

Zeiss LSM710 Confocal User Guide

2023-05-19 edition

- Users must be trained and approved. Work with others until you have mastered the system. Ask JM for help.
- Use iLab for reservations, maximum of 4-hours at a time.
- Do not use gloves to touch confocal switches or the computer.
- Please ask Dr. Murray immediately you notice any issues with the instrument or if you have forgotten any procedures (jwm2175@cumc.columbia.edu or slack workspace).

Here's a good but long video demonstration of a LSM710 microscope:

<https://www.youtube.com/watch?v=sCnAPIJGIQ>

Zeiss confocal knowledge base

<https://www.zeiss.com/microscopy/us/local/zen-knowledge-base-home/zen-knowledge-base-lsm-800-900-980.html>

AVOID THESE DAMAGING EVENTS:

- Oil dripping down objective lenses. Use small amount. Gently wipe off oil from lens when there is too much, also wipe sides of lens. Use only lens paper.
- Bumping Objective Lenses with the stage Insert or your sample (glass slide) always handle the microscope and slides gently.
- Getting nail polish or other damaging substances on the objectives microscope components (e.g. Oil on Dry lenses).
- Using very bright laser intensities (>20% will usually cause strong photobleaching).
- Frequently turning on and off the lasers.
- Turning off the power before fan has cooled the argon laser.

How to Start Up the LSM710

Remove dust cover from microscope, inspect the area. See me (8-801F black bldg.) or email jwm2175@ if you find problems.

Make sure "LASOS panel" little silver switch (6) is on standby (down)

Turn on: (see images below)

- (1) Main switch, (2) System/PC, (3), Components
- (4) Computer (if not already on).
- (5) LASOS argon key from off (0) to on (-)
- (6) LASOS panel silver switch to "run"
- (7) X-cite mercury lamp, green switch



- Login to computer with your UNI
- Double click ZEN BLACK (not blue!) software, and click "Start".
(Zen Blue is for analysis only)

It takes ~10min for the green LASOS light to turn on, indicating stabilization of the laser. Make sure the red light is not on, indicating laser power is too high. If red, turn counterclockwise to green.

