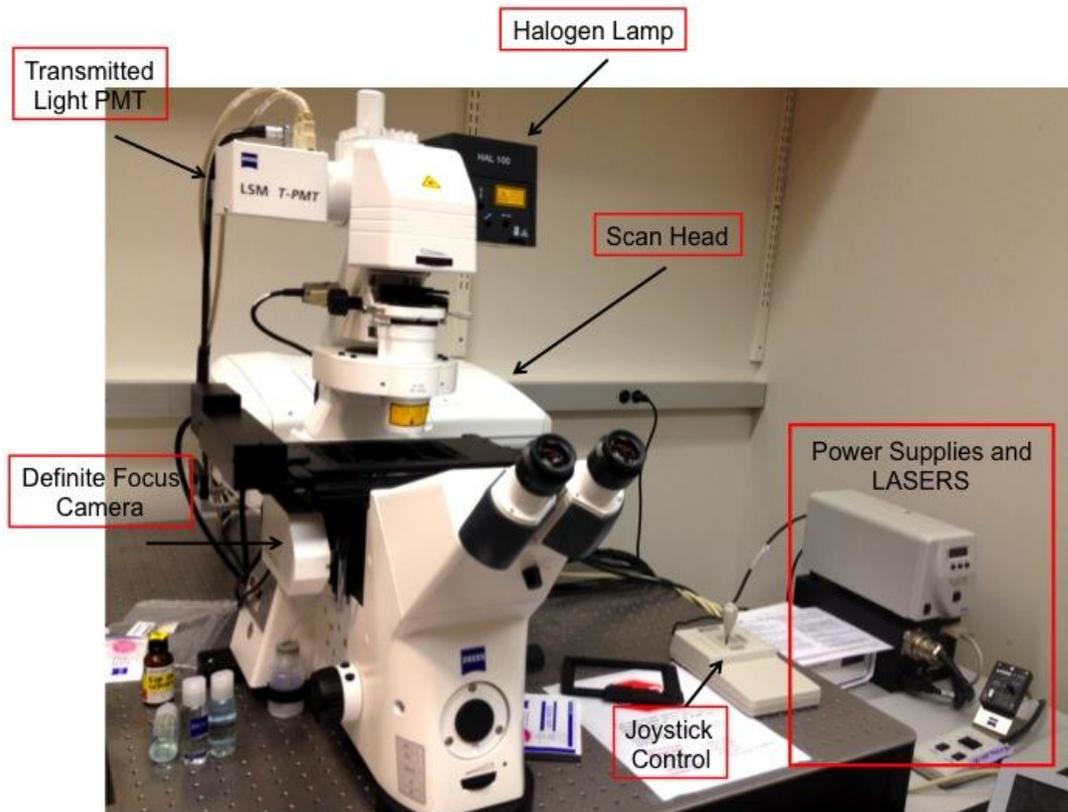


# NeuroTechnology Studio

## Zeiss LSM 710

### User Manual



*For questions or concerns contact:*

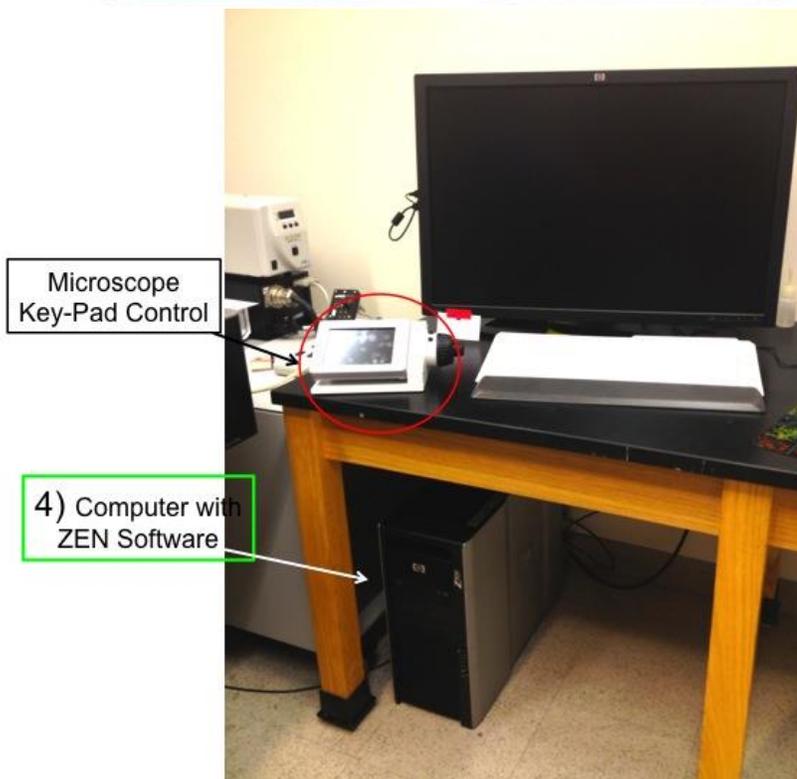
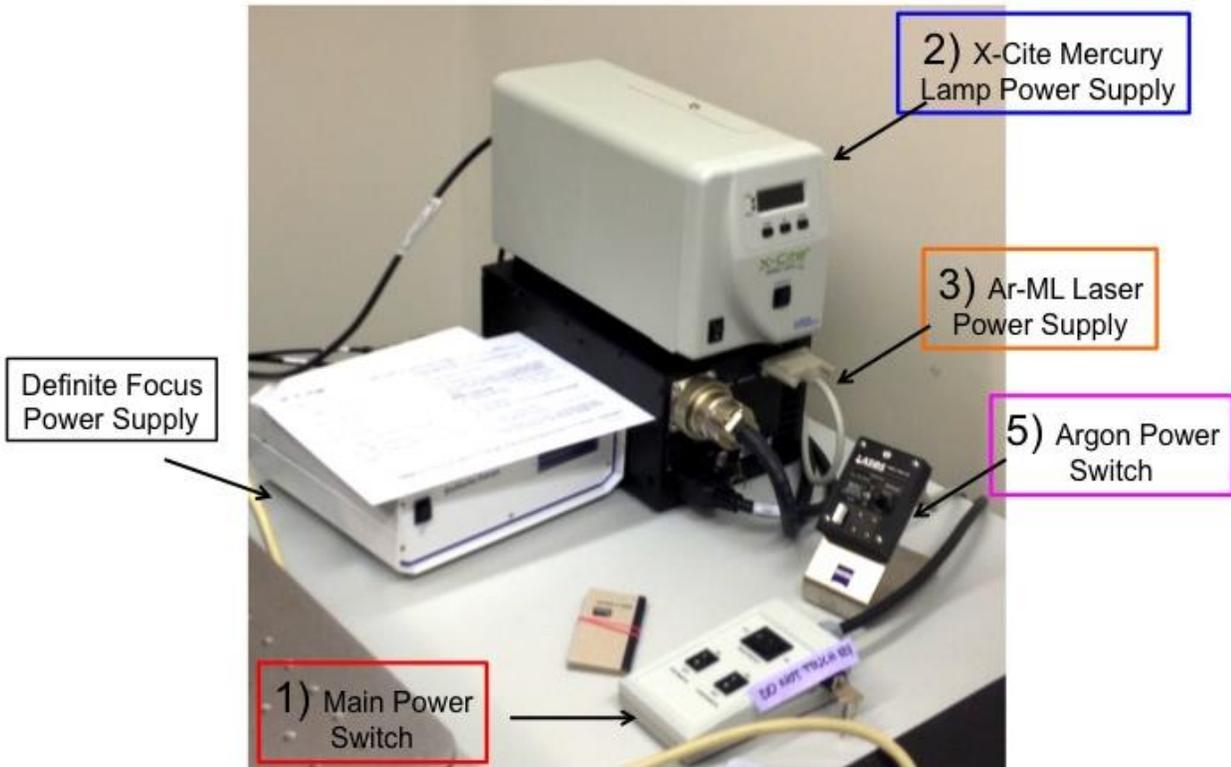
