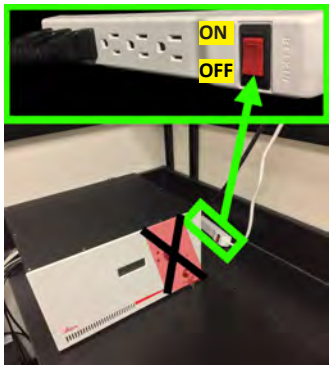
QUICK START - LEICA SP8 FALCON

MICROSYSTEMS

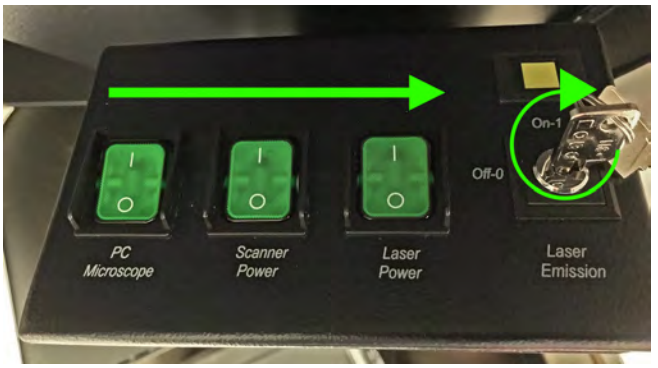
START UP

1. ***Carefully*** remove dust cover from microscope. **DO NOT LOAD YOUR SAMPLES YET.**
2. Check to see if table is correctly floated (contact staff if not)
3. Turn on instrument as follows:



If not already on, turn on red switch on power bar located behind laser box.
DO NOT TOUCH LASER CONTROL BOX

Turn on Sola Light Engine for widefield fluorescence imaging (Located on the floor between tables)



Turn on the microscope, scanner and lasers using the panel located ***under the workstation*** desk begining with the switch on the left and ending with key on the right.

3. If necessary, turn on Workstation PC on the floor to the right of desk.
4. If you are running a **FLIM** experiment or are using the **440 nm laser**, see next page for additional instructions, otherwise continue reading.
5. Turn on software by double-click the LAS X icon
6. When prompted for Configuration and Microscope, ensure that **machine.xlhw** and **DMI8** are selected. If using the resonant scanner turn **ON** the Resonant switch. Click **OK**.
7. *Wait for system to initialize completely before setting up sample on the stage.*

6. If using the 25x lens, first select the 10x lens before going back to the 25x OR go to **Configuration > Light Path** and select **Optics 2**

SHUT DOWN

1. Save your projects and transfer your data to an external drive. **THE PC HARD DRIVE WILL BE CLEANED EVERY 2 WEEKS - NO EXCEPTIONS**
2. Close the software. WAIT FOR SHUT DOWN SEQUENCE TO COMPLETE. Shut down the PC.
3. Shut down the system in *reverse order* as the startup above i.e. Laser Emission > Laser Power > Scanner Power > PC Microscope > Sola Light Engine. Leave Power bar switch **ON** next to laser control box. If using the 440 nm laser and/or Stage-top Incubator, turn off power bar under monitor (next page).
4. Clean objectives used using the supplied optical lens tissues (**NOT Kimwipes**). If using oil lens, apply small amount of alcohol to tissues to clean.

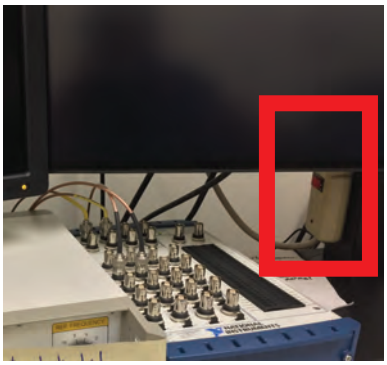
CAUTION! Laser light in objective plane from now on. User manual/instructions must be strictly observed.

440 nm Pulsed Laser

440 nm Pulsed Laser START UP

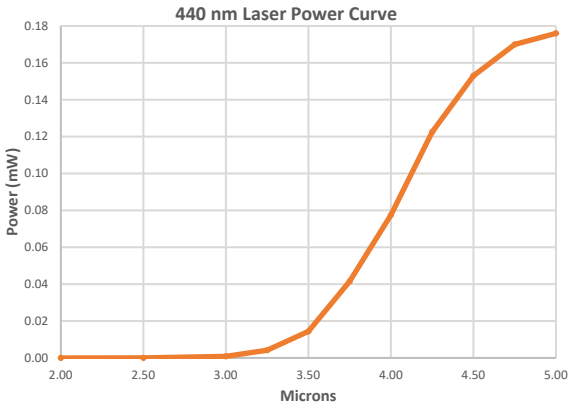
1. Turn on the red power switch located under second monitor shown in the **red** rectangle. This will also turn on power to the Tokai Hit Stage-top incubator

2. Turn key (shown in **green** rectangle) to the ON position on the PDL 800-D laser driver.
DO NOT TOUCH ANY OTHER DIALS OR BUTTONS.



