NeuroTechnology Studio
Zeiss LSM 880
User Manual

For questions or concerns contact:
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To Enter Room: Press Code 2-4 (at same time), then 3.

START-UP

NEVER TOUCH the key (***). The key remains in the horizontal position.

DO NOT TOUCH ANY BUTTON/SWITCH UNLESS IT IS SPECIFIED BELOW.
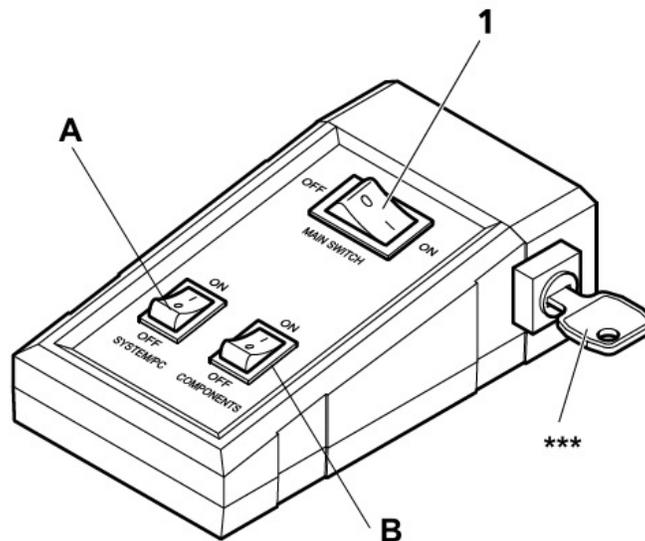
1. Turn on the Main Switch (1). It is located at the top of the rack left to the air-table.
2. Next, turn on switches A and B (SYSTEM/PC and COMPONENTS). Wait for 15 seconds between each switch turn-on.
3. Now turn on the X-Cite LED lamp with the black switch (below). The X-Cite is located at the top of the computer.

4. If Computer is not on, turn on the computer.
5. Log in to your lab account on the computer. Use the lab account and password you were given during your training. Remember to log off when you are finished with your session.
6. Next, open the software

Note that the ZEN software can only be opened after following all the prior steps

    a. Click on ZEN BLACK Acquisition.


    b. A dialog window will pop up. Click Start System.

    c. As the hardware configures, you will hear the microscope parts starting to activate. This is normal. If any error message shows up, take a picture and notify Lai Ding or Teri Zgoda immediately.
7. You are now ready to image! Please save the files to D: drive "User Image" folder and the corresponding lab folder.

***Before changing objectives, press <u>LOAD SAMPLE</u> on the control pad touch-screen (located at the right side of the computer table). This will lower the objectives and prevent accident! ***

Opening Zen

1. Once initialized, choose what objective you want using the microscope display screen and place your slide on the stage
2. Navigate to the ACQUISITION tab and click the SHOW ALL TOOLS box outlined in red below. Turn on the lasers if they are not already on. Pressing the "laser properties" dropdown will show the status of the lasers. Wait a few minutes for the lasers to reach full capacity.
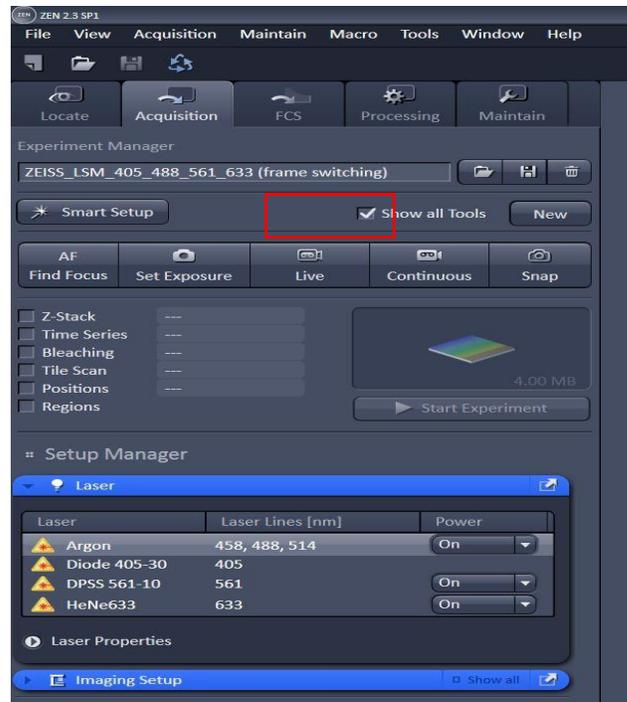
3. Use the LOCATE tab to view using the eyepieces.
   - Press the X-Cite Dial to illuminate
   - The incubation chamber can be set up in this tab.

