LEICA SP8 τSTED

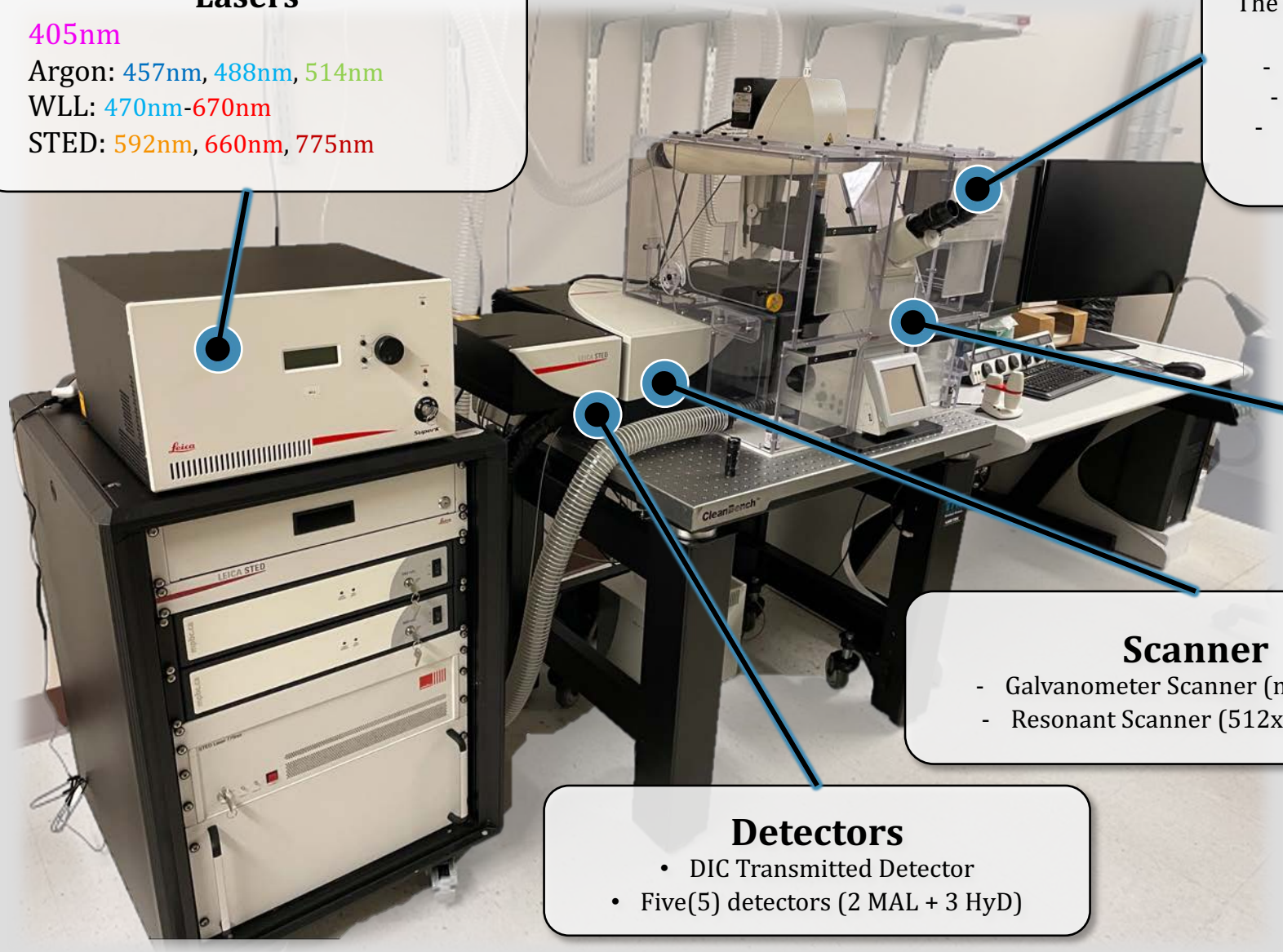
Basic Operation

1. System Basics
2. Software Start
3. Sample Finding and Focus
4. Scanning and Capture
5. Channel Settings
6. Z-Stacking
7. STED Acquisition

System Basics

Lasers

- 405nm
- Argon: 457nm, 488nm, 514nm
- WLL: 470nm-670nm
- STED: 592nm, 660nm, 775nm