3. Additional steps are required to enable the laser in the software before use (See next Page 3).

4. This 440 nm laser power is controlled using the Mitutoyo micrometer shown in the **blue** circle above. The power increases non-linearly between 2 - 5 microns as shown in the chart to the right. Power readings are approximate and dependent on alignment.



440 nm Pulsed Laser SHUTDOWN

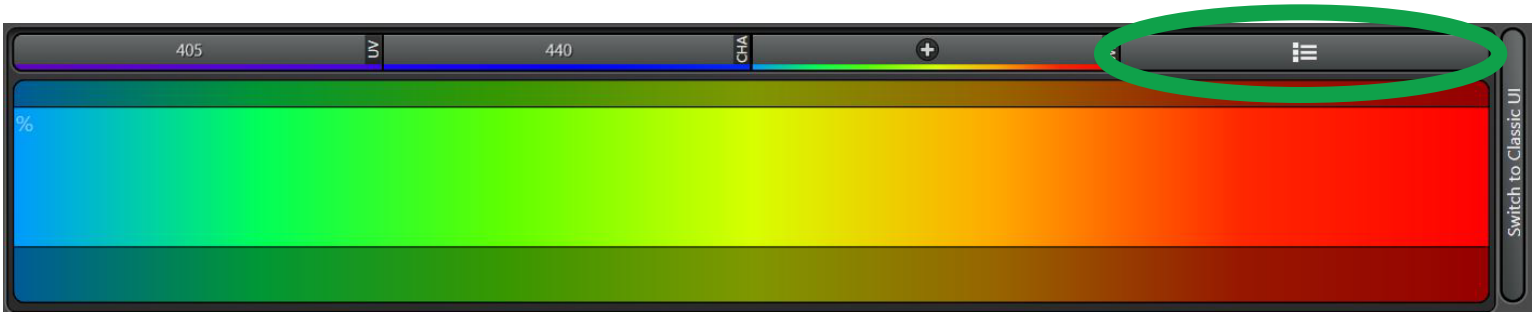
1. Return the Mitutoyo micrometer (**blue** circle above) to the ‘0 microns’ position.

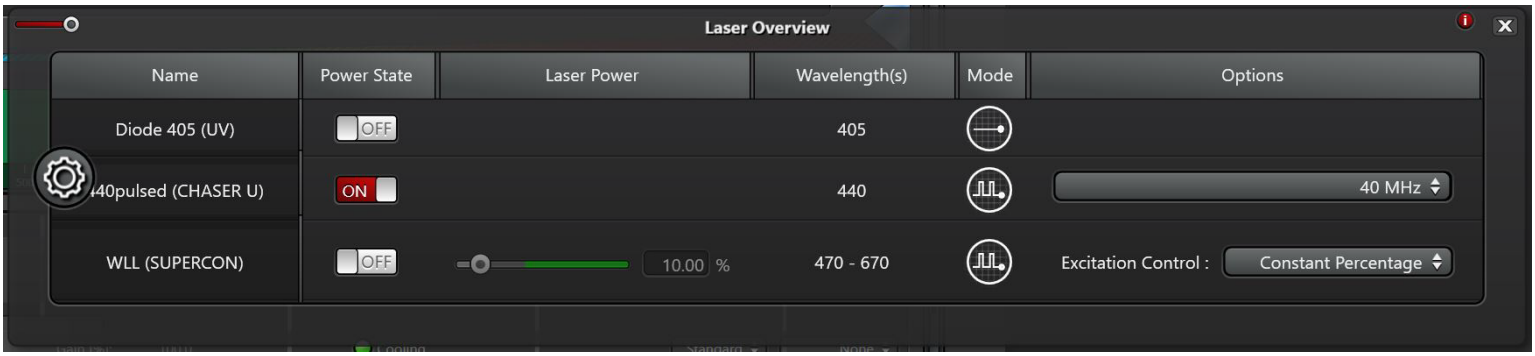
2. Turn key (shown in **green** rectangle) to the STANDBY position on the PDL 800-D laser driver.
DO NOT TOUCH any other dials or buttons.

