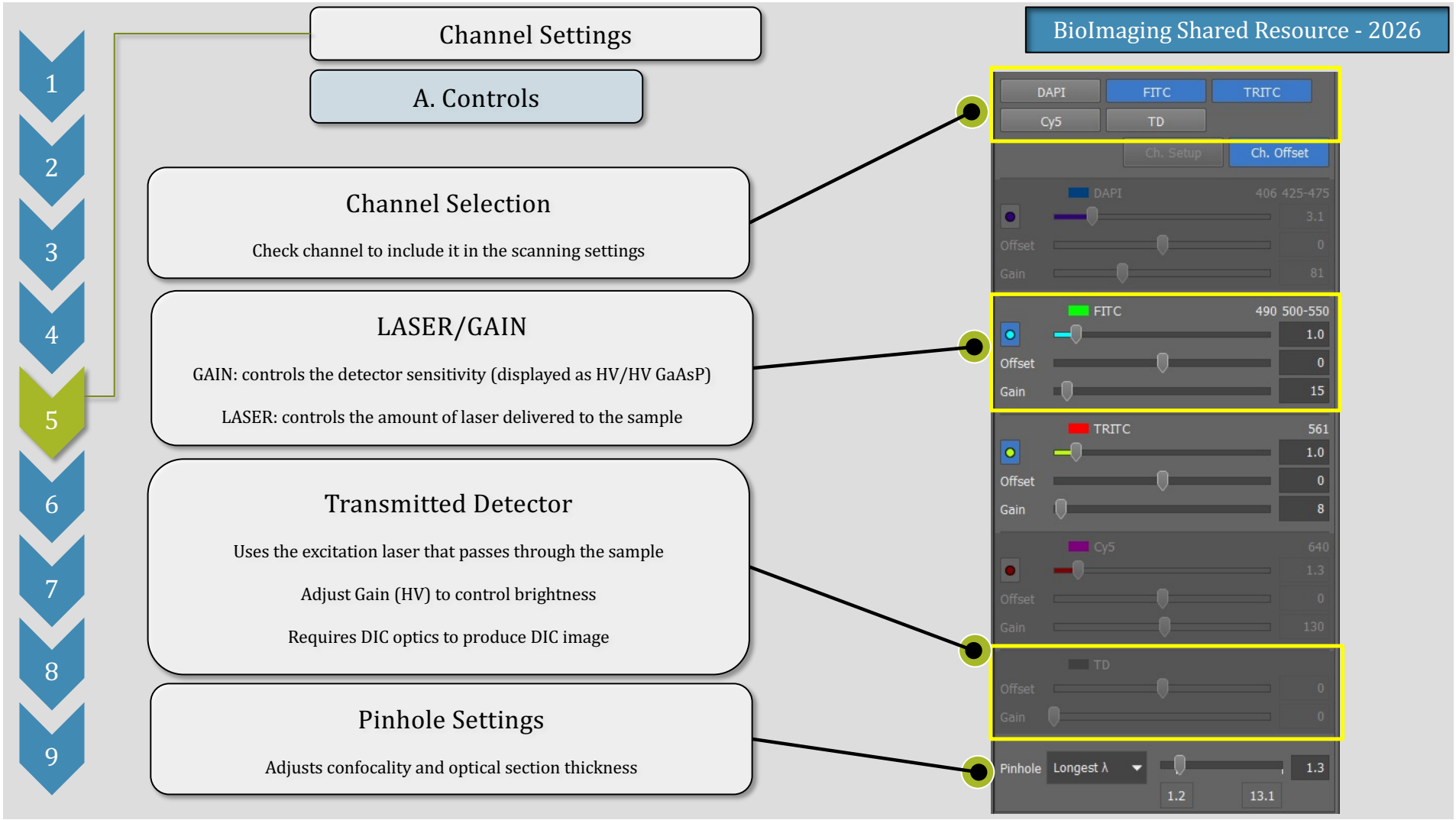
## Channel Series

Collect colors in separate scans to remove bleedthrough/crosstalk

## Scanner Mode

Choose between Resonant and Galvano Scanners

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Channel Settings
A. Controls
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4
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6
7
8
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Channel Selection
Check channel to include it in the scanning settings
LASER/GAIN
GAIN: controls the detector sensitivity (displayed as HV/HV GaAsP)
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
Pinhole Settings
Adjusts confocality and optical section thickness
DAPI
FITC
TRITC
Cy5
TD
Ch. Setup
Ch. Offset
DAPI 406 425-475
3.1
Offset 0
Gain 81
FITC 490 500-550
1.0
Offset 0
Gain 15
TRITC 561
1.0
Offset 0
Gain 8
Cy5 640
1.3
Offset 0
Gain 130
TD
Offset 0
Gain 0
Pinhole Longest λ 1.3
1.2
13.1

