

LSM 310 Instructions

Before you do anything, sign-in to the logbook.

Startup:

1. If you plan on viewing your sample with epifluorescence, turn on the **Mercury Arc Lamp** located on the left of the scope, and wait until the beam is steadily lit. If used previously in the day and still warm, the lamp should be allowed to fully cool before powering it on. It should left on for at least 20 minutes if used.
2. Turn on the Jaz and Zip drives with the **Power Strip** located on top of the rack.
3. Turn the **Computer Power Key** near the top of the rack to the right to start the computer. Allow the operating system and the LSM software to load completely.
4. Power up the ArKr laser by turning the **Laser Power Key** at the bottom of the system rack to the right. Wait about 30 seconds, then push the green **Power Button (5)** next to the power key to start the laser. Wait 2-3 minutes for the laser to warm up and stabilize.

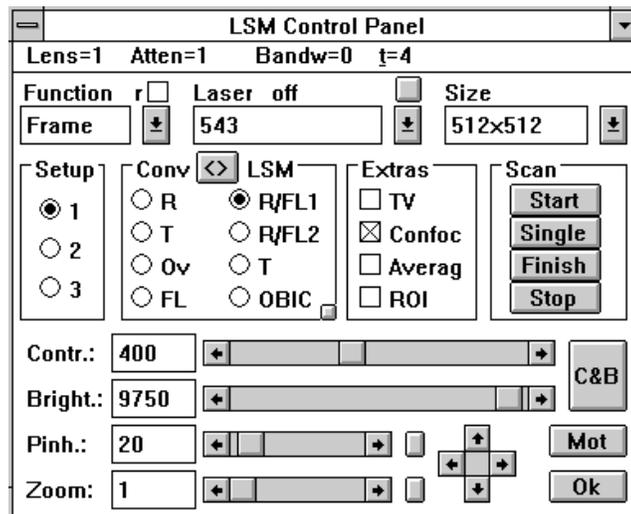


Shutdown procedure:

- Move your data from the computer with a Jaz or Zip drive using the **Windows File Manager**. This is very important! Drive space is limited and data more than a week old **will be deleted without warning!**
- Turn the **Laser Power Key** off (left position). Let the laser cool for 10 minutes before proceeding.
- Exit the LSM software, and exit Windows. Once in DOS, turn the **Computer Power Key** to the off position.
- Turn off the power strip to the Jaz and Zip drives.
- Switch off the **Mercury Arc Lamp**.
- Make sure objectives are clean and dry. Be gentle, and use lens paper with a little methanol if necessary. Don't apply direct pressure to the objective tip; hold the lens paper by the edges and lightly drag it over the surface until all oil has been removed.
- Cover the microscope and clean the work area.
- Finally, sign-out of the logbook.

Using the LSM software:

Most of the main features of the 310 are controlled via the **LSM control panel**.

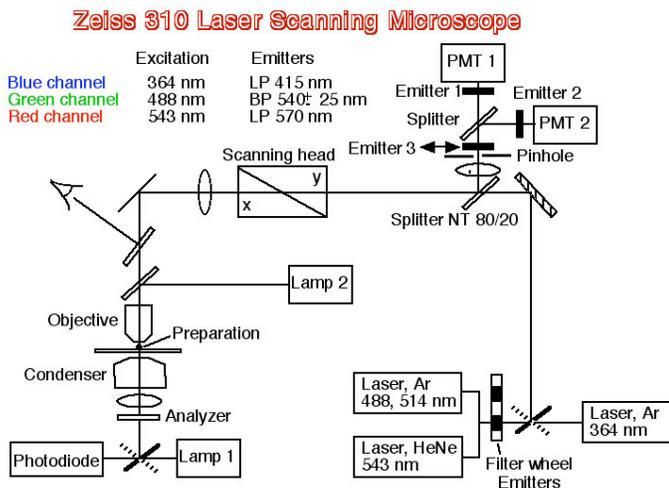


The Menu Bar:

- **Lens:** Choose an objective using under this menu. The 310's lens carriage can also be rotated manually. The lens setting in the software must match the objective being used for accurate measurement (i.e., microns per pixel).
- **Atten (attenuation):** In this menu, the checked number N reflects the laser line attenuation, where attenuation is $1/N$. Values of $1/100$ or $1/10$ generally yield good results depending on the strength of your fluorescence staining or expression. Lower attenuation (3 or 1) increases signal at the expense of more photobleaching.
- **Bandw (bandwidth):** This is a low-pass filter that will limit the bandwidth of the incoming signal. For highest quality imaging it should be left at 0 or 1.
- **t (time):** The pixel dwell time of the laser; the slower the laser scans, the more emission will occur. Lower it (**t~4**) to increase scan speed, and raise it (**t~8**) to increase picture quality.

To view your sample with reflected or transmitted light:

- Generally, you will position your sample and locate your imaging region under the scope using white light or epifluorescence.
- **On the microscope:** Move the reflective slider to the middle position (there is no detent, so you must approximate). The filter in the slider passes red and green light, but you must be using dyes that emit under the blue illumination, so some red dyes won't work here.
- **In the Control Panel:** Select **FL** (epifluorescence) or **T** (transmitted white light) under **Conv** (conventional). Select an objective manually or under **Lens** in the software.
- Position your slide on the stand and focus on your specimen.



