

Leica SP8 Confocal Quick Start Guide



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➔ These are procedures provided by Leica Microsystems that describe how to operate the instrument in its most simplest format.

➔ The Leica SP8 in the Keck lab has additional HyD detectors, resonant scanner, live cell imaging mode and light sheet. You must have additional training to use the advanced features on this system.

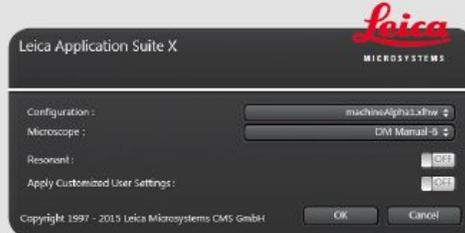
➔ Live cell imaging or light sheet mode that exceeds a 4-hour duration must be conducted at off peak hours. Notify Keck lab staff if you intend to use the live cell imaging or light sheet mode so the system can be modified for your use at least 24hrs in advance of use.

System Startup



For system configuration with FSU:
Turn on the power supply for the PC **1**, the scanner **2** and the lasers **3** by pushing the green buttons and turn the laser key **4**, to open the laser shutter.

Software Start Up



Start LAS X by double-clicking the LAS X icon.



Make sure there is the correct selection for **Configuration** and **Microscope**. Turn on **Apply Customized User Settings** to choose **User Settings** saved in a preceding session. Turn off **Apply Customized User Settings** to start LAS X with the default settings. Click **OK** and follow the instructions (i.e. **Initialize Stage**).

***for general use select machine.xlhw**

Laser Start Up

Go to **Configuration.** → **1** Activate lasers (**ON**)
2 Use the slider to set the laser power of the Argon laser (458 nm–514 nm) to 20–30 %.

- Our system also has the white light laser option. Laser power is set to ~1% when combined with HyD detectors.

Image Acquisition

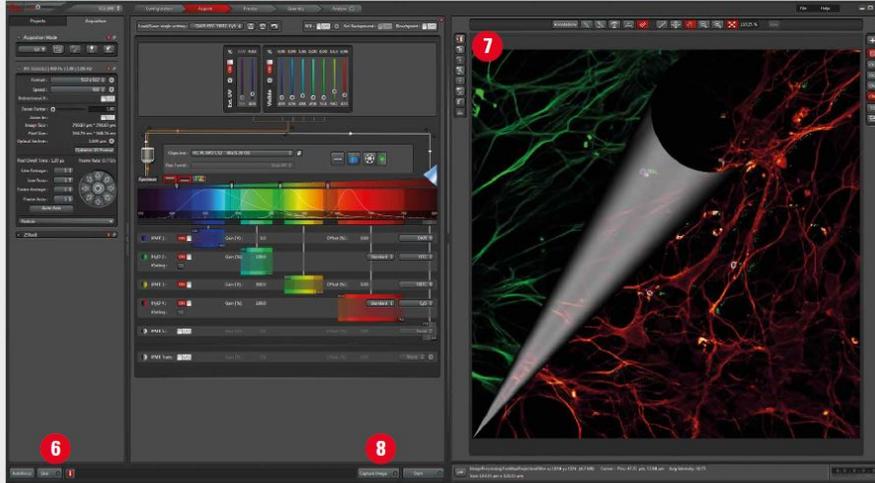
Go to **Acquire**

1 Choose a **single setting** from the list (i.e. FITC/TRITC) and continue with step 6.
 Steps 2 to 5 are automatically set.

If no appropriate **single setting** is available, proceed manually with steps **2** to **5**:

2 Activate lasers (**ON**).
3 Set excitation laser line(s).
4 Activate PMT/HyD (**ON**), define **Gain** and **Offset**.
5 Position PMT slider at maximum emission.

*Dye assistant is also very helpful for creating an optimal configuration for scanning.

