Zeiss LSM 800 Confocal Microscope Procedures

Please do:

Turn on/off carefully and properly!

Never use chem wipes on any part of microscope!

Carefully clean objectives lens if it is necessary by using lens paper only!

If you do not know how to do this, please ask!

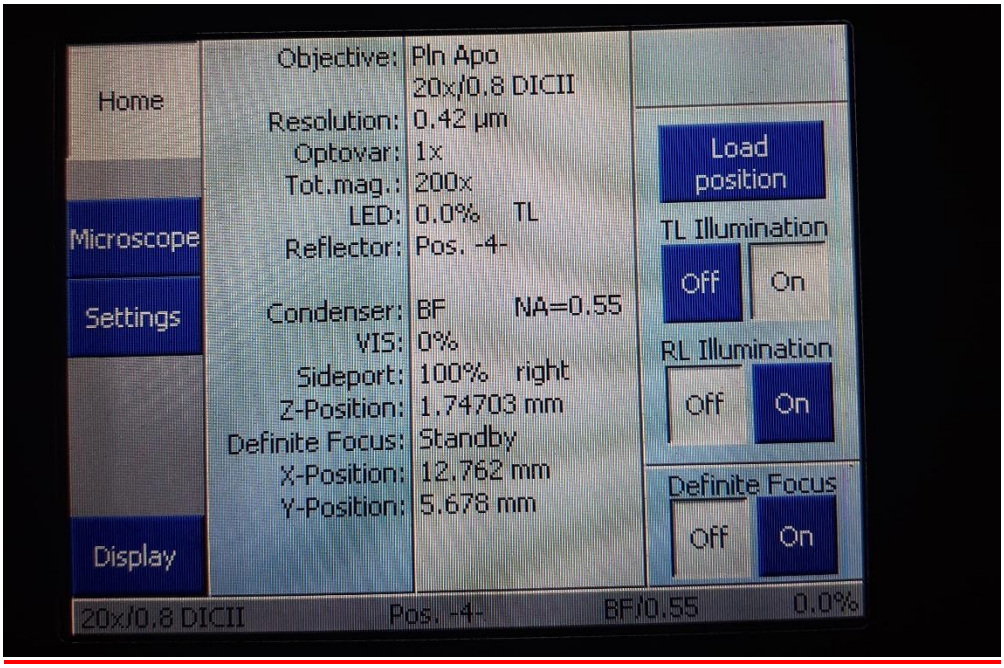
Clean up after yourself!

Please leave RAID back up disk station on at all times!

If you have any questions? Please contact Lori Cobani, cobanil@lafayette.edu or Jim Dearworth dearworj@lafayette.edu

Powering on and opening the software.

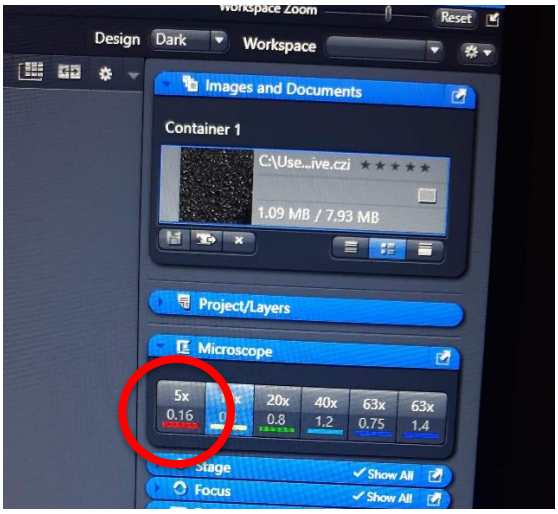
1. Power on the microscope by following the order below:
    a. Turn on Switch #1, located on the lower tower to the left of the microscope. Wait ~ 30 sec
    b. Turn on Switch #2, located directly adjacent to Switch #1.
    c. Wait ~ 30 sec
    d. Turn on Switch #3, located on the right side of the antivibration table.
    Wait patiently until touch pad has booted.



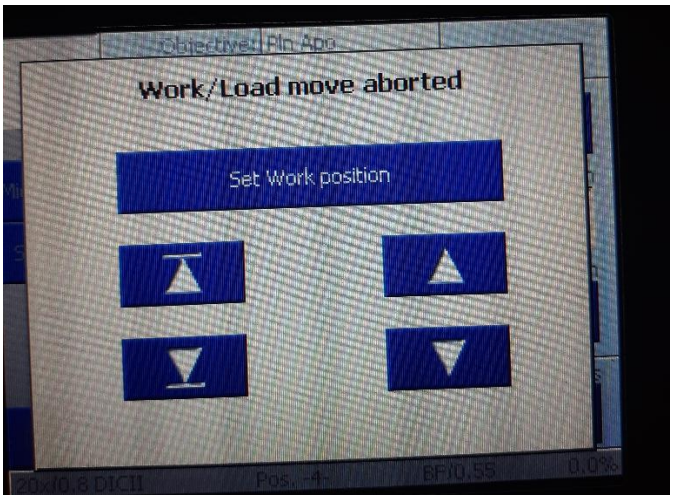
    e. Power on the computer tower labeled as #4
    Wait for software/drivers to be done loading.
2. Log in to the computer and open the Zen Blue software from the desktop, then select Zen System. **Click once and wait patiently.**
3. Turn on the TV monitor (if applicable).