Microscope

The SP8 is mounted on an inverted stand, DMi8 from Leica

- Motorized XY stage with encoders
- Motorized Z focus with encoders
- Motorized turret of widefield cubes
- Full incubation enclosure

Optics

HC PL Apo 10x/0.4
HC PL Apo 40x/1.3 (oil)
HC PL Apo 63x/1.4 (oil)
HC PL Apo 93x/1.3 (glycerol)
HC PL Apo 100x/1.4 (oil)

Scanner

- Galvanometer Scanner (max 8k x 8k)
- Resonant Scanner (512x512, 30fps)

Detectors

- DIC Transmitted Detector
- Five(5) detectors (2 MAL + 3 HyD)

Software Start

A. Overview

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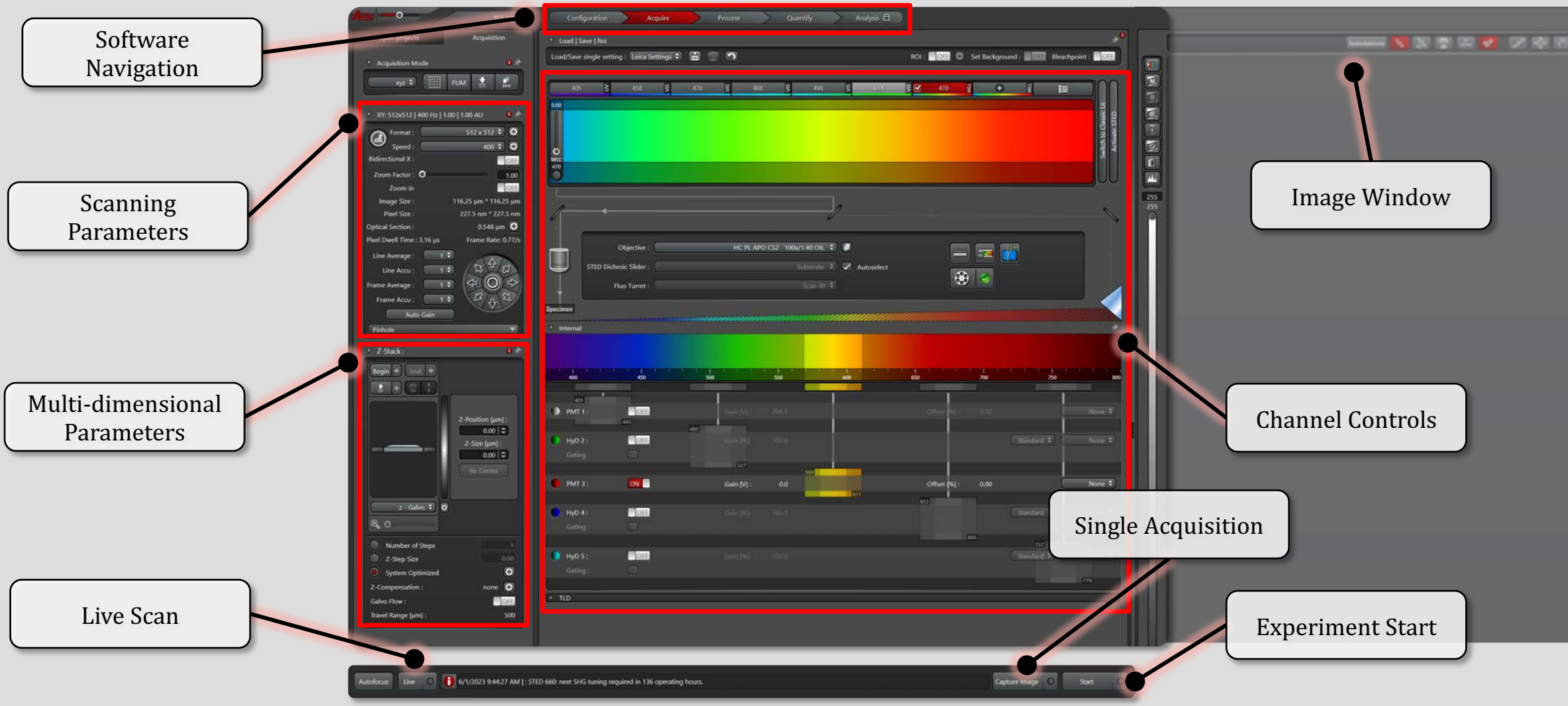
*The Leica SP8 STED runs in **LAS-X software**. The system starts in a standard configuration, but requires a few steps **each time** the system starts.*