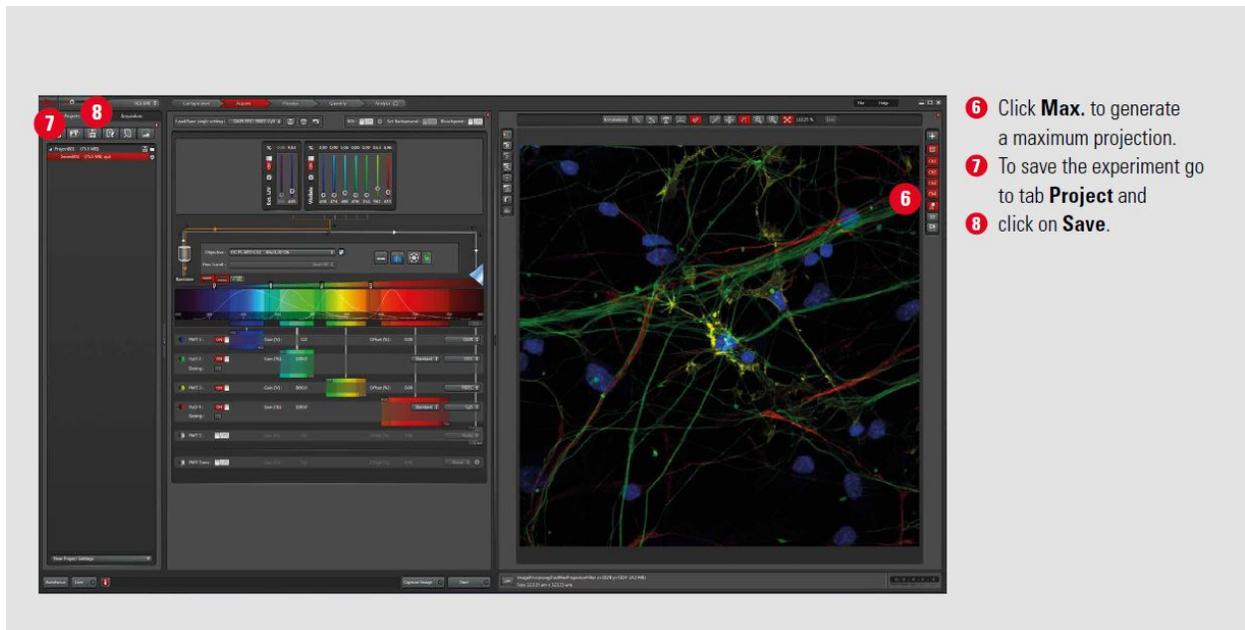


- 6 Start **Live scan***.
- 7 Click **QLUT** to switch to Glow. Change **Z Position** by turning the respective wheel on the control panel until you get the brightest signal (focal plane).
- 8 Optimize image settings manually by adjusting steps 3 to 5, until the image contains a few blue (saturated) pixels. Go back to original **LUT 7** by clicking twice.
- 8 **Capture Image***. **Save Project** (see below, Z-Series Acquisition).

Z-Series Acquisition



- 1 Open **Z-Stack** Dialog.
- 2 Set **Focal Plane**.
- 3 Set **Begin-** and **End-Position**.
- 4 Click **System Optimized** to define the number of slices.
- 5 Click **Start** to acquire the z-series*.



- 6 Click **Max.** to generate a maximum projection.
- 7 To save the experiment go to tab **Project** and
- 8 click on **Save.**

- Prior to shut down, check the asu.corefacilities.org iLab schedule to confirm there are no users following you. If someone is scheduled within 2 hours of your completion time, leave the system and lasers running in standby mode.

System Shut Down

Follow exactly the sequence described to shut down the system:

1. Deactivate all lasers in the laser configuration window (see Laser Start Up section), wait approx. 5 min. until ventilation of Argon laser has shut-off.
2. Close software.
3. Shut down PC.
4. Shut down CSU or FSU, respectively, in reverse order (see **System Start Up** section).