Channel Set up

Load preset settings. LSM_405_488_561_633 (frame switching) = basic 4 channel non-Airyscan

Select any of these boxes to bring up a corresponding window

IMAGING SET UP WINDOW

- o Press the dropdown to select your dye. The corresponding excitation graph will appear above. Edit the horizontal scroll bar to cover the peak of the line.
- o Select the TPMT box to use transmitted light (brightfield)

Acquisition Mode Window

- o Change your objective here.
- o Frame size: higher number = better resolution, but longer scan speed
- o Speed: slower speeds = better signal quality, higher chance of photobleaching
- o Averaging: smooth images
- o Scan area: Can 'zoom in' on a certain area

Channels Window

- o Create or delete a channel using the + and - buttons
- o Pick what laser you want to use
- o Adjust laser power: higher intensity = higher chance of photobleaching
- o Keep pinhole at 1 AU (airy unit)
- o Adjust gain (brightness): higher gain = stronger signal and more noise

Live Mode

CHANNEL WINDOW
- o Check the box for what channel you wish to view. If you have multiple checked, the system will scan each one continuously and make a composite.
- o The channel that is highlighted will be the editable channel
- o Adjust laser power and gain for each channel

1. Once in live mode, find focus of sample. The focus plane of scanning will be slightly different from the focus plane by eyepiece viewing.

2. The Range Indicator button, circled in red below, can help find focus and determine exposure. The red pixels in the image indicate signal saturation, and the blue pixels indicates zero intensity.
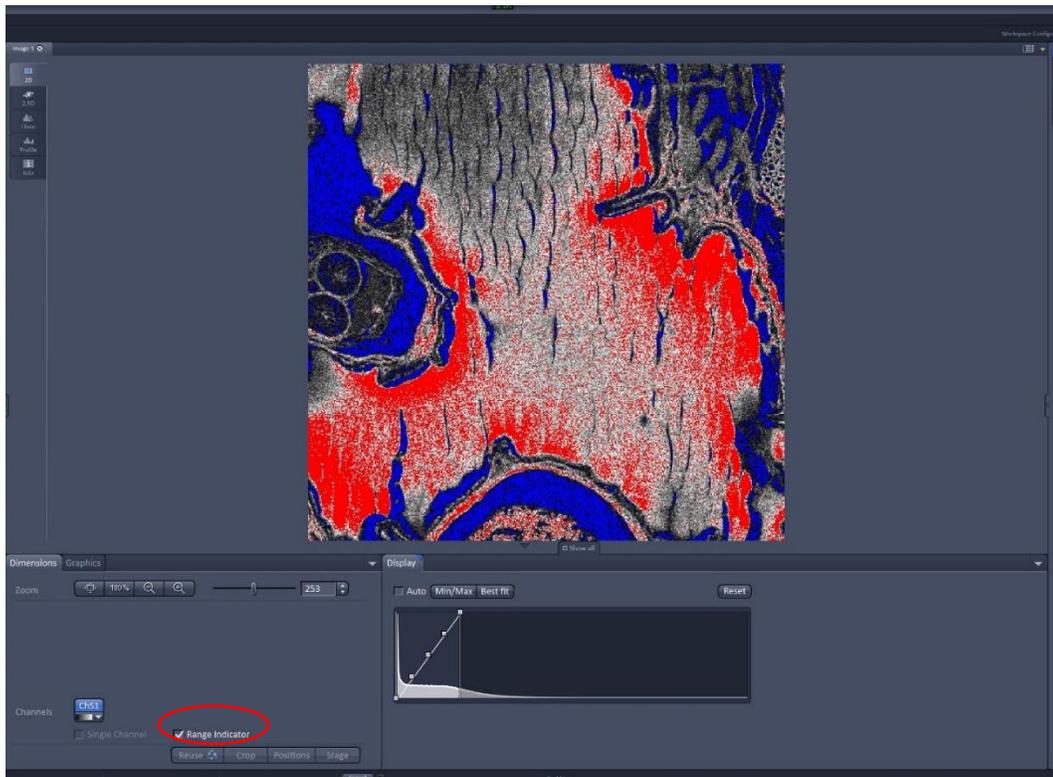
3. Once you have found focus and adjusted the exposure for each channel, check the boxes of the channels you wish to capture.
4. Press CAPTURE to take a single image of all the channels.
5. Press START EXPERIMENT if you have a Z-stack or Time-lapse.
6. Use the Min/Max or Best Fit on the display histogram to edit contrast.
7. Press the REUSE (recycle) button on an open image to apply the settings of that image.
8. Press the CROP button on a still image to select a smaller area of interest. Pressing LIVE again after this will automatically zoom the scan area in to that specified area.

