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



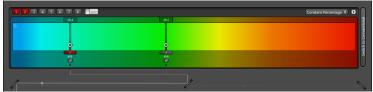


Part 2: Software interface

Acquisition	Excitation and Emission	Image Viewing
Create Projects Create		
Autolocus Live O 🚺	Capture Image O Start O	

Part 2: Software interface – Excitation Emission

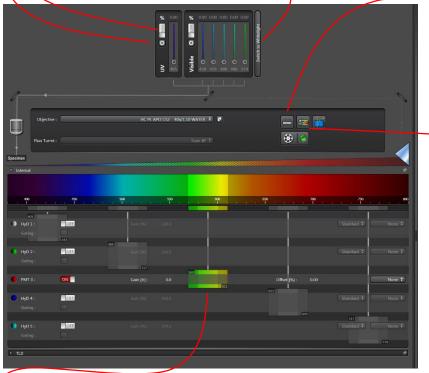
- Click + button to access laser power menu
- Toggle from off to on for each laser
- Visible argon laser is mostly for bleaching
- Click "Switch to Whitelight" to see WLL interface



- 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)

USB Control Panel

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

- <u>Format</u>: 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.
- <u>**Bidirectional**</u>: doubles scan speed, phase may need adjustment if image appears blurry.
- <u>Pixel size</u>: spatial size of pixels. To achieve best resolution zoom or increase pixel count to Nyquist limit. Button to the left of <u>Format</u> will set pixel count to Nyquist.
- <u>Line Average</u>: 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 "<u>Begin</u>" and "<u>End</u>" to desired positions
- <u>System optimized</u>: Nyquist z-spacing
- <u>Z-Compensation</u>: 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

Format:	512 x 512 🗘 🕀
Speed :	400 🗘 🛇
Bidirectional X :	OFF
Zoom Factor : O	(a)
Zoom in :	- OH
Image Size :	290.62 µm * 290.62 µm
Pixel Size :	568.74 nm * 568.74 nm
Optical Section :	1.271 μm 💽 μs Frame Rate: 0.773/
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5126512 | 400 Hz | 1.00 | 1.00 AU 🛛 🕕 🕅

Part 2: Software interface – Timelapse, Sequential, Autofocus

Stage

- <u>Tilescan</u>: 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.

	▼ Stage 🚯 🖈
	X: 57.27 \$ Y: 36.84 \$ [mm]
	0.0 25.0 50.0 75.0 100.0 125
	9.9
	121
	9 8 9
	510
	89
	Zoom : O 1 x 🔀 🛃
	Positions :
	Calibration
	Field size :
	Merge Images : ON
	Auto Stitching ON
	Overlap blending Smooth \$
	Linear blending ON
	Advanced
	▼ Autofocus: AFC + Best Focus ●
1	
	Focus-System : AFC + Best Focus \$
	AFC Settings Timelapse Stage
	AFC Pos, Objective: 10x
	AFC On/Off
	Operation mode
	Continuous mode
	On demand mode
	Use experiment
	Hold plane in Z-stacks
	Store AFC Pos Recall AFC Pos
h	
	▼ Sequential Scan 0 ∓
	Seq. 1
	Between Lines
	ž —
	Between Frames Load
	ž –

Part 3: Heat and CO₂ incubation stage

- Turn on switch on Okolab touch screen.
- Turn on switch on Lauda water bath.
- Open CO₂ valve (if needed).
- Set desired temperature and CO₂ percentage.
 - Turn off airflow if CO₂ 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 zstack 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