Leica SP8 Resonant Confocal

Quick-Start Guide

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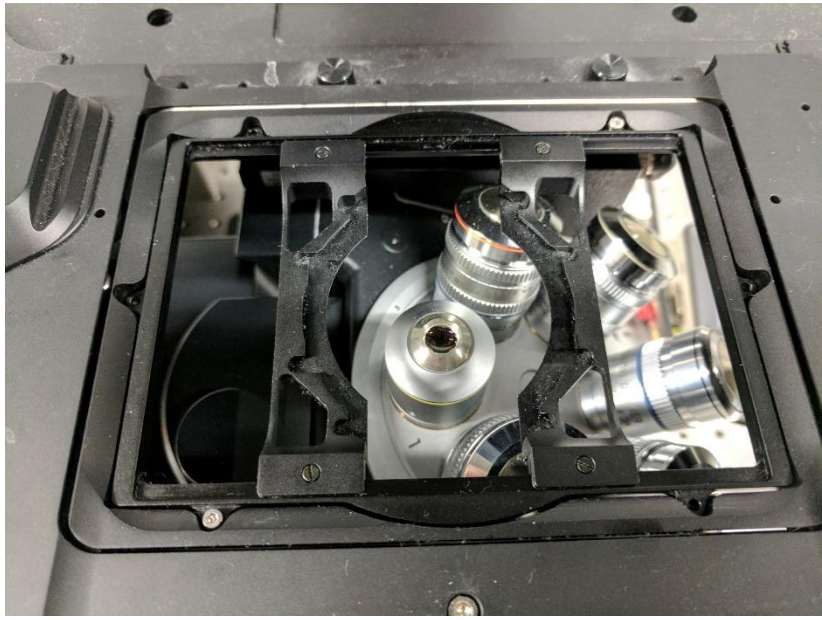
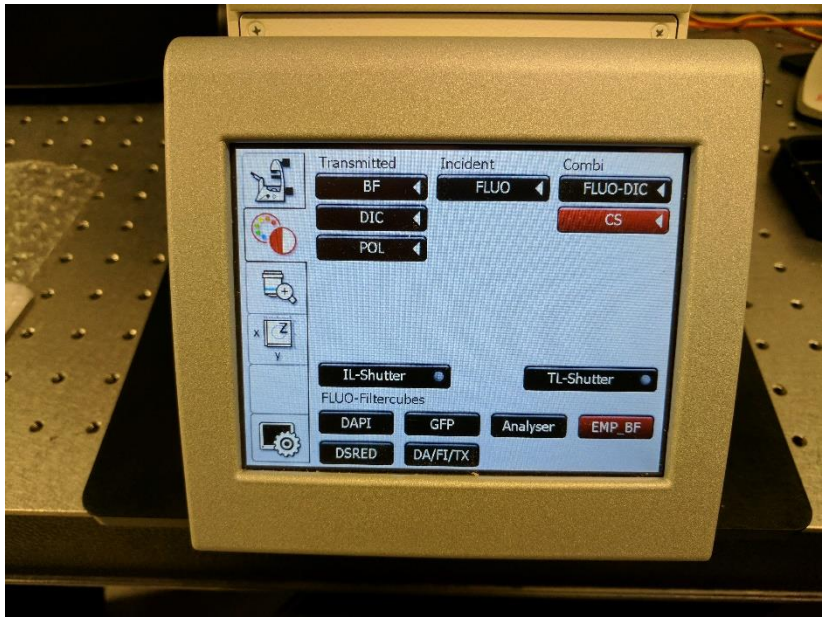
Start-up: Turn on the Microscope / Computer

- Turn on 3 green switches from bottom to top
- Turn key to on position
- Turn on fluorescence excitation source for the eyepieces.
- Log-in using your ADS account name and password.
- For access to the network drive, select Run and then type: \\microscopy-nas1.nri.ucsb.edu
- Open LASX software
  - Choose resonant scanner to be on or off