Lai Ding: 781.901.6116 or [lding@bwh.harvard.edu](mailto:lding@bwh.harvard.edu)

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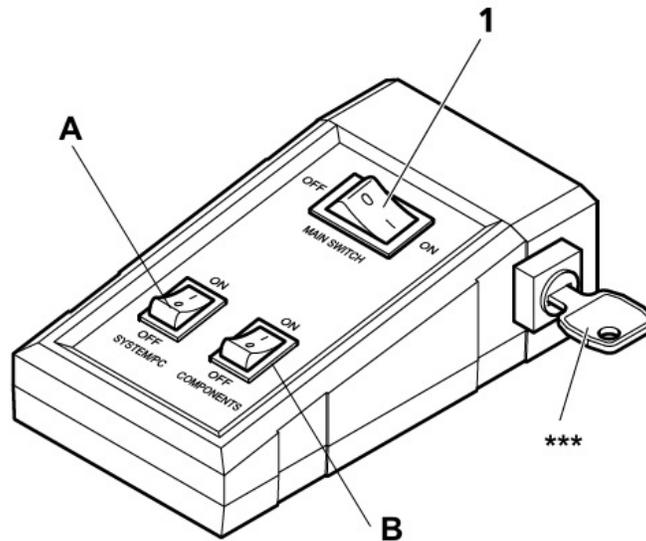
# Startup

## Power Supplies and LASERS



## To START the Confocal System:

1) Turn on the Main Switch (1).