How to use equipped lasers

This microscope is equipped with three types of lasers: a **405** nm CW laser, a **440** nm pulsed laser and a tuneable (**470 - 670** nm) white light laser.

1. To begin, open the Laser Overview options shown in the **green** circle. Turn on the lasers you wish to use under the 'Power State' column. Note: The 440 nm laser requires a separate startup sequence described in the previous page. DO NOT change any other settings. Close (x) window when done.





2. To add the 405 or 440 nm laser to your selection, click the respective buttons in the top row of the laser controls shown in the **red** rectangle below. To add (up to 8) lines from the white light laser, click the ⊕ symbol. Laser power for the 405nm and white light lasers can be adjusted by dragging the slider, using the mouse scroll wheel (with cursor positioned over slider) or double clicking the % value and entering a value between 0.01 - 100. To use the 440 nm laser, drag slider to max and use external micrometer to adjust power (instructions on previous Page 2).

3. Before using the laser, the shutter needs to be opened. Open the shutter by click the button on the respective laser (grey is shutter closed, red is shutter open). For the white light laser lines, a check box in the top row must also be ticked before use.

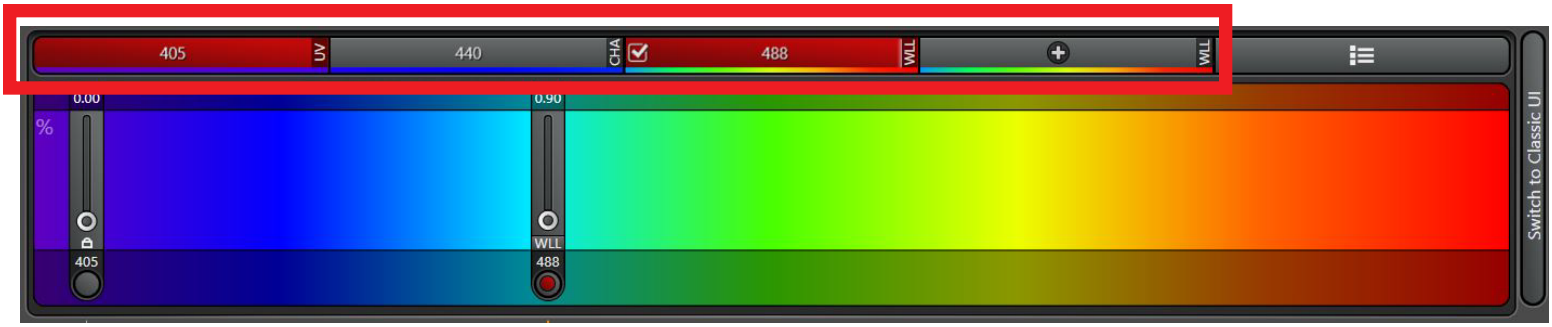
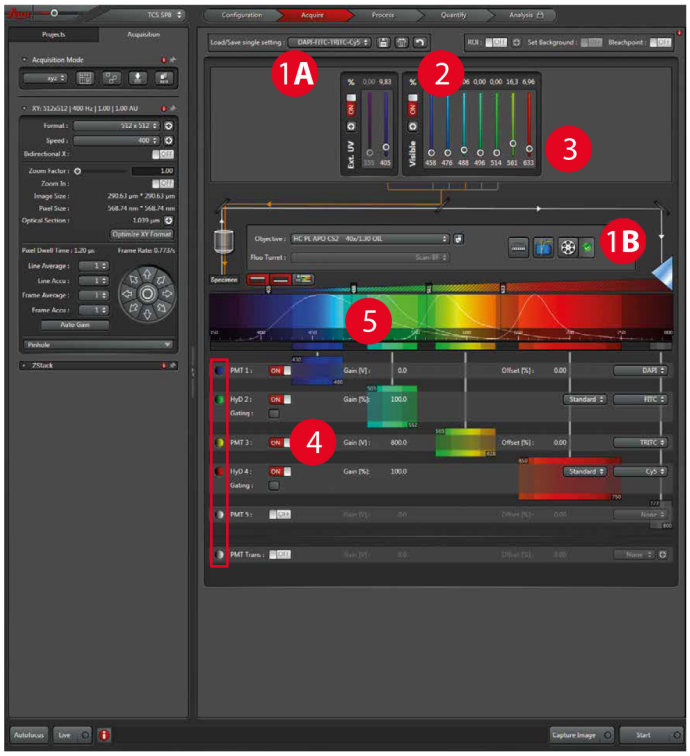


Image Acquisition: **single plane**

* 8-bit / 12-bit / 16-bit images: Go to Configuration/Hardware/Resolution



1 **A)** Choose dye from settings list & Continue with step 6
Steps 2 to 5 are automatically set.