Part 1: on the scope

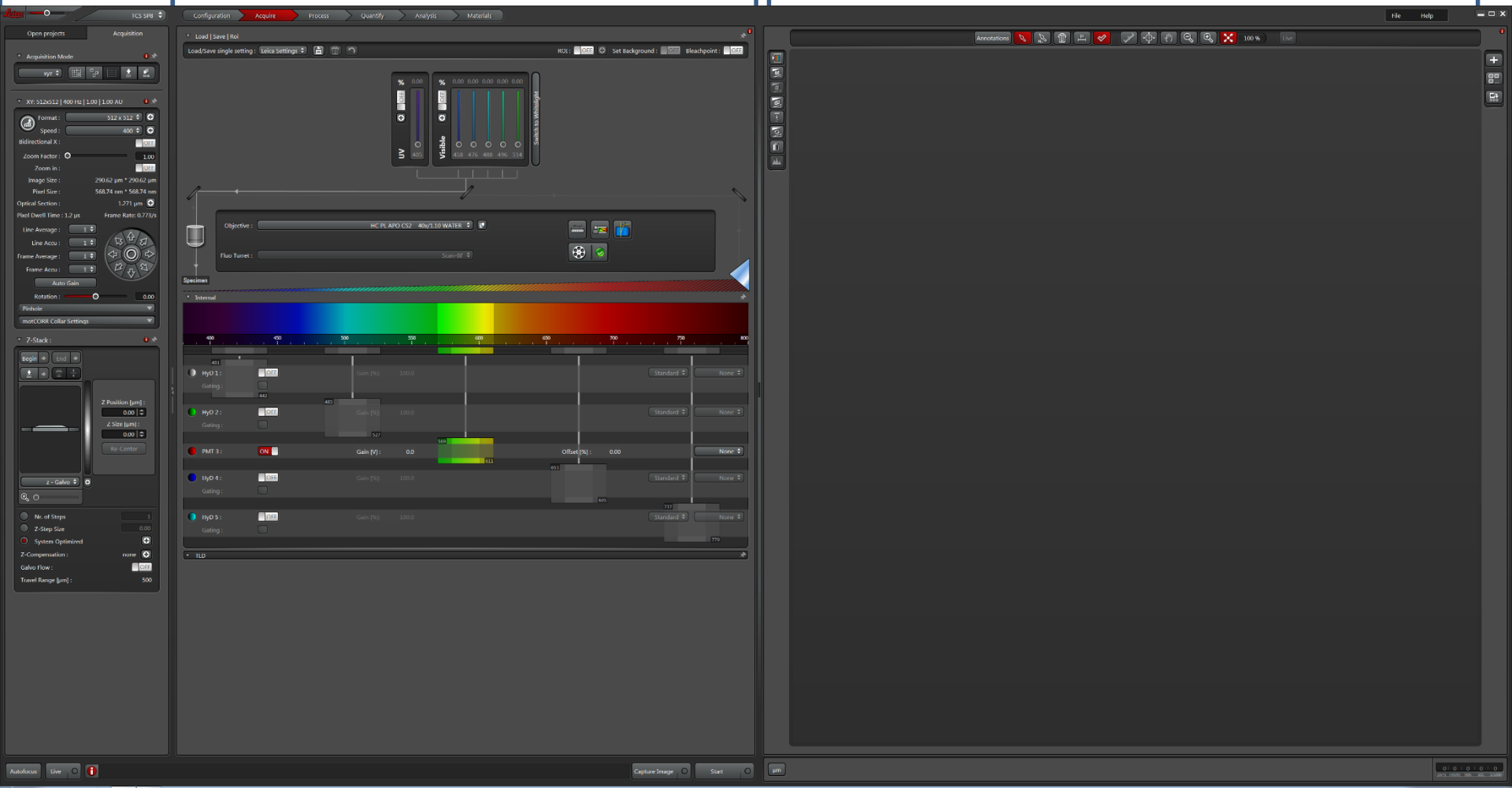


- Lean condenser/bright-field arm back
- Choose objective from LASX software
  - Pay attention to immersion medium
- Place slide or dish in sample holder
- Use scope touchscreen to choose brightfield or fluorescence
  - Be sure to turn off fluorescence shutter when not observing through eyepiece
  - Small knob on left side will adjust fluorescence excitation intensity