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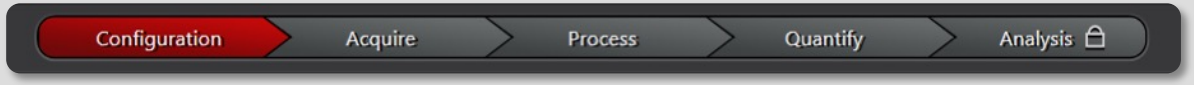
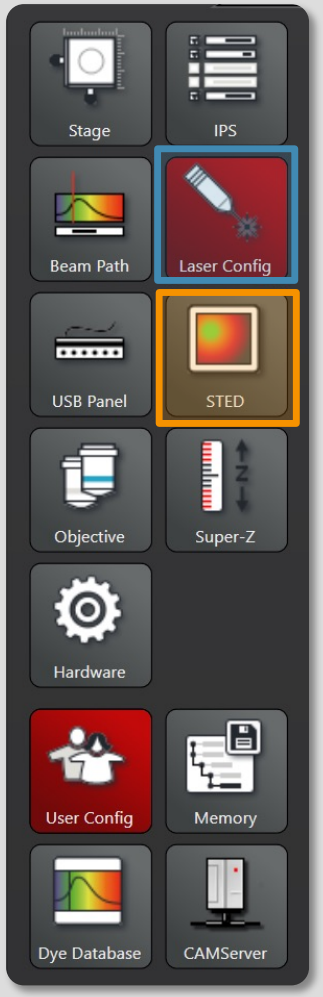
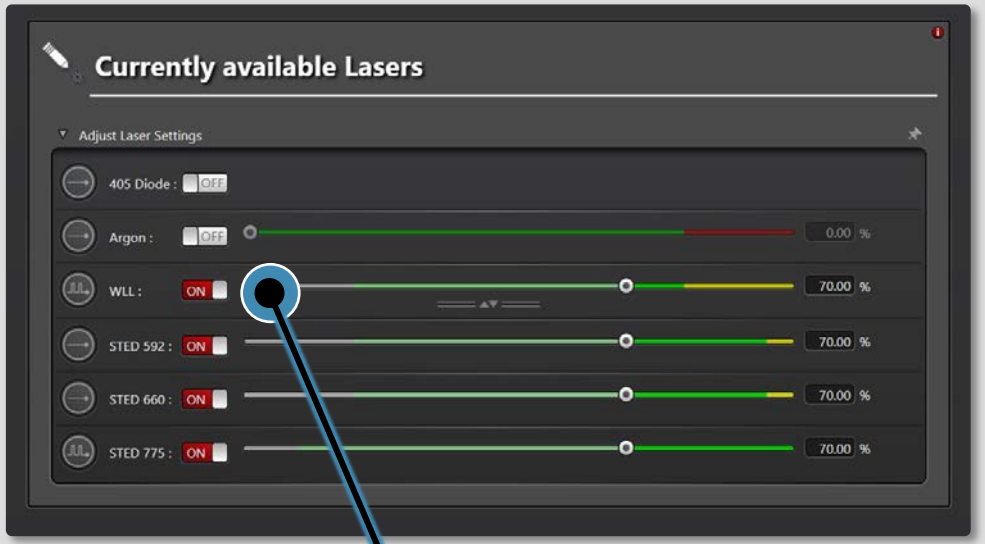
Software Start