B) Choose dies in the Dye Finder, Apply & Continue with step 6
Steps 2 to 5 are automatically set.

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

2 Activate lasers (ON).
3 Set excitation laser line(s) to 0.01%.
4 Activate PMT/HyD (ON) detector(s) and define:
Gain: PMT 700-800 V; HyD 10-100%
Offset: 0.00
5 Position PMT/HyD slider(s) at maximum emission,
AT LEAST 10 nm away from laser line(s)!

* Change channel pseudo color by clicking color LUTs
* Average: increases SNR, decreases photobleaching/toxicity
* Accumulation: use with HyD, increases signal by adding all passes
* Live: use line accumulation & frame average

6 StartLive scan*.
7 Click QLUT to switch to Glow (blue shows saturated pixels).
Change Z position with the control knob until you get the brightest signal (focal plane).

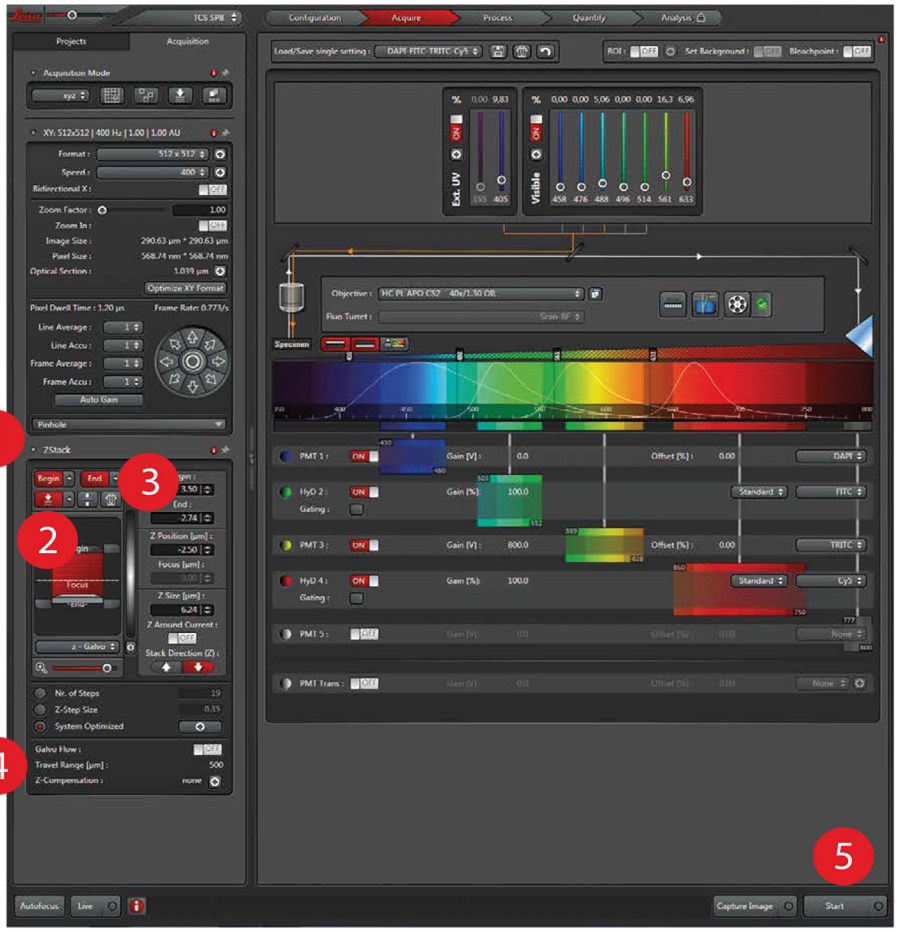
Optimize image by adjusting steps 3 to 5, until image contains a few blue (saturated) pixels.
*scan speed (pixel dwell) & pinhole size ("confocality") can also be adjusted
Go back to original color LUT 7 by clicking twice.