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# Channel Settings

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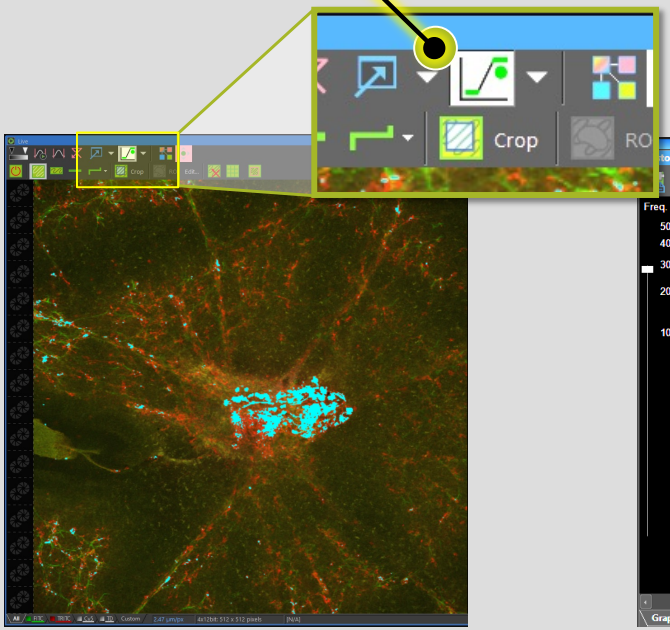
## B. Oversaturation

Oversaturation refers to signal that is beyond the quantifiable range of the detector.
If intensity-based measurements are needed, avoid oversaturation

