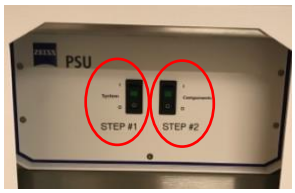
Zeiss LSM 800 Laser Scanning Confocal Microscope SOP

Preparation

1. Be sure to bring the excitation and emissions for your dyes you are using.
2. Turn off lights in the back only

START THE MICROSCOPE

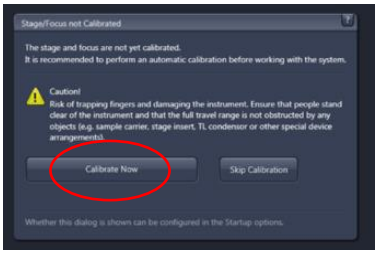


1. Turn on scope by flipping the STEP 1 (System) switch, then, STEP 2 (Components) switch.
    a. Note, to turn off, this must be done in the opposite order, turn off step 2, then step 1.

START THE ZEN SOFTWARE

1. Log into computer using your FOM ID and password
2. Once on, open the ZEN 2.0 program on desktop

3. Click “ZEN system” to access the confocal



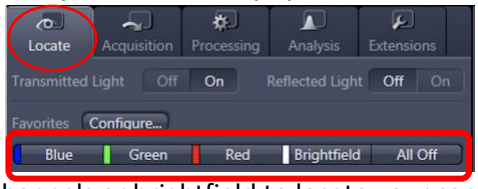
4. A “stage focus” dialog box will open, and asking you to calibrate the slide. Hit “calibrate now”

LOAD SAMPLE

1. Once calibration is done, open all four doors
2. Gently tilt and lift the scope head up
3. Load your sample on the stage of the microscope
    a. Note: If the stage is not secure, sure the stage is securely in place with the stage springs in the bottom left corner

LOCATE SAMPLE

1. The locate tab will allow you to use the eye piece and fluorescent channels to find your sample.



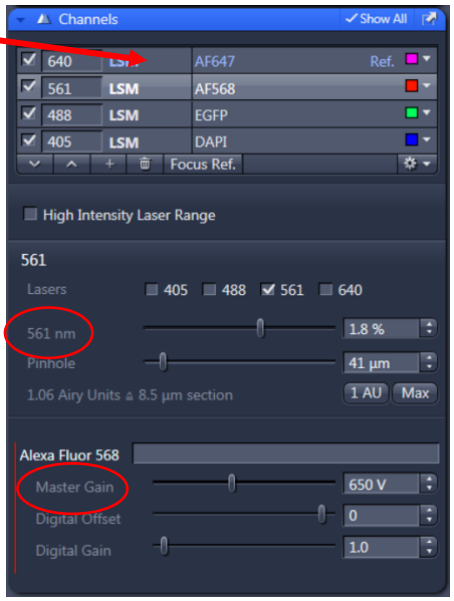
2. Use the florescent channels or brightfield to locate your sample
    a. Note: The focusing depth is larger with fluorescent microscopy; if you cannot find your sample, you can refer to this "Locate" tab if you cannot find your sample in the "Accusation" confocal-mode.

CONFIGURE SETTINGS

1. Use the "Accusation" tab to begin the confocal laser imaging
2. Use the first dropdown to set the experiment type
    a. Usually you can use the "**Confocal-DJL_405_488_555_640(frame)"**
3. Select or deselect the setting/experiments you will be using such as "tile" and Z-stack"
4. Set the channels you are using to stimulate your dye in the "channels" dropdown
5. Set your emission in the "imaging setup"

PREPARATORY IMAGE

1. Press Live to view your sample
2. Increase the laser intensity (example in image bellow: 561 nm) and master gain to around 75% of the bar
3. Adjust the <u>fine focus</u> to your sample to the camera, make the sample as bright as possible
    a. Focus is usually very bright and overexposed when in focus with the parameters listed above
4. Readjust the laser intensity and master gain for all lasers
    a. Toggle through the different lasers by clicking the here
        i. each laser will save intensity and gain value you set
    b. Use “range finder” if necessary
5. **Snap** (at top) to take an image



Changing Magnification

1. Make sure you are switching to a dry lens or else removing your sample and applying the right solution
2. Use the fine focus to create the brightest image
    a. If you cannot find your sample, go to the locate tab and manually find the sample with the fluorescent channels.
3. Adjust the laser intensity mand master gain
    a. Use “range finder” if necessary

Turn off

1. Set focus back to 5x
2. Lower the objectives till the lowest z has been reached
3. Turn off the scope by flipping the step 2 switch, then, step 1.