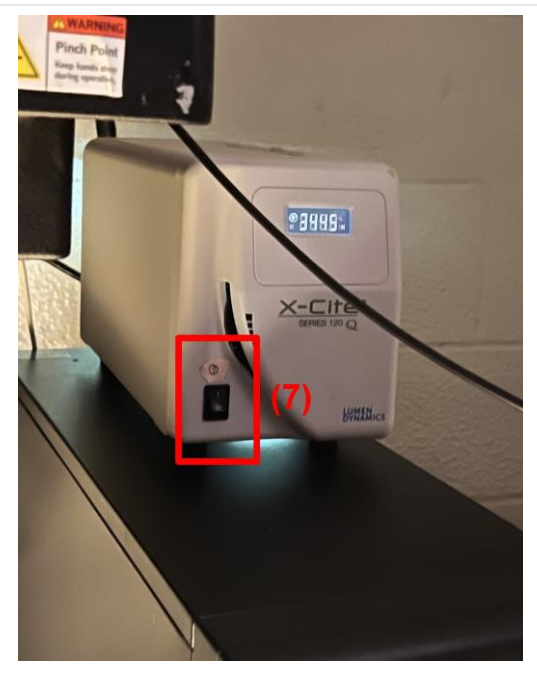
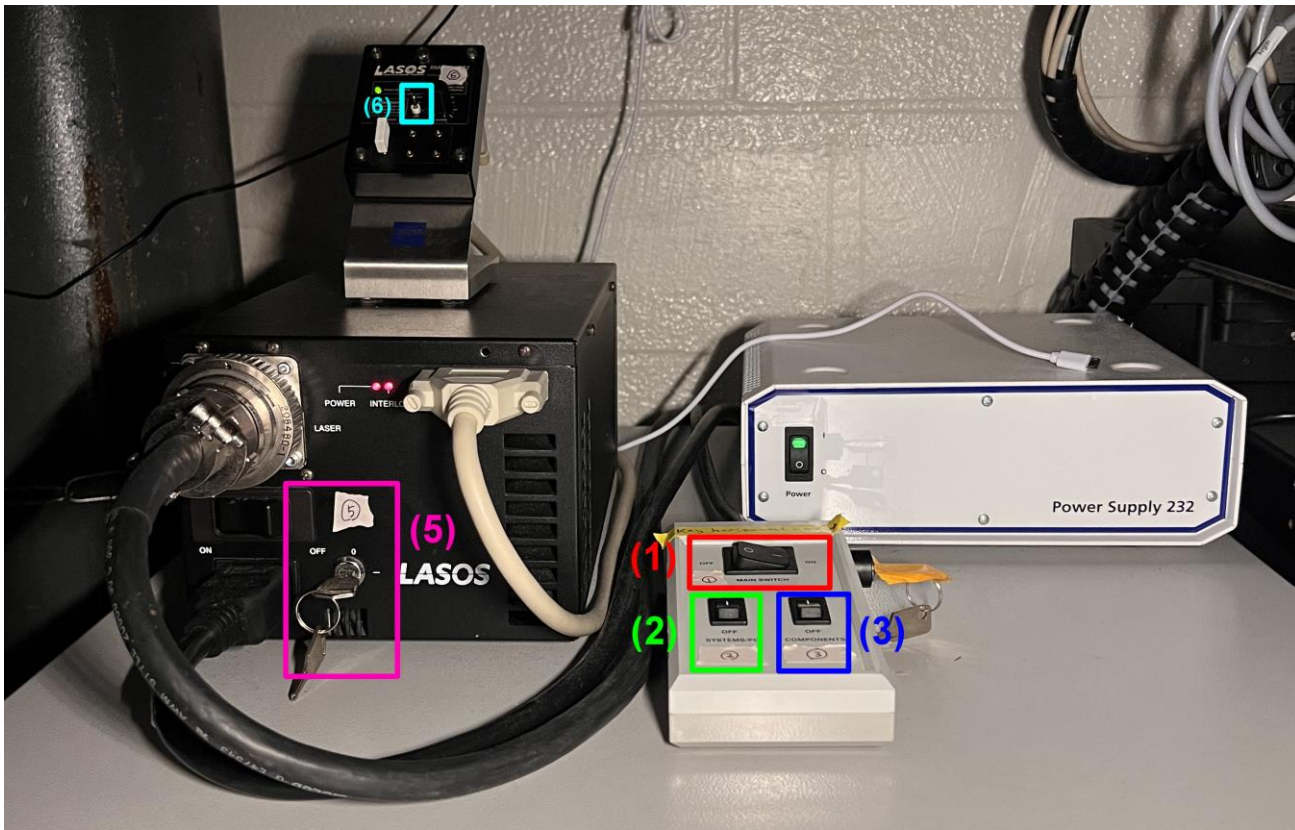
Save your images to the D: Drive (this is temporary storage and will be deleted)

Transfer your data to your own external hard drive*No data is allowed on the C: drive and will be deleted. You can temporarily store images in your folder such as, D: \ USER\ "your folder". All data may be deleted after 2 weeks. *Use multiple backups or cloud-based data storage.

Location of the On / Off Switches

(leave all other switches as is)

- (1) Main switch
- (2) System/PC
- (3) Components.
- (4) Computer (not shown).
- (5) LASOS argon key from off (0) to on (-).
- (6) LASOS panel “standby/idle” tiny silver switch



(7) X-cite mercury lamp

Shut Down the Zeiss LSM710 Confocal

THE ARGON LASOS LASER NEEDS TO COOL DOWN FOR ~5 MIN WITH FAN ON.

Without appropriate cool-down, the LASOS Argon laser will break or laser life will be reduced.

1. Put LASOS panel silver switch (6) to “idle / standby” position.
 - a. If you are certain that another user is arriving within 30 min, you can leave in idle mode.

Continue with complete shutdown:

2. Turn LASOS argon key (5) from on (-) to off (0).

In ~5 min the fan will turn off, indicating that all other switches can be turned off.

3. Clean the area.

If oil was used, gently remove oil from the objective lens (including the sides) with lens paper. Do not use a kimwipe (additional solvent or detergent cleaning of the lenses can be done by the manager).

4. Transfer your data using the USB extension cord.
5. Shut down the computer (4). Don't turn off the power switch (2) until the computer has shut down.
6. Turn off X-cite mercury lamp (7).
7. Cover the confocal microscope with the blue dust cover.