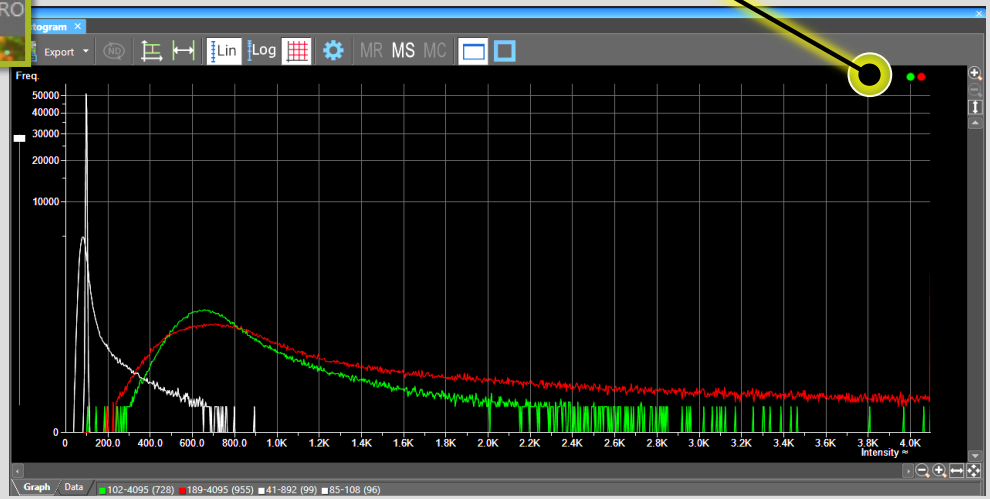
### Oversaturation Indicator

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

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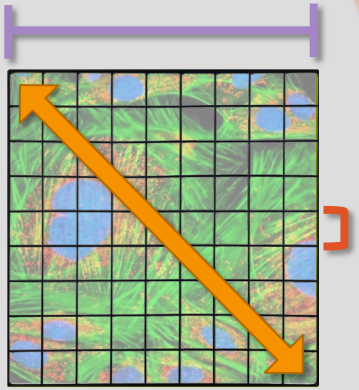
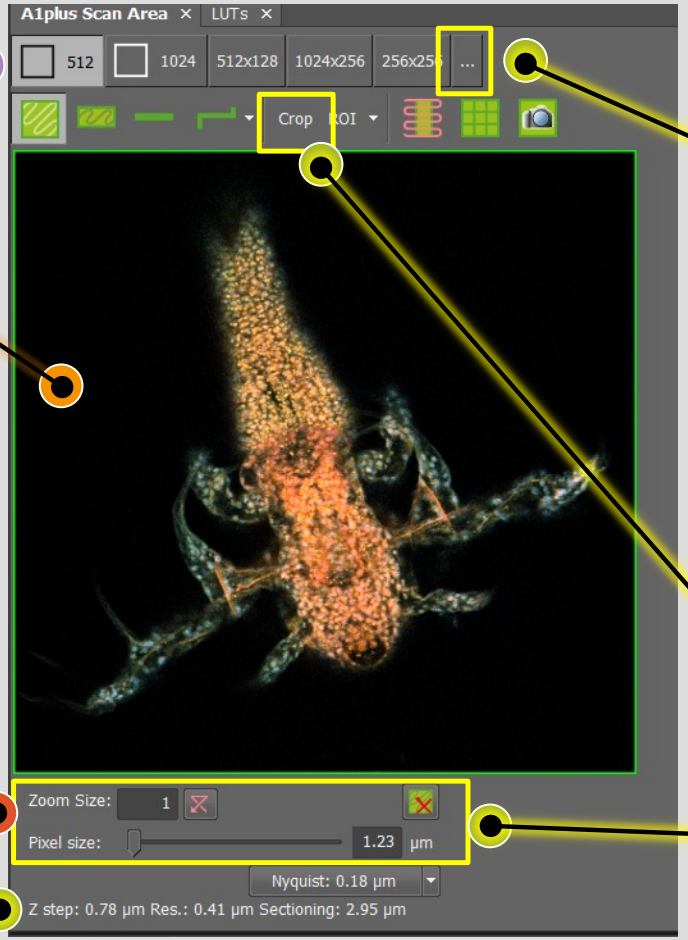
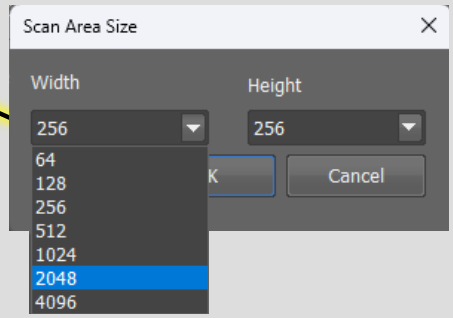
**Array Size**

**FOV Size**

**Pixel 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

1 2 3 4 5 6 7 8 9

# High Speed Scanning

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The Nikon A1R HD confocal can also be run in **RESONANT MODE**

The faster frame rate has several application benefits, but users may face limiting tradeoffs.


## Frame Rate (512x512)

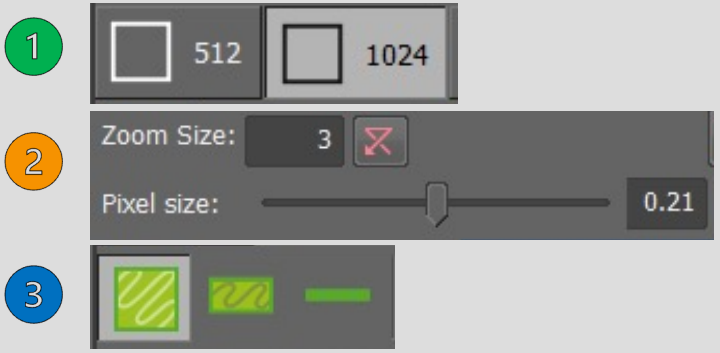
1fps → **30fps**
No speed choices
Bidirectional Scanning
***Fast Acquisition, Live cells, High-speed dynamics***

## Signal:Noise

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


## HD Resolution

1. Array options of 256x256, 512x512, and 1024x1024
2. Resolution increase by Zooming also
3. "Band Scanning" provides additional speed