Z-Stack
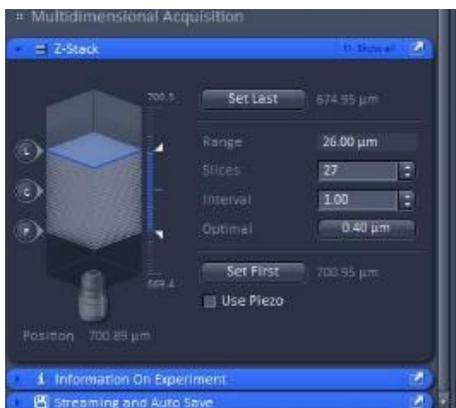
- o Scroll through the Z to find the intended scan range. Click "Set First" and "Set Last" to set the begin and end position
- o The system will automatically provide the optimized number of steps and Z-step size. You can change the size of the stack by changing the number of steps "Slices", or the size of the step "Interval".
- o Press START EXPERIMENT to image a Z-stack.

Process Tab
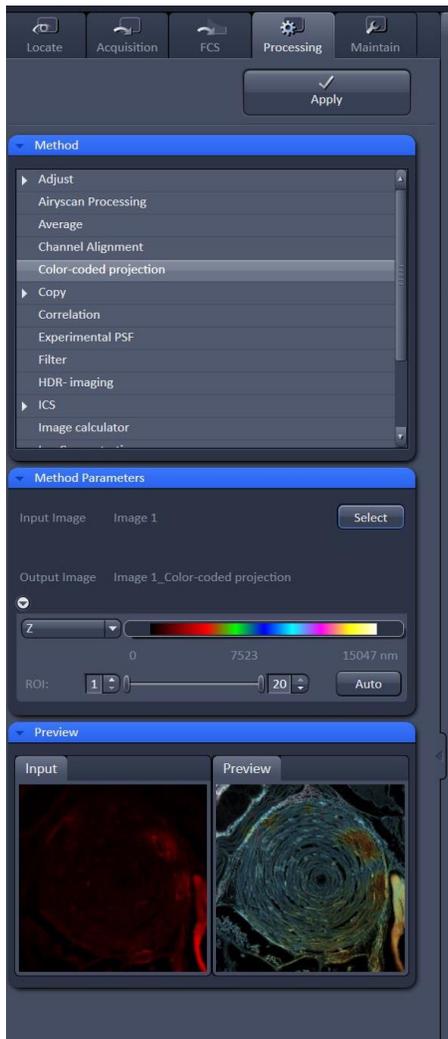
- o There are many options here to process your images, which can also be done in programs such as ImageJ.

- o

- o Airyscan Processing must be done using Zen, there is a computer located on 9016BB can also be used for Airyscan process

- o Highlight what image you want to process on the right-hand side, then press SELECT on the left side under method parameters. Choose which method you wish to use and hit APPLY.

Shut Down

When you finish your session, check on the calendar again. If you are the last user of the day, go to Procedure A. If you are not the last user, go to Procedure B.

Do NOT clean the objective! NTS staff will clean them routinely. We only ask user who use oil objectives to wipe off excessive oil left on the objectives.

PROCEDURE A (SHUT DOWN)

1. After imaging, make sure to save and copy your data!
2. Shut down ZEN and, when prompted, turn off all lasers. Then, <u>log off your lab account</u>. Now shut down the computer.
3. If the Argon laser was on, wait 5 minutes for the cooling fan to stop.
4. Turn off the X-Cite lamp.
5. MAKE SURE the LASOS power supply has finished cooling before shutting off power switches!!! (You will hear the fan shut off or wait approximately 5 minutes.)
6. Then, on the main switch pad, turn off the 2 small switches (SYSTEM/PC and COMPONENTS). Labeled A and B in picture above.
7. Next, turn off the Main switch (#1) in the picture above.
8. Make sure to leave the confocal clean and tidy. Throw away any trash including used kimwipes, lens paper, etc. Take your slides, dishes, etc. with you and turn out the lights.

PROCEDURE B (STANDBY)

1. After imaging, make sure to save and copy your data!

2. Shut down ZEN and, when prompted, DO NOT turn off lasers. Then, <u>log off your lab account</u>.