Zeiss LSM 510 META

1. Turn on the mercury arc lamp if you intend to use it. It is the "HBO 50" box sitting on the desk with the monitor

2. Switch on the main power "Remote Control."

3. Log into iLABs and open the Zen2009 software. Click on Start.

Zen Software: OCULAR tab

1. Under this tab you can turn on and off light to the microscope. You can look through the microscope eyepieces to find your sample and focus on it.
2. To view your sample through the microscope click the "online" button at the top of the Ocular Tab
    a. Brightfield: You can change the brightness of light to your specimen by moving the dial on the lower front of the microscope.
    b. DAPI/CFP. Although the microscope has the filter cube for dapi epifluorescence viewing, the confocal microscope does not have the capabilities of imaging this wavelength.
    c. GFP/FITC
    d. RED.
3. I recommend clicking "ALL OFF" when you are done here.

Zen Software: ACQUISITION tab. Setting up to image

1. Under "experiment manager" at the top of the window, load a **configuration**. Select from the saved configuration files that are under the folder icon.
    a. If you know you will be acquiring a z-stack, time-series, multi-position, etc image, select that parameter now.
2. Under Lasers: A pop-up window should alert you to the lasers that must be turned on for each configuration. The lasers can also be selected from within the setup manager window -- double check that the appropriate lasers have started up. Laser choices on this microscope are:
    - 458, 477, 488 and 514 nm Argon Laser
    - 543 nm He/Ne laser
    - 633 nm He/Ne laser

    a. If the argon laser is need, it will require about 5 minutes to warm up. It will also require you to click on the triangle next to "laser properties" and increase the output % to no more than 50%.

1. Under "Imaging Setup" where you can see the visible light spectrum. Here you can modify each channel you intend to use including which lasers will turn on and which wavelengths are sent to the detector arrays. Common multichannel acquisition parameters have been set up, and when they are selected, there should be no need to modify these settings.
2. Under "light path". Again, once a configuration is selected, the light paths will automatically be set.
3. Under Acquisition Mode (second column)

a. Objective: here the objective can be selected. Objectives can also be selected the buttons near the focus knob on the microscope.
   - 10X: C-Apochromat 10x0.45 W M27 AIR
   - 40X: Plan Neuofluar 40x/1.3 oil DIC OIL (Immersol 518
   - 63X: Plan Apochromat 63X/1.4 DIC OIL (Immersol 518)

   i. Note: currently the 40x OIL objective has a heat collar so that temperature can be fully controlled for live cell imaging.

b. Other scan parameters should be automatically set when an acquisition parameter is loaded. It is good to glance and make sure that they are what you would like. Commonly these parameter are used:
   i. Scan mode: FRAME
   ii. Frame size: 1024x1024 (this can be loaded by clicking on the X*Y button or typing in the values)
   iii. Line step = 1
   iv. Speed: This is up to users. I would recommend a Speed of 7 to start with.
   v. Averaging: Typically some averaging (2X) is used. This can be done every line (recommended for most users) or every frame. Typically the method used is Mean rather than Sum.
   vi. Bit Depth: I would recommend 16 bit images.
   vii. Direction: For this microscope the single direction (-->) scanning is recommended.
   viii. Scan area: Check that this is where you want it. Because it can be saved and loaded with the acquisition parameters, it is possible to overlook this.