# ND Acquisition

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

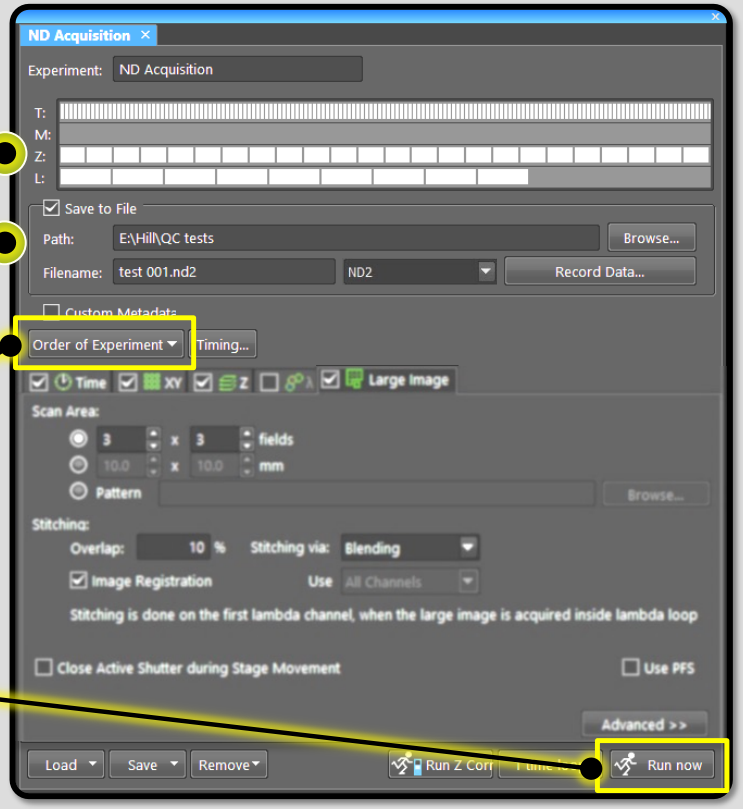
This dialogue allows you to set parameters for each dimension and control basic experimental logic

Selected Dimensions

Auto-Save Path

Experiment Order

Run Experiment



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# ND Acquisition

## A. Time Lapse

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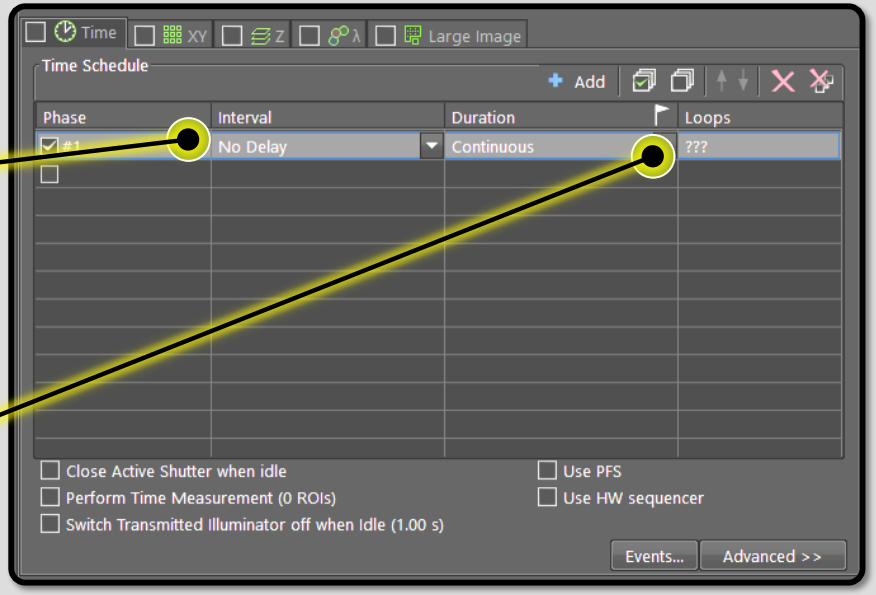
### Interval

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



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# ND Acquisition

## B. Multi XY

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### XY

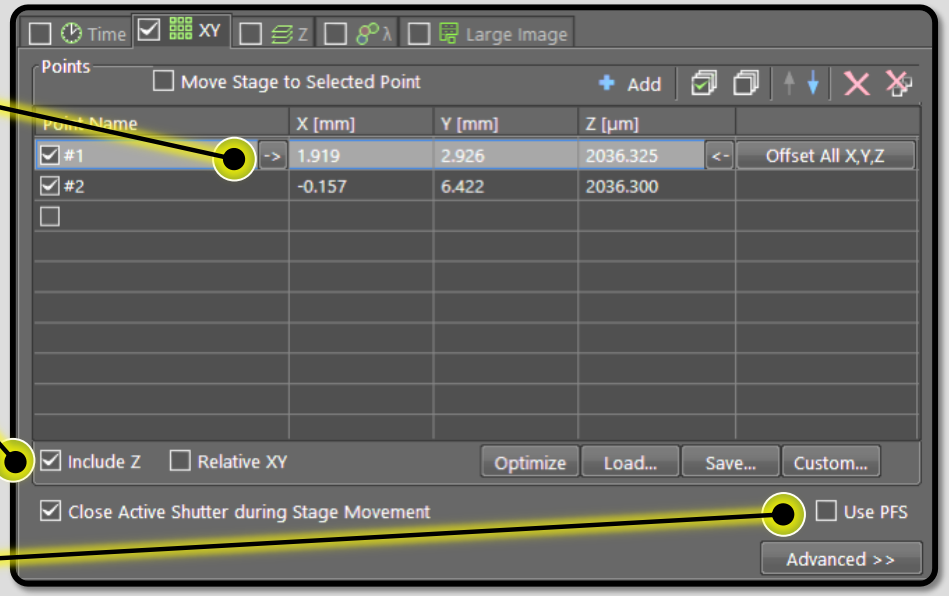
How OFTEN do you want to image
"No Delay" runs as as possible