B. Initial Steps





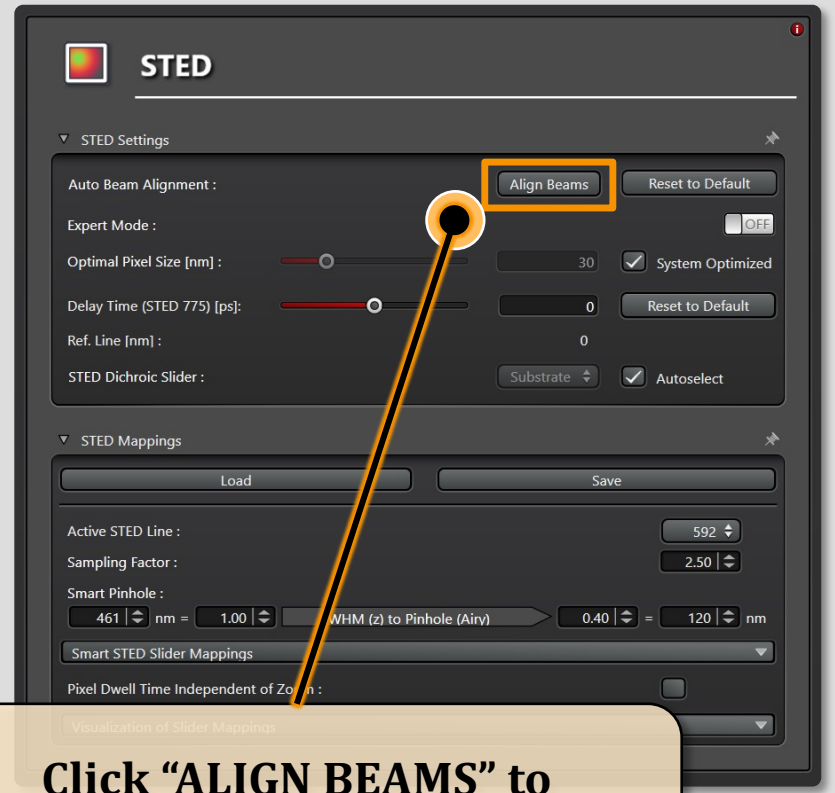
1 Turn on Lasers



WLL: Turn ON and set to 70%

STED Lasers: Turn on required lines and match WLL %

2 Align Beams



Click “ALIGN BEAMS” to calibrate the system for STED

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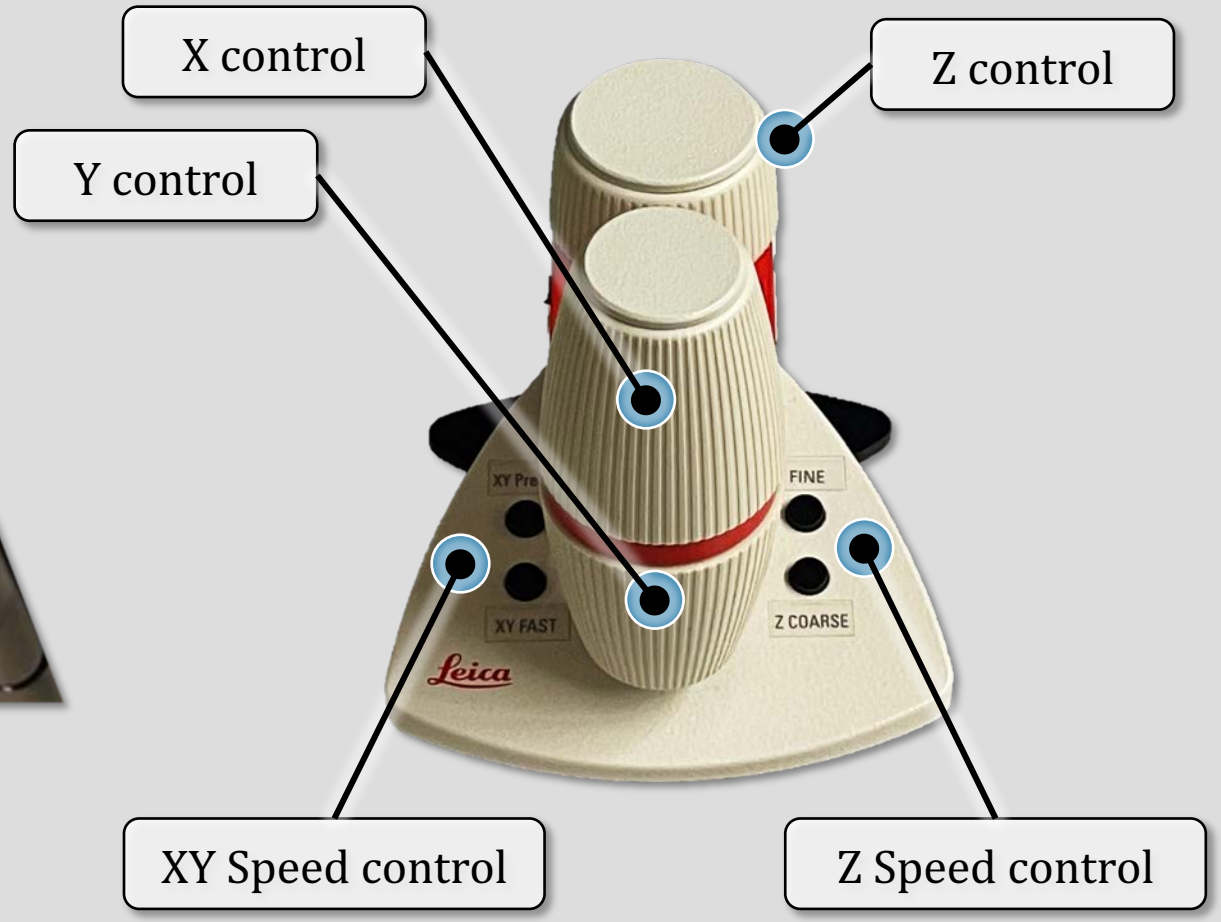
Sample Finding and Focus