**NEVER TOUCH the key (\*\*\*)**. The key remains in the horizontal position. Next, turn on switches A and B (SYSTEM/PC and COMPONENTS).

1.b) Turn on switch A (SYSTEM/PC)

**Wait until the PC is fully started, showing the login window. then**

1.c) Turn on switch B (COMPONENTS).

1.d) Turn on computer.

2) Now turn on the X-Cite Mercury lamp by the black switch (2)

Check the calendar for users after you--if someone will be using the microscope after you, please LEAVE the X-Cite ON!



3) For the Argon laser power supply, (LASOS) turn the Laser key to the right, to the horizontal position. This is the **ON** position.

Do not touch other switches on the power supply.

If the key is in the vertical position, then it is off.



4) Log in to your account on the computer:

Login to the computer using the lab account. Do not share account password with anyone outside your lab. Remember to log off when you are finished with your session.

## 5) Turn the Argon Laser (LASOS) on:

Wait 5 minutes after turning the power supply key on before you turn the Argon laser on. (A) Make sure the toggle switch is flipped up to “run”.

(B)-->To set the laser to 50% power, turn the dial clockwise until it lights up red. Then slightly turn the dial counter-clockwise until it changes from red to green. The light should remain green while imaging.



## 6) Next, open the software:

Click on **ZEN BLACK**.

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

A dialog window will pop up. Click the button: **Start System**. (Our system runs Zen 2011.)

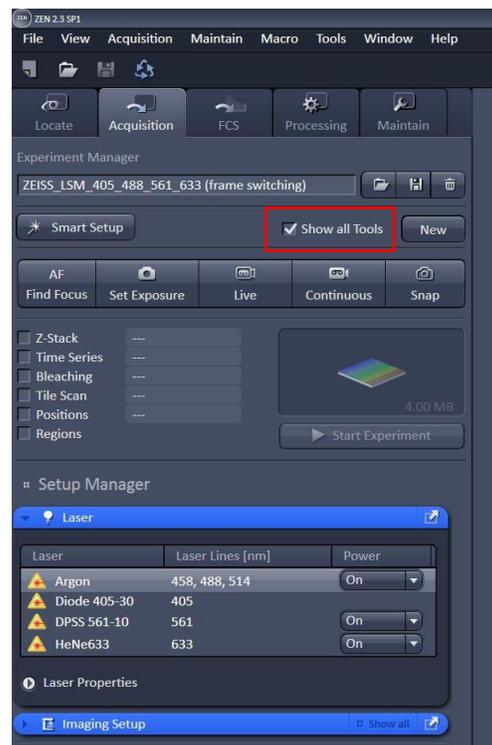


As the hardware configures, you will hear the microscope parts starting to activate. This is normal. You are now ready to image! Remember to only save files on the D: drive. **DO NOT save files on the C: drive!**

\*\*\*\*Before changing objectives press **LOAD SAMPLE** on the touch-screen. This will lower the objectives and prevent accidents!

# 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 current status of the lasers. Wait a few minutes for the lasers to reach full capacity.



3. Use the **LOCATE** tab to view sample using the eyepieces.

# Channel Set up



**Load preset settings.** LSM\_405\_488\_561\_633 (frame switching) = basic 4 channel

Select any of these boxes to bring up a corresponding window

## IMAGING SET UP WINDOW

- 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.
- Select **Ch1** for DAPI/Hoechst, **Ch2** for far-red dye like CY5, **ChS1** for all other dyes
- Select the **T-PMT** box to use transmitted light (brightfield)
- 