4. Under Channels. Here you can select which channels you would like to use. The acquisition parameter that you loaded (step 1) will likely make multiple channels available to you. By clicking the box next to each one you are free to turn on and off whichever channels here.
   a. It is possible to click on and off a channel, but unless you click within the center of the channel's row, you will not have "selected it." When a channel is selected, then its information will appear in the lower portion of the "Channels" window.
   b. When a channel is selected the following parameters can be modified. Using the "RANGE INDICATOR" setting rather than a green or red pseudo-colored image is recommended so that saturated pixels (appearing red) and true black pixels (appearing blue) are easy to see.
      i. Laser check boxes. These should be set automatically when an acquisition parameter is loaded. The check will indicate which laser will be used with the channel selected.
      ii. Laser Power: 0-100%. Ranges around ~10% are common.
      iii. Pinhole. Set this to 1 AU (airy unit).
         1. Select the pinhole for the channel that acquires the longest wavelength (633 or 561 typically) will mean that you are acquiring slightly more out of focus light in the shorter wavelength channels. Selecting the pinhole for the shortest wavelengths (488, typically) will mean that you will be acquiring less light (smaller than necessary pinhole) for the longer wavelengths. How you handle this pinhole selection is up to you and will be saved with the acquisition parameters.
         2. Please note: You will need to set the pinhole again if you change objectives.
      iv. GAIN: this changes the amplification of the signal by changing the voltage that is sent to the PMT detectors. Increasing the gain too much will increase the amount of non-signal background noise. A good place to start is 700.
      v. Digital Offset: Changing the offset will change the level at which the very low intensity pixels will be acquired as true black (0 pixel values). Beware: Having the digital offset set too high will cause very low intensity pixels to be acquired as black – essentially erasing them from the image.

vi. Digital Gain: Rarely used. Not recommended.

Zen Software: ACQUISITION tab. Scanning and acquiring an image

1. Under experiment manager:
    a. Clicking on the LIVE button will give you a fast, 512x512 scan of your sample. This function is preferred for finding the plane of focus, centering your specimen, setting the top and bottom of a z-stack.
    b. Continuous: Scanning under the continuous mode will give you the image yielded by the "acquisition settings" you have selected. For example: It will give you the image with averaging (if you have it selected) with whichever frame size you have selected using the scan speed that you have set.
        i. I recommend that after you have found and centered your sampled under "LIVE," that you check it by clicking on the "CONTINUOUS" mode. If you are acquiring a z-stack, do this both for the most intense z-level and the least intense. You should avoid saturation, but also be satisfied that the laser and GAIN settings are appropriate.
    c. SNAP: Like "continuous" SNAP uses your acquisition settings, but it will stop after a single scan of the frame.
    d. "START EXPERIMENT" click on this if you have want to acquire a z-stack, time series, multi-position etc image.

Zen Software: ACQUISITION tab. Setting up a z-stack

1. Clicking "z-stack "under the experiment manager will open up a "z-stack" window.
    a. Select the z-step interval that you would like to use or use the interval suggested by the software for the parameters you have selected.
    b. While scanning under "LIVE" use the focusing knob to focus through your specimen until you are above or below it. Click "set last." Focus through to the other side and "select first." The microscope always moves against gravity, so it doesn't matter if you click the top or bottom, just as long as you set the range that you want.
    c. Clicking "start experiment" will take this z-stack.

Zen Software: ACQUISITION tab. Saving your files

1. In the "images and documents" window, all scanned files will appear. When they are unsaved, there will be a caution icon next to each one. Selecting the image and clicking the obsolete diskette icon will save the file. Be sure to save the file within a folder you set up in the DATA D:/"LSM USER DATA" folder. Saving you files elsewhere can interfere with the software on the computer and is not allowed. If files saved in unauthorized folders are found they will be erased immediately. Backup and erase your files often, there is no long term storage of data on the acquisition computer and you should expect that it will be erased periodically.

Shutting down the system

1. Click and turn off each laser. If you used the argon laser **you must wait 5 minutes** before turning off the main power switch to the system. You will know that it is cool because the fan will shut down.
2. Turn off the arc lamp if you used it.
3. Shut down the ZEN software.
4. Log out of iLAB.
5. Remove your specimen from the stage and clean the lens using Zeiss cleaning solution and lens paper (not KIMWIPES!) while you wait for the lasers to cool down. You will hear the fan turn off when the laser is ready for shut down.
6. Put the dust cover back on the microscope
7. Once the lasers have had time to cool down, turn off the Main Power switch.