8 Capture Image *. Save Project (see Z-Series Acquisition).

*Scan Zoom: adjust if full FOV is not required or for Nyquist sampling (can also adjust scan format up to 8096x8096)
zoomed ROI can be moved with or use area in annotation and Zoom In (ON)

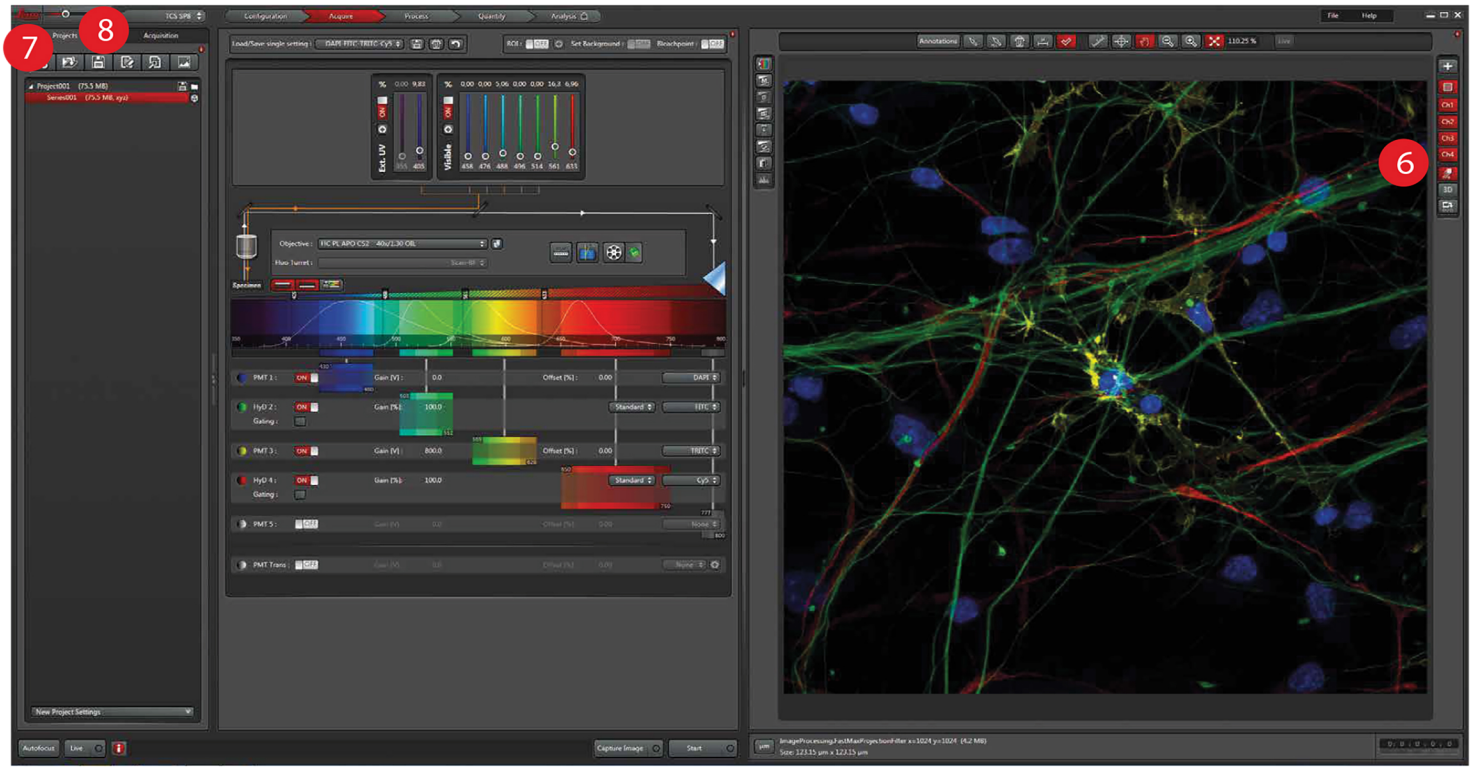
* **NYQUIST sampling for DECONVOLUTION**: adjust scan zoom & scan format (pixel at least < 2 x optical res) OR use optimize button.

Image Acquisition: **Z-Series**



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. ClickStart to acquire the z-series*.