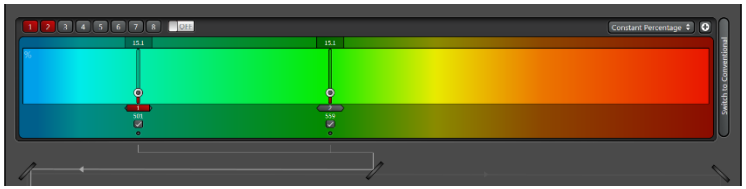
Part 2: Software interface

Acquisition	Excitation and Emission	Image Viewing

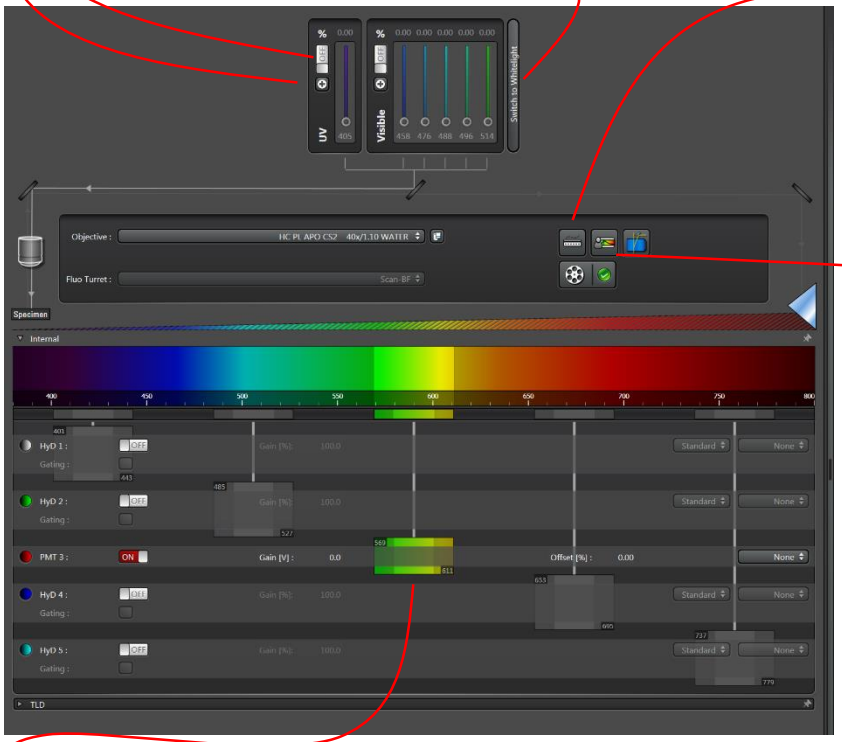


Part 2: Software interface – Excitation Emission

- Click + button to access laser power menu
- Toggle from off to on for each laser
- Visible argon laser is mostly for bleaching
- Click “Switch to Whitelight” to see WLL interface

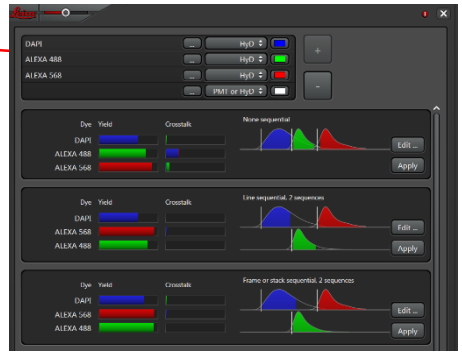


- Activate laser lines by clicking on 1,2,…,8
- Move line to desired wavelength
- Top number is laser % (5 is a good place to start)
- Click checkbox to turn on



You can set which properties the control panel controls, and how coarse or fine the control is

- Dye assistant will set excitation and emission for chosen fluorophores
- Use the crosstalk column to decide if sequential scan is necessary



- For each detector you can set the starting and ending position of the emission window
- The only limitation is that the channel windows have to be arranged in order from left to right, 1 is leftmost, then 2 to the right of 1, etc.