### 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



1 2 3 4 5 6 7 8 9

# ND Acquisition

## C. Z-Stack

### Stack Type

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

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

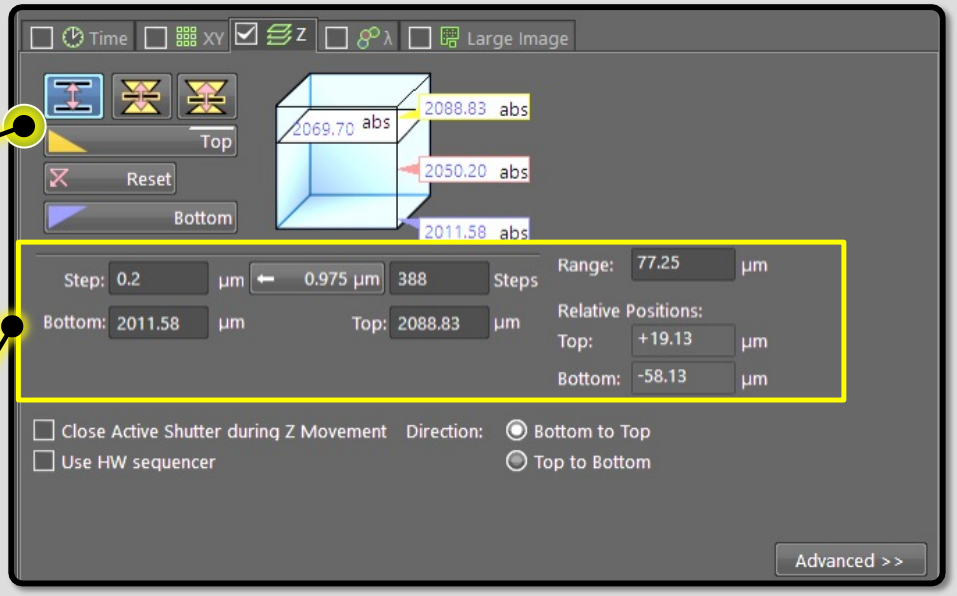
### Stack Parameters

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

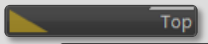
**Stack Range**: Distance between defined TOP and BOTTOM

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

**Limits**: Top and Bottom of Range




## 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 

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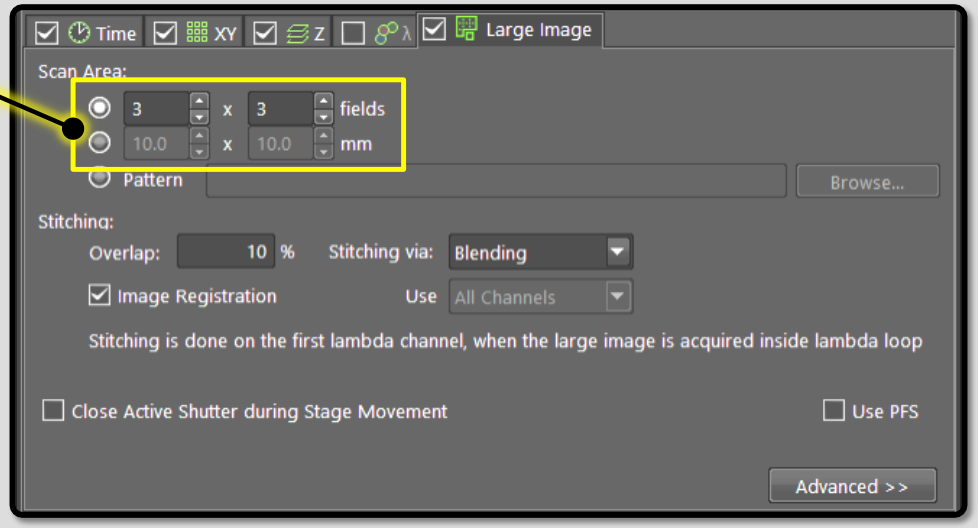
# ND Acquisition