* Z-Compensation:
adjust laser intensity with depth



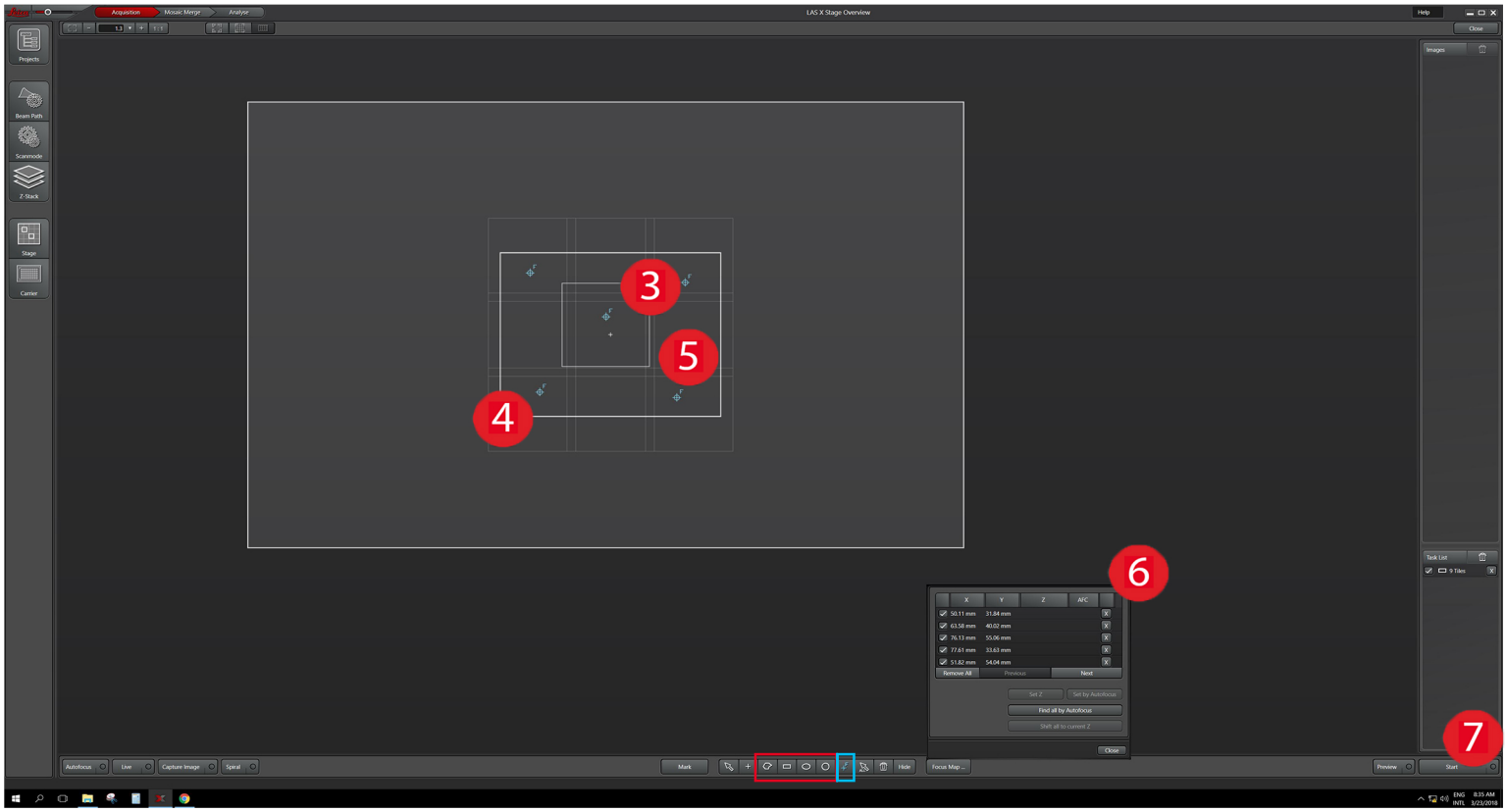
6. Click Max to generate a Maximum Intensity Projection (MIP) or 3D for volume rendering

To save: Go to Project tab 7 and click on Save 8 or right-click Save as (**please save on E drive**)

To use previous imaging parameters: right-click on Export parameters

Image Acquisition: **Tiling via Stage Overview**

1. Select Stage Overview.
   Zoom out by scrolling with mouse.
2. Select appropriate carrier if needed.



3. Locate sample by double-clicking or using Live
   !CAUTION! double-clicking moves the stage
4. Draw your ROI using Tools.
5. Define focus point positions.
6. Define focus map:
   Set all focus points by autofocus or focus manually for each point using Live and Set Z
7. Click Start to acquire*.

***All defined Channels / Z-stacks will be acquired! To change, adjust with Beam Path and/or Z-Stack icons**