A. Stage Placement

The Stage Adapter can accommodate

- Slides
- Multi-well chambers

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



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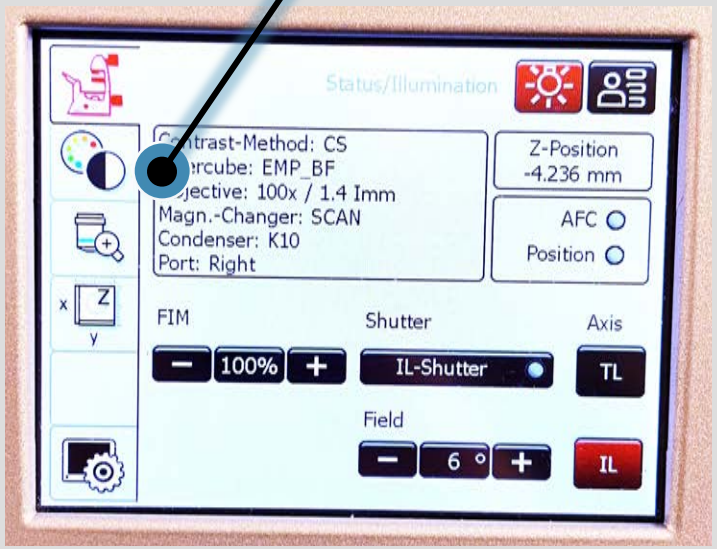
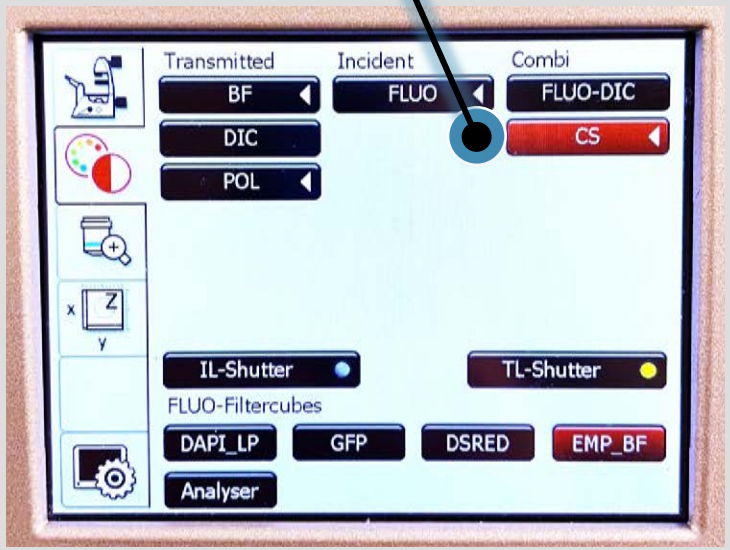
Sample Finding and Focus