## D. Large Image

**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

Advanced >>

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

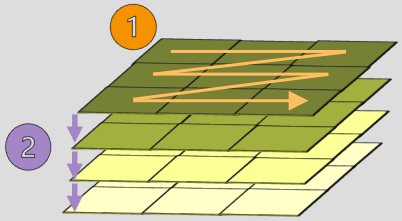
Use PFS

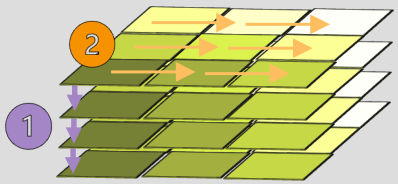
When collecting data that returns to a set location in Z, consider **USE PFS** to maintain a log of focus positions. This can be helpful when stitching over a large area, running a live-cell timelapse, or setting up multiple or recurrent Z-stacks.

Order of Experiment

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

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Advanced Routines

A. Sample Overview

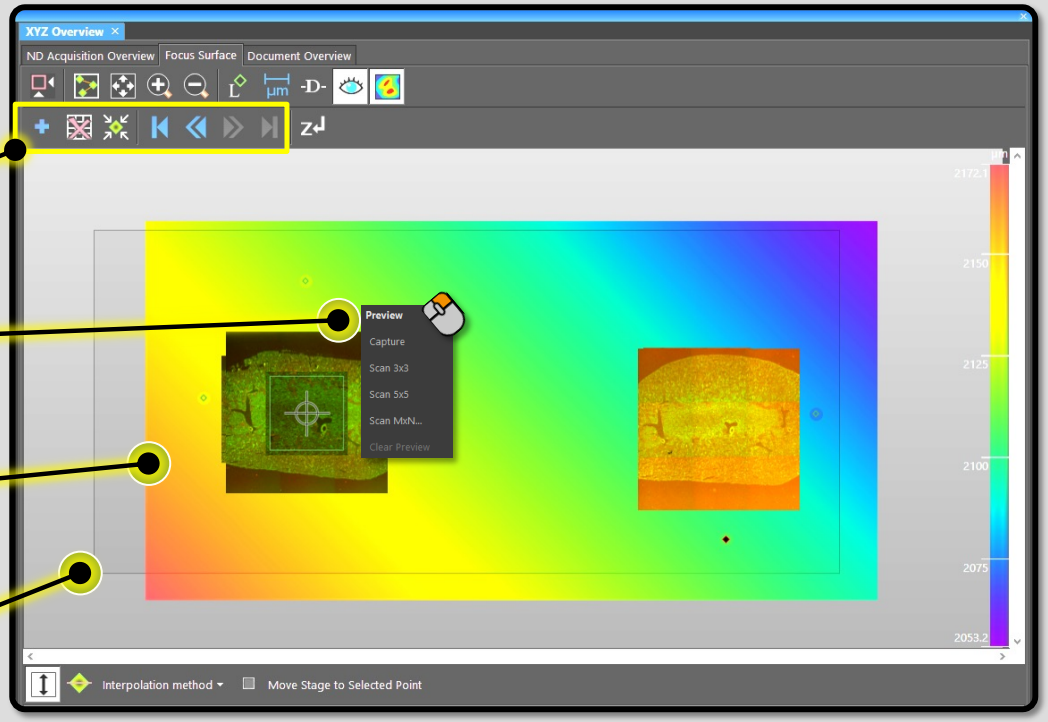
**XYZ OVERVIEW** is a control window that assists in sample navigation. It contains functions for XY point navigation, preview stitching, and can be used to create a virtual plane of focus for complex stitching routines