Once the LASOS fan is quiet (~5 min after laser key (5) was turned):

8. Turn off and Components (3) Systems/PC (2) and Main switches (1).

Available Zeiss LSM710 Excitation lasers in nanometers (with example dyes):

405 (DAPI)
458 (CFP)
488 (EGFP)
514 (YFP)
561 (Alexa 568)
633 (Alexa 647)

Common Problems with Zen Startup (2023-05-19)

- 1) RealTime computer not talking to the main computer.
Solution: Open the RealTime computer front door, press reset button, re-start Zen Software.
- 2) Error message indicating that devices being used by other software.
Solution: Ask JMurray for help, OR if you are familiar with Windows Task Manager, Close Zen software. Press CTL-ALT-DELETE to run the task manager. Look for whether a version of Zen Software is still running under “processes”. If you find a Zen still running, click it and click “end task”. Re-start Zen.

Using Zen software to run the LSM710

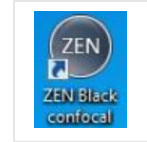
Refer to separate software guide: [Zeiss_LSM710_Software_Guide_JWM.pdf](#)

Double click Zen Black icon.

Select 'Start System' from the Zen login window.

Zen Black will open with many tabs visible

Oil: Use Zeiss oil for the LSM 710. Minimize the amount of oil used. Clean up oil from both your slides and the objective lenses (lens paper only). Use "splash" detergent to remove oil from your slides.



“Locate” tab is the “view by eye”, mercury lamp mode

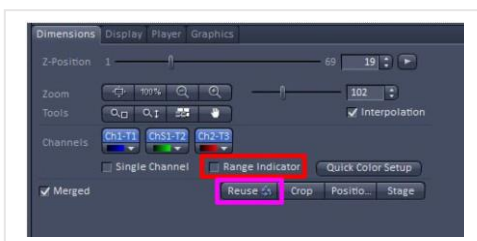
This mode will allow you to find your sample and focus. Three filter cubes are available (DAPI (violet), GFP (green) RFP (red) and 5 objective lenses: 4x, 10x, 20x, 40x (oil), 63x (oil).

“Acquisition” tab is the laser scanning confocal mode

Acquisition settings and practical tips

(green = initial recommended setting)

1. Click, “show all” at the top to see all settings.
2. **Frame size (=X-Y pixel dimensions of image).** **Set to 1024x124 or 2048x2048.** Best choice will depend on zoom factor and objective lens used. “Optimal” setting will give maximum theoretical size.
3. **Scanning speed.** **Set to 7.** Slower speeds will give better images but will take more time.
4. **Average imaging.** **Set to 4.** This will remove noise (it averages the pixel readings). For slower scans speed you can use less averaging.
5. **Bit depth.** **Use 8-bit for normal imaging, 12-bit for quantification** (use 12 bit for images with bright and dim pixels). Bit depth is the number of gray scale values for the pixels.
6. **Laser power.** **Avoid going beyond 20%.** Higher power can damage the detectors and will photobleach your samples. If you cannot see a good signal with 20% laser power and 700-gain setting, you need to improve your staining or fluorophores to have a better signal noise contrast.
7. **Gain.** **Less than 700.** High gains give pixelated images. Low gains look better but require brighter fluorescence signal.
8. **Use the Range indicator (red box below, bottom panel of Zen Black).** Check this button to view your saturated (red = 255) and black (blue = 0) pixels. Adjust the laser power and gain of each channel when you hit “continuous” or “live” imaging (“Continuous” uses your chosen settings while “live” gives fast live view).



Typical methods to get good image settings:

Set laser to 2% (default), press “Set Exposure” (upper left, auto adjusts image settings).

OR

Check Range Indicator, press “Live” (live = fast viewing) and manually adjust gain until pixels just begin to saturate. Confirm image settings by pressing “Continuous”. Adjust gain and laser settings for best image. Usually avoid saturation (a lot of red).

For lowest bleaching, use lowest laser power, slower scan speed, more averaging.