B. Eyeport Viewing

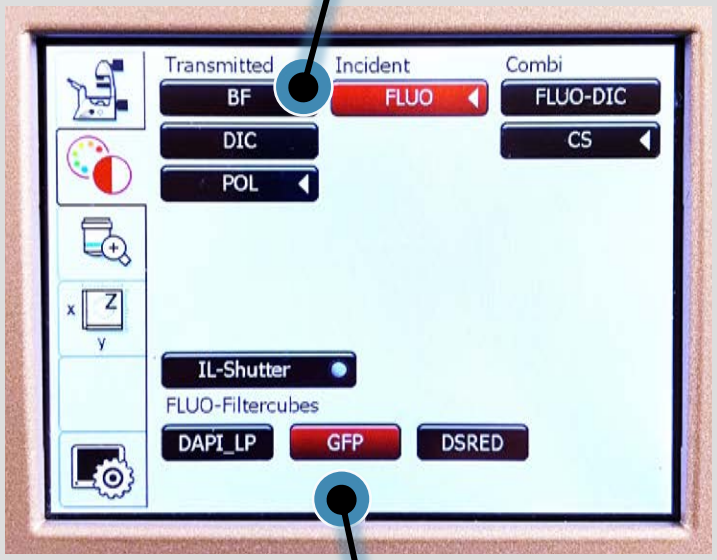
Filters and Lightpath



Confocal Scanning Mode



Eyepiece Widefield Mode



Current Filter Set

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Scanning and Capture

Scan Array Size

Image Data

Average/Accumulate

Collect image multiple time to improve signal to noise

Pinhole Size

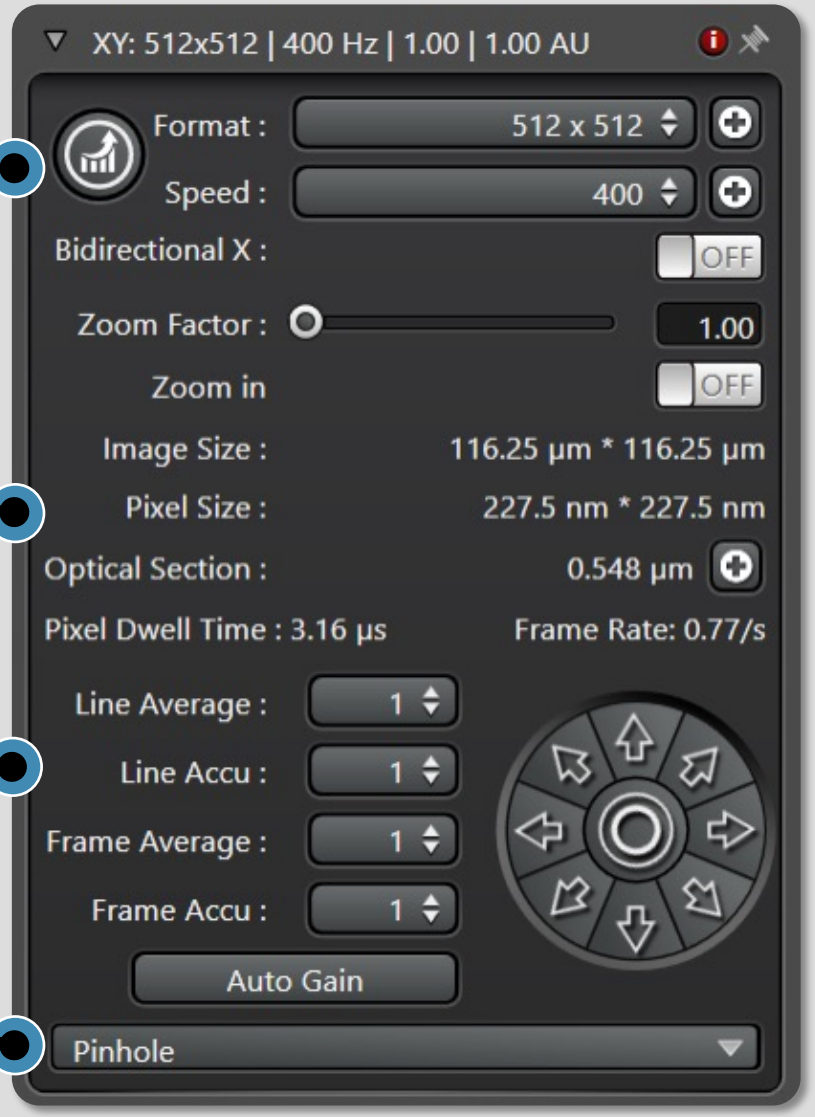
Controls optical section thickness and removes out of focus light