XY Location Navigation

Quick Preview Stitching

Interpolated Focus Surface

User-defined Sample Area



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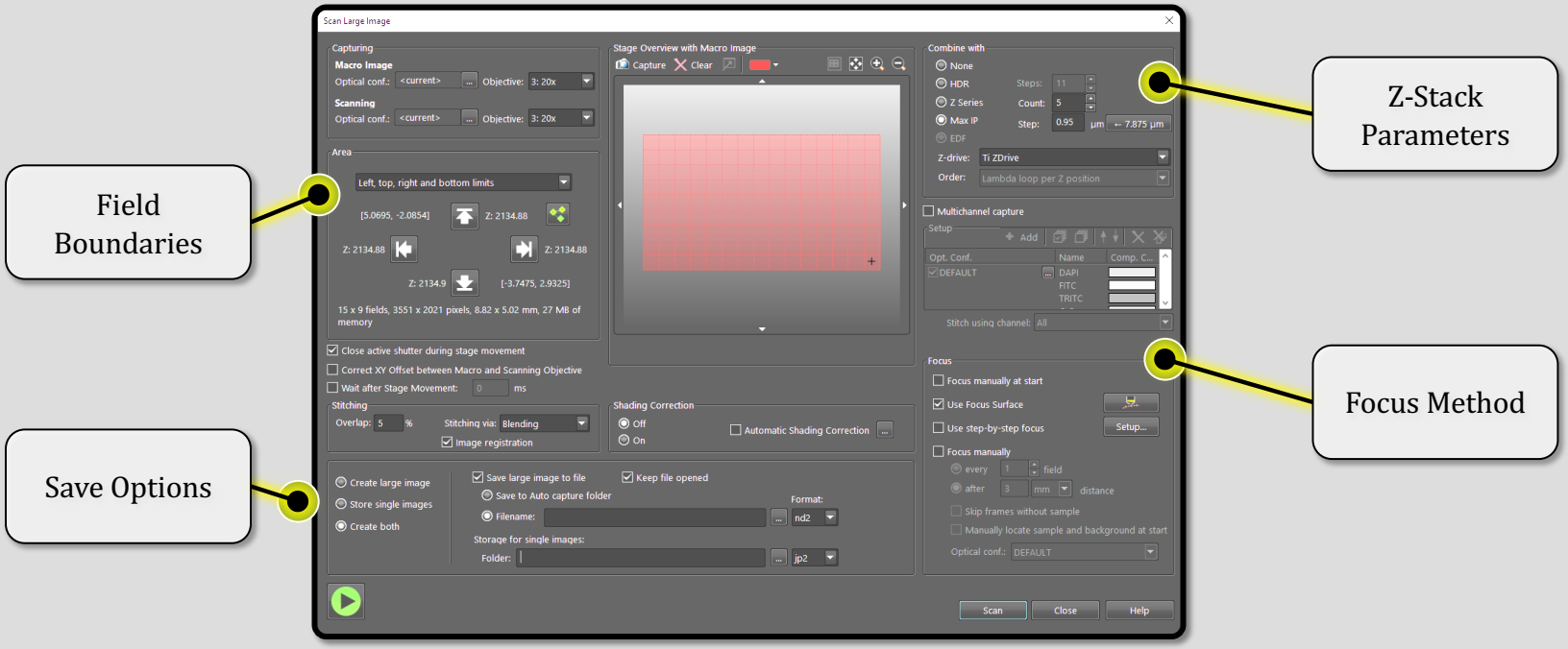
# Advanced Routines

## B. Large Image Stitching

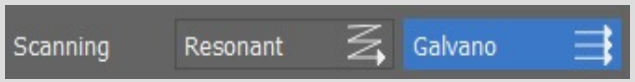
**SCAN LARGE IMAGE** is a control window dedicated to stitching multiple fields together into a single image. Stitched fields can be defined as # of fields (X by Y) or by the boundaries (TOP, LEFT, BOTTOM, RIGHT). This dialogue also allows for the collection of a Z-stack at each position, but unlike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



1 2 3 4 5 6 7 8 9

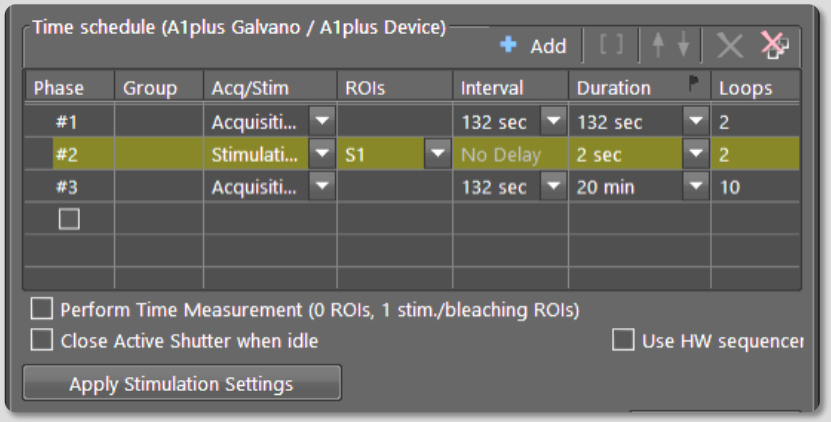
# Advanced Routines

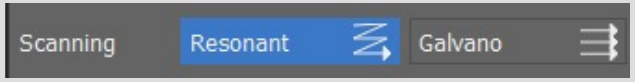
## C. Stimulation/Bleaching: Method Choice