Loading a slide and initializing and capturing an image with BrightField.

1. **If not already, set objective to 5x (the position does not have an objective so is most safe for loading slides and samples).**



2. Press the load position in the touchpad before placing the slide.
3. Gently push the arm back by pushing back on the top part of the arm past some resistance and slowly lower until it reaches the loading position.
4. Place the slide upside down and adjust the tray sliders so that the slide is secured but not locked into position, leaving a small amount of room on one side between the slide and the tray slider. (If for some reason the slide tray is not properly secured in the stage, please do so before placing the slide in the tray. Carefully insert the tray into the stage by pushing closest-left corner (red dots) against retaining springs and pushing firmly until it clicks into place. If not familiar with this, please ask someone who is.)
5. Firmly but carefully push the microscope arm down to the working position and close all the openings in the chamber.
6. Press the set work position on the touchpad.



7. With the mouse, from the blue *Microscope Control* tab on the left-center of the screen under the **Locate** main tab, make sure that the TL LED button below the lightbulb is

selected (Turn on the light to preferred light intensity). To change from T-PMT (if it is selected), click the box labeled T-PMT under the lightbulb. Ensure that the scope's shutter is open by toggling the button left of the TL LED button to open.

8. Set magnification to 10x by toggling the Motorized Nosepiece button, the third button down on the far left of the *Microscope control* tab.
9. Bring image into view through eyepieces by pressing the 100x eyepiece button near the bottom of the tab.
10. Increase the light intensity (if necessary) by toggling the lightbulb button at the top of the tab or using the manual light intensity control below the eyepieces on the microscope.
11. Bring image into focus by adjusting coarse and fine focus using knobs found on the right of the scope.
12. Bring image onto the computer screen by pressing the camera icon toward the bottom of the *Microscope Control* tab then clicking the **Live** button just above the tab.
13. Press the Set Exposure button just left of the **Live** button to set the image's exposure. If the image is still not clear, check the "Auto" box (or "Min/Max" "Best Fit" options) above the graph on the lower center portion of the screen, where the histogram is located.
14. Refocus image using fine focus knob, either on the microscope or on the control screen located separately from the computer monitor.
15. To capture an image, press the Snap button located to the right of the **Live** button, then save it as a .czi file by clicking "Save Image Data Here," then backup directory, e.g, then Dearworth Lab folder, then appropriate folder for project.

Widefield Acquisition Imaging

1. Click the **Acquisition** tab in the top left corner of the screen.
2. Click the "Widefield" button on the *Imaging Setup* portion on the left side of the screen and select the appropriate Dye or Contrasting method and click add or double click on the dye.
3. Click on the **Live** button on the top of the screen and adjust the image with the trackpad and the coarse and fine focus knobs.
4. Use the **Snap** button to capture images.
5. Remember to always save ALL images to the corresponding files. Save it as a .czi file by clicking "Save Image Data Here," then backup directory, then Dearworth Lab folder, then appropriate folder for project.

+LSM Confocal Acquisition Imaging

1. Click the **Acquisition** button in the top left corner of the screen.
2. Click the "+LSM Confocal" button on the *Imaging Setup* portion on the left side of the screen and select the appropriate Dye or Contrasting method and click add or double click on the dye. Go to *Channels* to ensure the correct laser(s) are on.
3. Click on the **Live** button on the top of the screen and adjust the image with the trackpad and the coarse and fine focus knobs.
   Minimize scanning except for when actually capturing images to reduce bleaching
4. Use the **Snap** button to capture images.
5. Remember to always save ALL images to the corresponding files. Then save it as a .czi file by clicking "Save Image Data Here," then backup directory, then Dearworth Lab folder, then appropriate folder for project.

Performing tiling experiments.

1. Start in the **Locate** tab.
2. Set objective to 20x by toggling the nosepiece icon found in the *Microscope Control* tab.
3. Refocus the image by repeating Steps 8-13 from the Loading a Slide procedure above.
4. Toggle the Motorized Path button (second from top, under lightbulb icon) in the *Microscope Control* tab to T-PMT and toggle the shutter button (directly to left) to Open.
5. In the same tab, open the RL Manual Fieldstop button to 50%.
6. Ensure the image is focused and ready to be tiled by repeating Steps 8-13 from the Loading a Slide procedure above.
7. Select the **Acquisition** tab from the top left of the screen, then check the box for "Tiles" on the left of the screen.
8. Under the blue *Imaging Setup* tab, select "+LSM Confocal" from the dropdown menu.
9. Under the gray *Smart Setup* tab on the upper left of the screen, press the plus button and select appropriate dyes and set the Proposal to "Smartest (Line)", then click OK.
10. Under the blue *Imaging Setup* tab, note which tracks each dye is found to be able to select them for tiling. Use the histogram to adjust dye overlaps to fit the experiment's needs.
11. Under the blue *Acquisition Mode* tab, press the "Optimal" button to optimize the frame size of the tiled image. The common frame size is 512x512 pixels. Set averaging to 4x.
12. Under the blue *Focus Strategy* tab, select "Use Focus Surface/Z Values Defined by Tile Setup" in the first drop down menu or select "By Software Autofocus" from the dropdown menu and check the "Adapt Focus Surface/Z Values" box
13. From the <<Multidimensional Acquisition>> menu, open the blue *Tiles* tab to set the dimensions of the tiling. Once an X/Y value is selected, press the plus (+) button.
14. Click the "Advanced Setup" button at the upper right of the *Tiles* tab then press the "Bring Navigator Into View" button at the bottom middle under the "Tiles" tab.
15. Use "Preview Scan" to check the location being imaged.
16. Under the gray *Display* tab, check the Auto box.

