Zeiss LSM 880

Starting up the system

1. Switch on the Main Switch then Systems/PC and Components. Pause between each one and don't switch them all on at the same time
2. Check that the HXP 120V is on if you would like to use the brightlight/epifluorescence on the microscope. It is located on the lower shelf under the computer. If you intend to use it and do not turn it on before launching the software, the Zen software will likely produce an error and the in-software controls will not work.
3. Log into iLABs
4. Launch ZEN black.
    a. Click: "Start System" The computer/Zen software is performing diagnostics with all the components of the microscope. This will take ~1-2 minutes to start up.

Zen Software: LOCATE 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.
    a. BF: brightfield. You can change the brightness of light to your specimen by moving the dial on the lower front of the microscope.
    b. DAPI
    c. GFP
    d. dsRED. For each of the three epifluorescence options, the intensity of the light can be controlled through the software OR by turning the dial on the power source (the HXP 120V)
2. 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 an **acquisition parameter**. Select from the saved acquisition parameters available in the dropdown menu that are accessed by clicking on 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: The lasers should start automatically with the selection of the acquisition parameter, but double check that the appropriate lasers have started up. Laser choices on this microscope are:
    - Argon laser (458, 488, 514nm),
    - 405 diode laser
    - 561 diode laser
    - 633 HeNe laser
    - Tunable Chameleon multiphoton laser (690-1040)

    a. If the argon laser is need, it will require about 5 minutes to warm up.
    b. If the Chameleon multiphoton laser is required, it will also need a few minutes to warm up.
3. 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.

4. Under Acquisition Mode
    a. Objective: here the objective can be selected. Objectives can also be selected using the touchscreen remote.
        - 10X: C-Apochromat 10x0.45 W M27 AIR
        - 25X: LCI Plan-Neofluar 25x/0.8Imm Korr DIC WATER (or Zeiss Immersol W)
        - 40X: LD C-Apochromat 40x/1.1 W Korr WATER (or Zeiss Immersol W)
        - 63X: LCI Plan-Neofluar 63x1.3 Imm Korr DIC WATER
        - 63X: Plan Apochromat 63X/1.4 DIC OIL (Immersol 518)

        i. Note: only the 63x 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 dual direction (<-->) scanning is faster and recommended for most users. Older microscopes had alignment problems that I have not seen on this model.
        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.

5. 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%. It is not uncommon to use lasers below 2%.
        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 (405, 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: drive. Saving you files elsewhere can interfere with the software on the computer and is not allowed. If these files 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.

Zen Software: ACQUISITION tab. Other windows

1. Focus: if you would rather focus using the software, this is where you can do it. It is not as convenient as the focus knob on touchpad remote or the microscope itself.

2. Focus Devices and Strategy: Here you can select “definite focus.” This setting can be useful for time lapse experiments to correct for the slight changes that occur overtime due to gravity and changing temperature. Before an acquisition, the microscope will send an infrared beam to measure the distance to the coverslip, and then make corrections if needed. You can set the interval that you would like to use
3. Time series: this window will appear if you check “time series” under the experiment manager.
4. Tile Scan: For very large specimen, it is possible to set up an automatic scan to collect a series of images in a grid pattern. You control how much overlap you would like the images to have. It will create a single montaged image for you.
5. Positions: The microscope will save XYZ positions. Scan and move to a region of interest and click “Add” under this window. After you select the positions, I recommend that you save them before you begin the experiment in case the computer crashes.
6. REGIONs: This allows you to target regions within the frame. Useful for photoactivation and bleaching experiments.
7. Stage: the microscope stage can be moved with the arrows on the field represented in this window. The stage can also be moved with the joystick controller on the table next to the microscope.

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.
2. Shut down the ZEN software.
3. Log out of iLAB.
4. 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
5. Put the dust cover back on the microscope
6. Once the lasers have had time to cool down, Turn off the power to the Systems/PC and Components before the main switch. Pause a second between each one.