Live/Capture/Start

LIVE: constant scanning for optimization and setup

CAPTURE: collects a single image with the current settings

START: Begin Experiment



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Channel Settings

A. Setup

Configure Channels

Add Channel from Dye List

CHOOSE PMT or HYD

Select Multi-channel Method

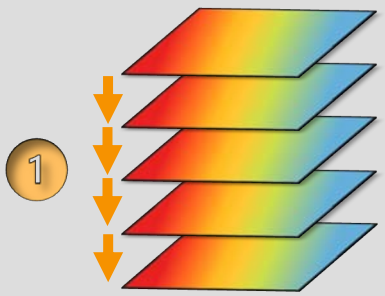
More information about methods on next slide

1 2 3 4 5 6 7

Channel Settings

B. Method

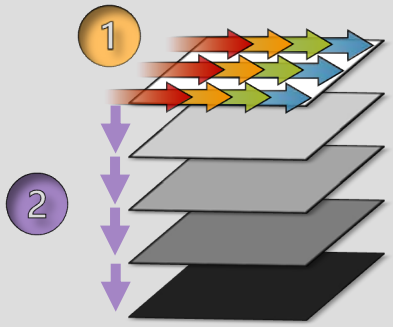
Simultaneous



All channels collected at the same time

- FASTEST METHOD
- HIGH BLEEDTHROUGH
- Incompatible with multi-color STED

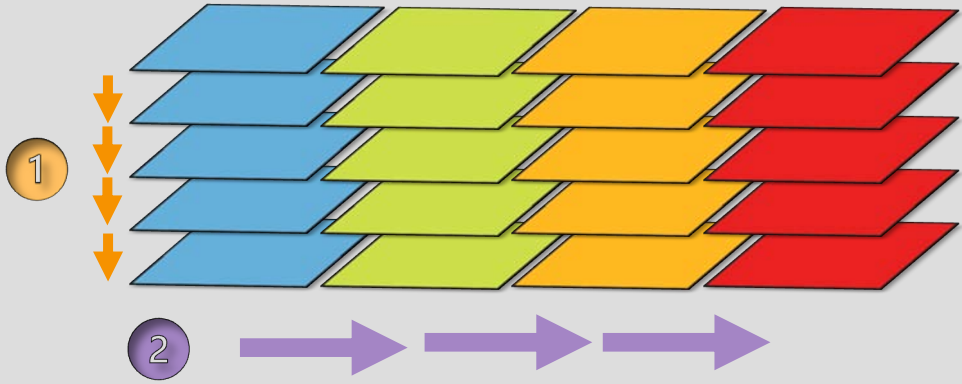
Line Sequential



Each channel collected separately, one line of the image at a time.

- NO BLEEDTHROUGH
- SLOWER SCANNING
- Incompatible with multi-color STED

Frame/Stack Sequential



Each channel collected separately, one frame/stack of the image at a time.

- NO BLEEDTHROUGH
- MULTI-COLOR STED
- SLOWEST SCANNING

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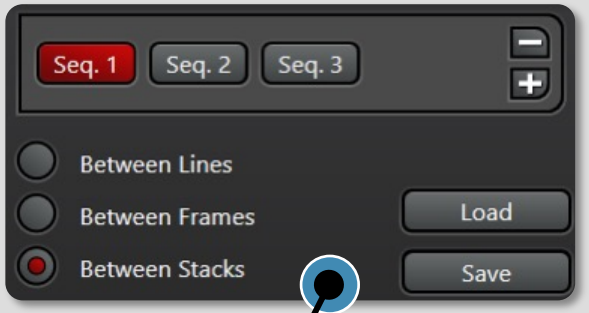
Channel Settings

C. Controls

Active Laser(s) and Power

Active Detector(s) and Gain

*The control windows above show three simultaneous channels being active



Multi-Channel Mode and Channel Selection*

- If a sequential mode is chosen, Select the specific channel for live viewing (e.g. SEQ 1)