**SEQUENTIAL STIMULATION**.

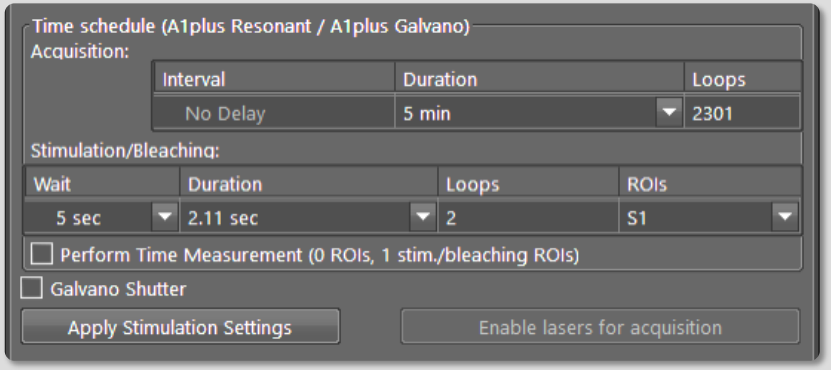
This method defines the conditions for
1) Prestimulation, 2) Stimulation, 3) Poststimulation.




**SIMULTANEOUS STIMULATION**.

This method defines the conditions for
1) Total Experiment Duration, 2) Stimulation Timing



# Advanced Routines

## C. Stimulation/Bleaching: Region Definition

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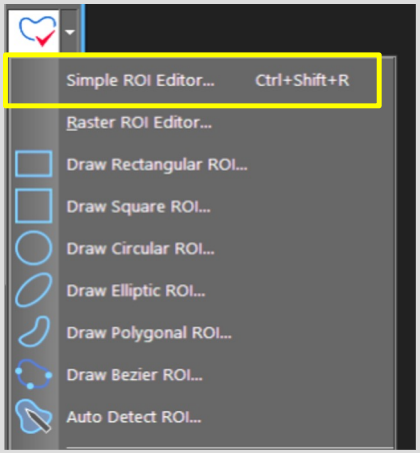
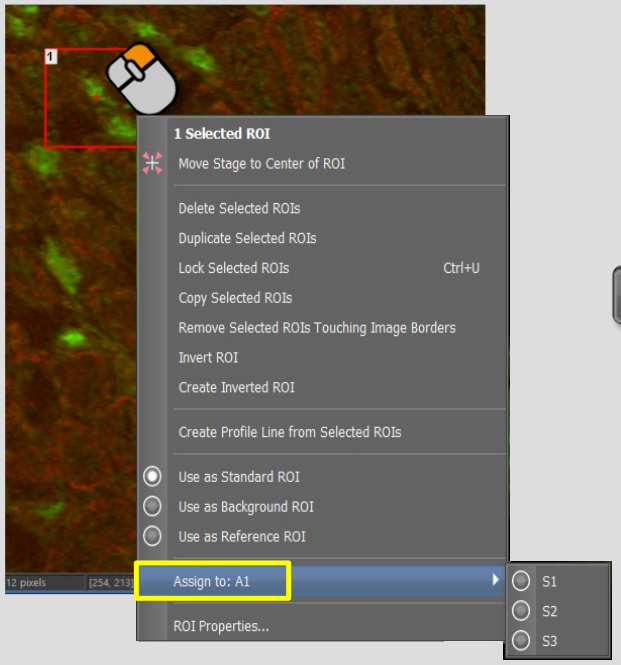
### 1 Create Region of Interest

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*

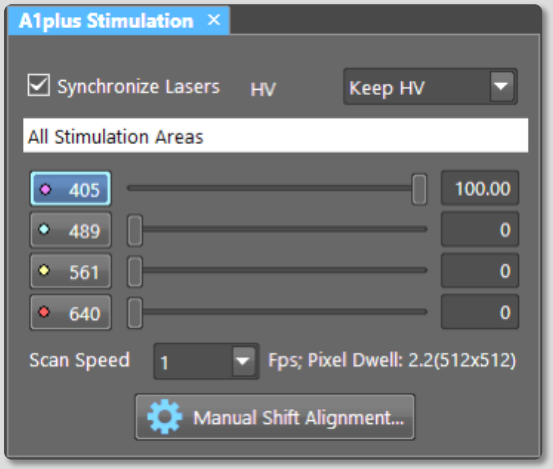
Apply Stimulation Settings

1 2 3 4 5 6 7 8 9

Advanced Routines

A1

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