## Acquisition Mode Window

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

## Channels Window

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

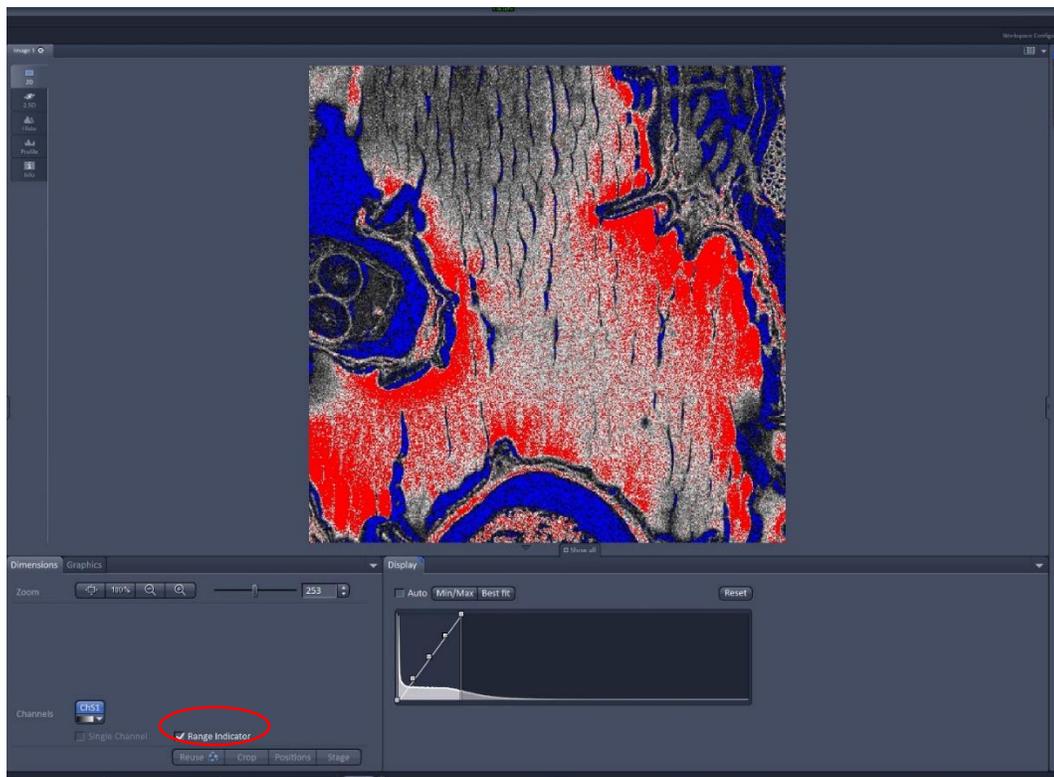
# Live Mode



## CHANNEL WINDOW

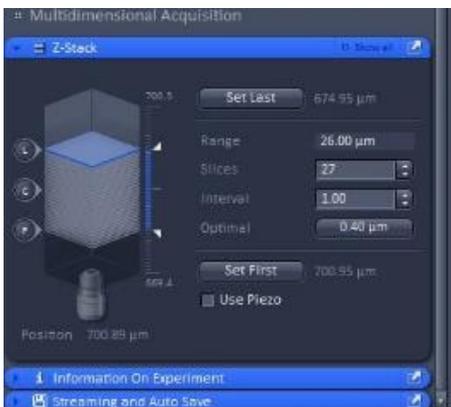
- Check the box for what channel you wish to view. If you have multiple checked, the system will scan each one continuously and make a composite.
- The channel that is highlighted will be the editable channel
- Adjust laser power and gain for each channel

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



- **Scroll through the Z** to find the intended scan range. Click "**Set First**" and "**Set Last**" to set the begin and end position
- 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**".
- Press **START EXPERIMENT** to image a Z-stack.

## **To SHUT DOWN the Confocal System:**

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) Switch off all lasers in “**Laser**” tab.
- 3) Shut down ZEN and **log off of your account**. Now shut down the computer.
- 4) Flip the Argon laser power toggle switch to idle (#5A in picture above)
- 5) Turn the key on the LASOS power supply to the left (counter-clockwise) and wait 5 minutes for the cooling fan to stop (#3 in picture above)
- 6) MAKE SURE LASOS POWER SUPPLY has finished cooling before shutting off power switches!! (You will hear the fan shut off, or wait approximately 5 minute)
- 7) On main control panel, turn off “COMPONENTS”, turn off “SYSTEM/PC”, turn off the Main Switch (#1)
- 8) Turn off the X-Cite lamp by using the switch (#2)
- 9) Make sure to leave the confocal clean and tidy. Throw away any trash including used Kimwipes, lens paper, etc. Take your slides, dishes with you and turn off the room lights

### **Procedure B (Standby)**

- 1) After imaging, make sure to save and copy your data!
- 2) Shut down ZEN and then **log off of your user account**.