17. Ensure the image is ready to be tiled by briefly switching to **Live** view.
18. Under the *Preview Scan* tab, click the "Start Preview Scan" button.
19. Begin the tiling procedure by pressing the gray "Start Experiment" button. If there are multiple tiling dimensions in the blue *Tiles* tab, check that only the desired dimension is selected
20. To save an image, save it as a .czi file by clicking "Save Image Data Here," then backup directory, then Dearworth Lab folder, then appropriate folder for project.

Time Series

1. Set objective to 20x by toggling the nosepiece icon found in the *Microscope Control* tab.
2. Toggle the Motorized Path button (second from top) in the *Microscope Control* tab to T-PMT and toggle the shutter button (directly to left) to Open.
3. In the same tab, open the Manual Fieldstop button (to the right of the cube) to 50%.
4. Ensure the image is focused and ready to be tiled by repeating Steps 8-13 from the Loading a Slide procedure above.
5. Select the **Acquisition** tab from the top left of the screen, then check box for "Time Series" from the left of the screen.
6. Follow +LSM Confocal Acquisition Imaging procedure above.
7. Under the blue *Focus Strategy* tab, select "Definite Focus" from the dropdown menu
8. Click the *Time Series* tab and select the proper time unit and duration (seconds or minutes are often most useful); adjust interval if necessary. [Eg, duration 3 minutes, interval 2 seconds]
9. Begin the time series procedure by pressing the gray "Start Experiment" button.
10. To save an image, save it as a .czi file by clicking "Save Image Data Here," then backup directory, then Dearworth Lab folder, then appropriate folder for project.

Z-Stack

1. Set objective to 20x by toggling the nosepiece icon found in the *Microscope Control* tab.
2. Follow either Widefield Acquisition Imaging procedure above or +LSM Confocal Acquisition Imaging procedure above as appropriate.
3. Toggle the Motorized Path button (second from top) in the *Microscope Control* tab to T-PMT and toggle the shutter button (directly to left) to Open.
4. In the same tab, open the Manual Fieldstop button to 50%.
5. Ensure the image is focused and ready to be tiled by repeating Steps 8-13 from the Loading a Slide procedure above, **if a slide has not been loaded.**
6. Select the **Acquisition** tab from the top left of the screen, then check the box for "Z-Stack" from the left of the screen.
7. Under the blue *Focus Strategy* tab, select "By Software Autofocus" from the dropdown menu.

8. Turn on **Live** and open the *Z-Stack* tab. Use the fine focus to locate the lowest point of the sample and click 'Set First'; this will be the bottom of the z-stack. Use the fine focus to locate the highest point of the sample and click 'Set Last'; this will be the top of the z-stack.
9. Turn off **Live**.
10. Adjust the Interval, or select the "Optimal" button or the appropriate number of slices.
11. Click the "Start Experiment" button and don't forget to save your data.
12. To save an image, save it as a .czi file by clicking "Save Image Data Here," then backup directory, then Dearworth Lab folder, then appropriate folder for project.

Removing Slide

1. **Set objective to 5x (the position does not have an objective so is most safe for removing and loading slides).**
2. Press load position on the touchpad.
3. Gently push the arm back by pushing back on the top part of the arm past some resistance and slowly lower until it reaches the loading position.
4. Remove the slide.
5. Firmly but carefully push the microscope arm down to the working position and close all the openings in the chamber.

Closing the software and Powering off

If someone is signed up after you, please leave system on.

Sign the log book, indicate if there were any problems.

If no one is using after you, power off the microscope by following the order below
Turn off the TV monitor (if applicable)

1. Close/Exit Zen Blue software
   Wait until program has shut down!
2. Shut down the computer by clicking on the Windows Icon and choosing option "Shut down".
   Wait until PC has completely shut down!
   a. Turn off Switch #3, located on the right side of the antivibration table.
   Wait a few seconds
   b. Turn off Switch #2, located directly adjacent to Switch #1.
   Wait a few seconds
   c. Turn off Switch #1, located on the lower tower to the left of the microscope.
3. **Sign the log book, indicate if there were any problems.**