9. **Smart Setup** (dye selection wizard) to set your dyes. Separate tracks for less bleed thru, combine dyes in tracks for faster image.
10. **Pin-hole.** **1AU** unless you have specific reason, such as very low signal.
11. **Scanning direction.** **Use unidirectional mode.** Bidirectional mode requires setting of XY-direction between each channel every time and will often create noise.
12. **Light path.** After you click, “show all” the light path column will show up. Our confocal has three detectors. Adjust spectrum width to **limit “bleed through”** (cross talk) to other channels. ‘No primary Ab’ controls are necessary to ensure that bleed through is not occurring.
13. **Tile scan.** **Use at least 10% overlap** between each image. Generally better to avoid online-stitching. Instead, after acquisition > Processing tab (upper left) > Method > Stich (see below, tile scanning).
14. **Z-stack settings.** **Use theoretical “optimal” Z-slice separation or chose your own.** 0.25 um is the minimum thickness with the 40x objective lens. For individual cells use 0.25 um and about ~30 Z-slices. **Temperature can affect Z-sectioning** (e.g. samples coming from refrigerator to confocal). Bring samples into the confocal room ~1 hr before scanning if precise Z-stack is required.
15. **T-PMT mode.** Transmitted (~bright field/phase contrast) imaging. Click ON the PMT to use this.
16. **Auto-focus.** **Hopefully not needed.** If you do use it, make sure the focus is already close to optimal.
17. **Imaging mode.** **Line mode is faster**, but “**Frame Mode**” allows gain, offset, pinhole setting and other adjustments to be made between tracks.
18. **Objective lenses.** **Clean oil from slide and lens when not using oil objectives!!**
Tissue imaging: Use 20x, 10x, or 4x
Cellular imaging: Use 20x, 40x, 40x+ digital zoom, 63x
19. **Sample preparation.** **Slides should contact the stage holder / insert so that they are absolutely flat.** Do not mount your coverslips so that they extend to the edge of your slides. This is critically important when you use oil lenses (40x, 63x objective lenses) as this can cause Z-focus to change as you move across your slide.
20. **Oil.** **Use Zeiss oil. Minimize the amount of oil.** Clean up the oil on you slides as well as the lens when you are finished. If you image an oily slide with a dry lens, you can damage the lens. Excess oil can damage other lenses and the microscope.
21. **Scan order and tips for different fluorophores.** **Fluorophores should have similar brightness to minimize “cross talk”.** Reduce DAPI stain so that it doesn’t overwhelm the other Fluors. Technically you should also image longer wavelength (e.g. A647) dyes first, as the blue laser (405nm) can damage and bleach the dyes. In practice, for slides mounted with anti-fade medium, this usually doesn’t matter. But you may want to image longer wavelength dyes first.

Tile scanning using Zen software

Here’s a good video demonstrating tile scanning using Zen software:

<https://www.youtube.com/watch?v=3nGjluUORR0>

1. Activate “tile scan” option in upper left menu
2. In Tile Scan menu, use Bounding grid (or Convex hull, which shaves off the corners) to mark the area to scan. You can also use “centered grid” to perform tile scan with the current position in the center.
3. Select 10% overlap. Optionally check online stitching (but to ensure best results, do the stitching later in the “processing” tab).
4. Find the boundaries of your sample, either using “Live” or going to “Locate” mode and finding by eye. “Add” the boundaries of your sample in the Bounding grid menu.
5. “Start Experiment”

Stich your images together in the Processing menu

1. Processing tab (upper left) > Method > Stich (down the list)
2. Select your image > Set Loose, Medium, or Strict setting
3. Apply (left near the top)
4. Save your image.

Analysis of your data

Zen software Blue edition can be downloaded and used on your PC, but does not work for Apple/Mac.
http://www.zeiss.com/microscopy/en_us/products/microscope-software/zen-lite.html.

Recommended image analysis programs (free to download, except Aivia):

Fiji/Image J (Mac or PC)	(free, heavy user base, Google “imagej fiji”)
CellProfiler	(automated nuclei and cell selection, batch processing)
QuPath	(pathology, whole slide image analysis)
<u>*Aivia (Leica)</u>	(3D image quantification, available on the HIVE server and room 905. *We have a permanent, single-user license (one instance at a time). <u>Please use Google calendar)</u>

I have many macros and automated ImageJ and CellProfiler image analysis and processing tips and am very happy to collaborate on image analysis projects.

Let me know if you notice any issues with the instrument or if you have forgotten any procedures. There is also a Slack workspace that you can use to text me.

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