Part 2: Software interface – Scan Settings, Z-stack, timelapse

Scan Settings

- **Format**: number of pixels, drop down menu gives choices, “+” button gives exact control.
- Speed: For resonant it is fixed at 8000. For galvo there are many options, speeds greater than 600 will limit field-of-view.
- **Bidirectional**: doubles scan speed, phase may need adjustment if image appears blurry.
- **Pixel size**: spatial size of pixels. To achieve best resolution zoom or increase pixel count to Nyquist limit. Button to the left of **Format** will set pixel count to Nyquist.
- **Line Average**: If low signal or noisy image increase line average to improve signal/noise. You will almost always need to use this with the resonant scanner.

Z-stack

- Set “**Begin**” and “**End**” to desired positions
- **System optimized**: Nyquist z-spacing
- **Z-Compensation**: adjust excitation or detection gain to compensate for brightness changes with focal depth changes.

Time

- **Minimize**: sets minimum time interval limited by image acquisition time
- Set length of timelapse in number of stacks, duration, or acquire until stopped

Part 2: Software interface – Timelapse, Sequential, Autofocus

Stage

- **Tilescan**: add a few positions with that button, then it will set a rectangular grid to include all of those positions.
- **Mark and Find**: track multiple fields-of-view for timelapses.
- For tilescan choose autostitching and blending method to have immediate stitching of grid of fields-of-view.

Autofocus

- AFC tracks position of coverslip to eliminate z-drift over time.
- "Best focus" searches for most contrast within a certain z-range. Use this for samples that can move over time.

Sequential

- Add sequences to separate excitation and emission for different fluorophores. Use this to eliminate crosstalk.

Part 3: Heat and CO_2 incubation stage

- Turn on switch on Okolab touch screen.
- Turn on switch on Lauda water bath.
- Open CO_2 valve (if needed).
- Set desired temperature and CO_2 percentage.
  - Turn off airflow if CO_2 is not needed .
- Remove z-galvo stage, set aside.
  - Two thumbscrews and one hex screw (red screwdriver).
- Move objective to lowest possible position.
- Slide in incubation stage so that spring contacts are used.

- Remember z-galvo stage is no longer attached so for your z-stack be sure to switch from z-galvo to z-wide.

Part 4: Other options

- HyD 1 is a special cooled detector. It can be used in any circumstance, but should be your first choice for low signal conditions.
- Auto immersion dispenser is available for long timelapses with the 40x water objective.
- Live Data Mode is available for automating more complicated tasks involving several different imaging jobs.
- Hyvolution is the deconvolution software. It can be run in automatic mode or manual mode. Manual mode will require some training.

Shut-Down Procedure

- Check the online schedule
  - Shut-down if nobody is scheduled for the rest of the day.
  - Leave the system on if somebody is using the system todaybut do the following.
    - Log-off the computer
    - Remove sample
    - Wipe immersion oil off of objectives with lens paper
    - Return to the 10x objective
- Adjust your online reservation end-time if you finished early or late
- Shut off the computer
- Turn off key, then each switch from top to bottom
- Turn off eyepiece excitation light.
- Put dust cover over microscope

Specifications

- 5 objectives installed
  - 10x/0.3 air
  - 20x/0.75 Multi-Immersion
  - 40x/1.1 water motorized correction collar
  - 63x/1.3 glycerol
  - 63x/1.4 oil
  - Available on request: 5x/0.15 air and 40x/1.3 oil
- 3 laser sources
  - UV: 405 nm
  - Argon:
  - White Light Laser: 470-670 nm
- 4 HyD and 1 PMT detectors
  - HyD 1 is cooled for better low signal detection