To begin scanning:

- **On the microscope:** Move the reflective slider to the rightmost position.
- **In the Control Panel:** Choose an objective manually or under **Lens**. Select **R/FL1** under **LSM** for red channel imaging and **R/FL2** for green channel imaging. Select the **Laser** line appropriate for your dye. Choose an image **Size**, and select **Confoc** (confocal) in the **Extras** box.
- Click on the **Start** button in the **Scan** box. Then, click the **AUTOBC** button in the upper-left corner of the main LSM screen to gather signal intensity data and automatically select contrast and brightness settings.
- Fine-tune the quality of your image with the **Contr** (contrast) and **Bright** (brightness) controls. Contrast affects the laser emission gain, while brightness affects the photomultiplier gain.
- Check for pixel saturation, which indicates a loss of image quality, by clicking on the **Color** button in the separate **Display Control** window. This applies the **Range Indicator** LUT (Look-Up-Table, a pseudocolor palette), in which red pixels mark high-end saturation and blue pixels mark low-end saturation. Adjust the contrast/brightness settings until all or most pixels are in a displayable range. Clicking on the **Color** button again cycles to a Glowscale LUT, and one more click returns you to the original image (grayscale LUT).
- Save your images with **File**→**Save** from the main LSM window. The **Save** dialog box allows creation of new folders.

310 Light paths

Conventional brightfield (tungsten lamp to eye piece):

- Click on **T** button in lefthand (Conv) column
- adjust brightness using filters in scope stand base

Conventional epifluorescence (Hg to eyepiece)

- move dichroic carriage to either the **Blue** or **Green** epifluorescence position
- click **FL** button in lefthand (Conv) column

LSM brightfield (Laser to T detector) [NOTE: this may not be available for class]

move dichroic carriage to middle (LSM) position

Click on T button in righthand (LSM) column

*LSM epifluorescence for Red fluors (Laser to **R/FL1** detector)*

- Select 543nm laser line
- move dichroic carriage to middle (LSM) position
- Select **R/FL1** detector button in righthand (LSM) column

*LSM epifluorescence for Green fluors (Laser to **R/FL2** detector)*

- Select 488nm laser line
- move dichroic carriage to middle (LSM) position
- Select **R/FL2** detector button in righthand (LSM) column

Objective Selection note:

Try to select objectives from the Objective Lens menu on the control window. Moving the nose wheel by hand is permissible but not recommended since the automated system will drop the stage for safety clearance.

If moving by hand, be careful not to get oil on non-oil lenses.

File destination note:

Always save files to the Jaz disk! Storage space in the RAM or harddrive is very limited and should not be used. Any image files found left on the system will be deleted.

Z Sectioning

Z Interval- Step size in microns

Number of Sections: User defined based on sample size.

Current Section: Take care always make sure that you are at the beginning of your stack

Refractive Correction: N_2/N_1
(Example: Oil/Water = $1.518/1.0 = 1.518$)

Destination

Video Memory: internal video memory buffer, do not use.

File: **Use this!!!!**

Screen: Display only one image on the image screen

Host Memory: Save into computer's RAM, must be saved through the File menu.

Z-Scan: Line Scan through your sample at the center of the image window. Users can define boundaries for z-sectioning through the use of this function.

Move to X: Single scan at first, middle, or last positions.

The 'z Sectioning' dialog box features a title bar with a red background and the text 'z Sectioning'. It contains several input fields and controls: 'z Interval (µm)' with a value of 1 and a slider; 'Number of Sections' with a value of 10, a slider, and a 'Max' button; 'Current Section Pos' with a value of 5 and a slider; and 'Refractive Correction' with a value of 1 and a slider. A 'Destination' group box contains four radio buttons: 'Video Mem.' (selected), 'File', 'Screen', and 'Host Mem.'. Below this is an unchecked checkbox for 'Interpolation'. To the right of the destination group are three buttons: 'z Scan', 'Move to 1', 'Move to Mid', and 'Move to End'. At the bottom are three buttons: 'Ok', 'Append', and 'Cancel'.

Time Series

Time Interval: User defined length of time in seconds

Number of Images: AKA number of time points

Destination

Video Memory: internal video memory buffer, do not use.

File: **Use this!!!!**

Screen: Display only one image on the image screen

Host Memory: Save into computer's RAM, must be saved through the File menu.

The 'Time Series' dialog box features a title bar with a red background and the text 'Time Series'. It contains several input fields and controls: 'Time Interval (s)' with a value of 5 and a slider; 'Number of Images' with a value of 10, a slider, and a 'Max' button; and 'Anisotropic Lines' with a value of 0. A 'Destination' group box contains four radio buttons: 'Video Mem.' (selected), 'File', 'Screen', and 'Host Mem.'. At the bottom are three buttons: 'Ok', 'Append', and 'Cancel'.