

Instructions for Using the Zeiss LSM 510 Confocal

1. Bring a USB stick to transport your files from the confocal computer back to your computer.
3. Use the main electrical switch labeled “**Remote Control**” to turn on all the equipment (including microscope and computer)
4. On the desktop, open “**LSM 510**”
5. Select: “**Scan New Images**” and “**Start Expert Mode**”
6. Find your sample using either DIC or epi-fluorescent microscopy

Select “**Microscope**” from the menu bar:

- A. **DIC**: To do DIC with transmitted light on the Axiovert microscope
 1. Turn **ON** the transmitted light – 10% level is enough
 2. Arrange the slider on the bottom of the microscope to “**Visible**” (push the LSM slider “in” and the VIS slider “out”; this will let you see through the eyepieces)
 3. Push in the **Analyzer**
 4. Rotate the **Polarizer** to 0 degrees (note: you do not need to follow this set-up to get DIC images from the laser scan)
- B. **EPI-FLUORESCENCE**:
 1. Epi-fluorescent cubes (cubes are automatically inserted into the light path based upon the settings from the “Microscope” window (note: there are no neutral density filters for the fluorescent light; however, inserting the DIC analyzer into the light path works well)
 2. Shutter for epi-fluorescences is located behind the fluorescent cube rack; out is closed, in opens the light path

7. Configure the confocal settings

Select “**Laser**” from the menu bar.

Select Argon laser for GFP fluorescence (power about 50-60%)

Select HeNe laser for rhodamine fluorescence

Select “**Configuration Control**” from the menu bar

A. “**Config**” button on the right side shows preset configurations

B. **Single-tract**: for single-label images

Find one which says GFP

If you can't get DIC to work with this, then use the multi-track settings
(To get a DIC image: turn on Channel D)

Select "**Scan Control**" from the menu bar

A. Mode

1. Choose objectives
2. Choose frame size (512 x 512 is where it should be I think)
3. Choose scan speed:

Advantage of a slow speed: increase the signal to noise ratio

Advantage of a fast speed: reduce photo-bleaching

(This window is also where you will control single scan, fast XY, continuous scan and Line Averaging)

B. Channel

1. Select the **pin hole** size
-Optimal pinhole size: 1 Airy Unit

2. **Amplifier gain and detector gain**

Advantage: increases a weak signal

Disadvantage: increases the noise as well

(Note: you should manipulate these in conjunction with pin hole size and the amount of line averaging to obtain the optimal image)

8. A suggested strategy for obtaining images from the Confocal:

1. Once you have selected an area and obtained good focus with the DIC or epi-fluorescence, switch over to laser scanning

- Remove the polarizer and the analyzer
- Switch the sliders from VIS to LSM (this will remove the cubes)
- Close the fluorescent shutter

2. Under **Mode**, do a **Single** scan

- If you do not see your image following the scan, check these things:

1. All DIC and epi-fluorescence are out of the laser path
2. Check the size of the scan area (you can see this under **Mode**, in a bottom panel that graphically shows the size and shape of the scanned area)

3. That you are using the right laser configuration to excite your sample
4. That you are in the right focal plane to see your image (check this following the directions indicated for “**Obtaining the Best Focal Plane**”)

3. Once you have an image of your sample, refine this image before saving it.

•**First, Obtain the Best Focal Plane** for the image

1. Select **Continuous** scan (make sure that there is no line averaging and that the scan speed is fairly fast)
2. Manually adjust the focal plane by turning the focus knobs on the microscope. (You will see the scanned image getting brighter or darker as you do this)
3. Stop the scan when you have found the brightest focal plane.

• **Crop** or **Zoom** to a region of interest or to eliminate unwanted staining

1. Select “**Crop**” from the menu bar on the side of the image.
2. A box will appear on the image that you can adjust for size and rotation
3. Once you have selected the refined scanning area, do another single scan before proceeding to optimizing the intensity of the image.

•**Optimize the intensity of the image**

1. Select “**Palette**” from the menu bar on the side of the image
2. A new box will open - Select **Range Indicator** (When you do this, the previously scanned image will turn to a black and white image. Red dots will indicate where the pixels are completely saturated)

• Lots of red and the image is too bright, no red and the signal is too weak

3. multiple ways to optimize the strength of the signal (under **Channel**)

- Adjust the detector gain
- Adjust the amplifier gain
- Adjust the pin hole size (last resort)

4. Once you have refined the image, you will want to scan it for saving

• Under **Mode**, you will want to select **line averaging** to help eliminate noise and background from the image. Usually 2 works well

• Do a **Single** scan and then save the image into your database.

12. A suggested way to obtain a Z-stack

1. Follow the steps above regarding “Obtaining the Best Focal Plane, Cropping/Zooming the Image, Optimizing Image Intensity and Selecting Line Averaging”

2. Select **Z-stack** from the Menu within **Mode**

3. Mark the first and the last focal plane to be scanned.
 - Select **XY Continuous** and adjust the focal plane using the focus knob on the microscope until you have focused all the way through the image in one direction.
 - At the one end of the image, click the button "**Mark First**"
 - Now adjust the focal plane back through the image to the other end of the image
 - At the other end, click the button "**Mark Last**" and **Stop** the scan.

4. Click the button called "**Z-slice**" to see the **optimal slice interval** and graphically how much overlap there will be between the slices scanned. Determination of the slice interval will decide how many slices will be scanned.
 - While the optimal interval is best, often it requires too many scans to be taken (for time and storage space reasons).
 - Adjust the slice interval so that 20-30 scans will be taken (over 30 is often too many).
 - Make sure that there will be some overlap between the slice intervals

5. When you are ready, click "**Start**". You can watch the individual scans by selecting "**Gallery**" from the menu bar on the side of the image.

6. With a Z-stack, you can collapse the images or make a movie from the images
 - Select **3-D view** from the main menu bar
 - To collapse all the images, select "1" projection and make the angle 0°
 - To make a movie, select the number of projections that you want (32 is usually good) and select the angle of rotation that you want for your image. For 360°, select panorama.