Using the ZEISS LSM 510 META

1. **Start-up** – Follow the start-up instructions.
   - 1 – Mercury Lamp
   - 2 – Main Switch (left of microscope)
   - 3 – Computer

2. **Kombi FCS-LSM META Icon** – double click on this icon to start the software.

3. **Scan New Images – Start Expert Mode** – click these options

4. **Acquire** - should be indented on the main menu.

5. **Laser** - click to view laser options.

6. **Argon, HeNe1, and/or HeNe2** – click on which laser you need for your application (excitation wavelength of the dye(s) you are using).
   - **Argon** – click **Standby** and wait until **Status:** reads **Ready or Connected**, then click **On**. Move **Output(%)** slider up until **Tube Current:** reads **6.1 A**. Once this laser is turned off, it should not be turned on again for 2 hours.
   - **HeNe1 and HeNe2** – click **On**

7. **VIS** - click on this before adjusting microscope settings. Allows for use of the oculars.
8. Micro - click to open the microscope settings window.

9. Objective - click to see a list of available objectives, choose the one you wish to use.

10. Set-up Microscope - instead of n.n, your buttons will have words representing microscope settings. Click on the setting you wish to use and the reflector, light, condenser, etc. will be put into place for you.

FITC – viewing green fluorescence
Rhod – viewing red fluorescence
DAPI – viewing blue fluorescence
DIC – Differential Interference Contrast
Phase – phase contrast
Transmission (BF) – bright-field

11. Load Specimen – The 10x objective uses NO oil or water. There is a 40x oil, 63x oil, and 100x oil objective – these all need a drop of oil on them before loading a slide. There is also one 40x water objective which needs a drop of distilled water before loading a slide. Be careful not to put oil on the water objective or water on the oil objective.

Place your slide upside down into the stage – the width of the holder can be adjusted.

Raise the objective (by moving the coarse focus knob away from you) until the objective just touches your slide.

12. Focus – push the top button (FL on/off) closest to you on your right side to use the mercury light to see your fluorescence through the oculars. Push the bottom button (HAL on/off) to use the halogen light for bright-field settings (BF, transmission, phase contrast, DIC). Focus on your specimen and turn the light off.
13. **Config** - click from the acquire submenu to open the configuration control window.

14. - choose if using only one dye or one dye and DIC.
- choose if using two or more dyes.
- choose if using linear unmixing/emission fingerprinting.

15. **Set-up Configuration** - click to view a list of configurations. This list varies between single track, multi track, or lambda mode. Choose the configuration that you wish to use and click **Apply**. If there is not a configuration that applies to your sample, a new configuration can be manually set up. If you are not comfortable doing this, please ask the confocal technician for assistance.
16. **Scan** - click from the acquire submenu to open the scan control window.

17. **Frame Size** (512 x 512 is standard).

Choose **Scan Speed** (8 or 9 is average) – the lower the number, the slower the scan. Use **12 Bit** and → for publishable images.

**Line** should be chosen for **Mode**, and **Mean** for **Method**. The higher the number you choose for **Number**, the smoother the image will appear and the longer the scan will take.

**Zoom** should be 1 and **Rotation** 0. The zoom can be further adjusted once the image is acquired.
18. **Channels** - Click on each of the channels **Ch1** and make sure that **Pinhole Ø = 1.00 Airy Units**. A click on the **1** will set it to 1.00.

- will scan your image one time using optimal Detector Gain, Amplifier Offset, and Amplifier Gain settings as determined by the computer.

- will continually scan your image until you click stop **Stop**. While scanning, you can adjust the Detector Gain, Amplifier Offset, and Amplifier Gain and watch the effects in real time. You can also change the laser intensity and center the specimen by clicking on the arrow buttons **Fast XY** in the mode window.

To zoom an area of your image click the Crop **Crop** button in the toolbar to the right of the image window. Choose your area of interest, and click Single **Single** in the scan control window.

You can check your pixel saturation by clicking the Palette **Palette** button in the toolbar to the right of the image window.

Click on Range Indicator **Range Indicator** to see saturated pixels in red and black pixels in blue.

To adjust, click **Fast XY** **Fast XY**, then the left arrow of the Detector Gain slider until all of the red is gone. Click the left arrow of the Amplifier Gain to increase the black pixels (less background), or the right arrow to decrease black pixels (more background). Usually, Amplifier Offset is left at 1.
Click Stop to end the scan and remove the Palette by choosing No Palette in the Color Palette window.

19. Final Image – click Mode in the Scan Control window, then choose a slower scan speed (≈ 7) and increase Number depending on the amount of noise in the image (the more noise, the higher the number should be). When you are happy with all of your settings, click Single.

20. Save – click Save from the toolbar on the right side of the image window.

If you do not have a database, choose New MDB.

In the Create New Database window choose Local Disk (D): from the drop down menu. Type in a File name (the name of your database, not the image). Click Create. Now type in the Name, Description, and any Notes for the image and click OK.

21. Shut Down – If someone is signed up to use the scope within two hours after you are finished, leave the Argon laser ON and log-off – leaving everything else ON. If you are the last person using the microscope, exit the software (remember to shut the lasers OFF) and follow the shut-down procedure.

   1 – Click Start on the bottom left hand side of the computer and choose the Shut Down option. Wait until the screens go black and the fan for the Argon laser shuts off before proceeding to 2.

   2 – Main Switch (left of microscope)

   3 – Mercury Lamp (underneath microscope and to the left)