Channel Look Up Table Adjustment

Z-Stack Acquisition

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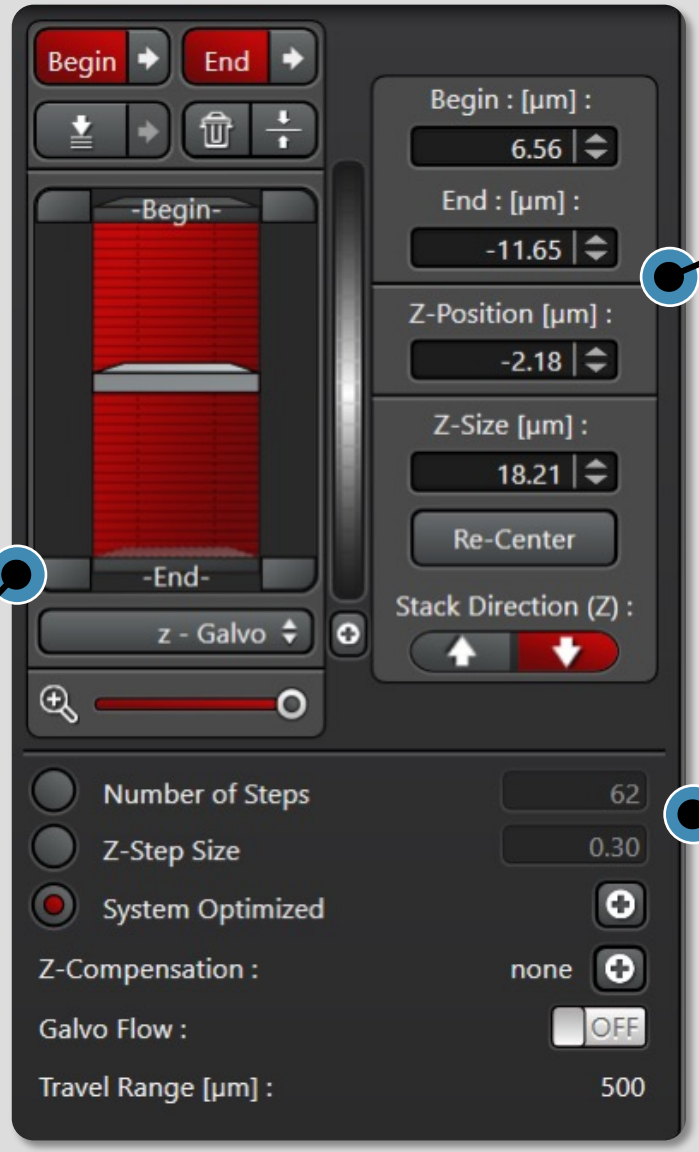
To set stack parameters:

1. Focus to top; click Begin
2. Focus to bottom; click End
3. Confirm step size and adjust if needed



Stack Details

Visual Navigation
Slide to see full stack

Step Size/Number

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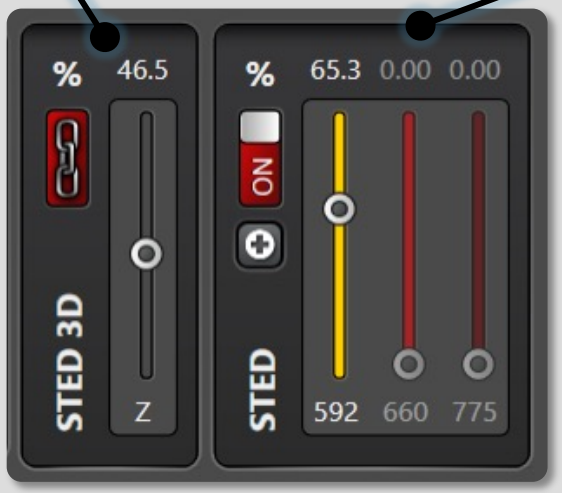
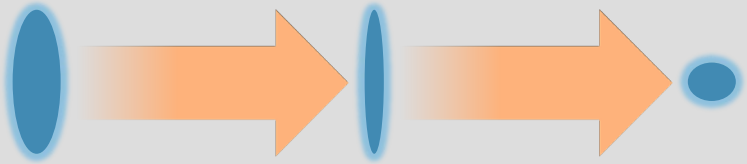
STED

% of Depletion Line diverted to 3D beam shaping

Depletion Line and Power





Confocal

2D STED (high power)

3D STED (100%)

STED Multicolor Tips

1. STED Depletion Lines will photobleach any dyes that emit near that wavelength

   Example: STED 592nm will bleach dyes like ATTO568, and mCherry

2. Optimize depletion power settings in a field of the sample you **DO NOT** plan to image with other wavelengths

3. Collect multiple colors using 

4. Configure your collection oder from furthest RED to furthest BLUE

   Example: Collect AlexaFluor 647 before ATTO568 before GFP