How to turn on/off Zeiss 880 confocal in Microscopy Core
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Yan.Deng@med.nyu.edu or Michael.Cammer@med.nyu.edu
Call (do not text) Michael at 914-309-3270 or call or text Yan at 347-788-8212

DO NOT GET OIL ON SIDE OF BOTTLE.

DO NOT PLACE OIL BOTTLES ANYWHERE OTHER THAN THE PETRI DISHES WHERE THEY LIVE.

Shutdown You are expected to follow these instructions as a checklist.

1. If oil or water lens, clean by gently moving taught paper across the lens top. Each swipe in one direction with a clean area of the tissue.
2. Set lens to 10X.
3. Turn focus on lens to lowest position. The touchpad will display a message when it is at the lowest position.
4. Check the schedule. If somebody is using immediately after you,
   * leave the power switches on,
   * leave the Ar laser in "Standby" mode, but follow the other instructions below.
   If somebody is not coming in immediately after you, turn all lasers off.
5. If environmental chamber on and next person does not need it, turn off.
6. Quit Zen.
7. Save all your data. http://microscopynotes.com/how-to-connect-to-server.html, other Internet location. *Do not use USB device unless it has been virus scanned by NYU MCIT.*
8. If no-one after you, switches 4, 3, 1 off.
9. Clean up. Clean up means look at all surfaces including the floor and pick up Kimwipes etc.
   Clean up means thoroughly cleaning any oil on the sides of bottles.
10. iLab Kiosk signoff.
11. Log out of computer.

How To Turn On System

Follow these instructions, not the numbered order of the switches. The wait step before switch 4 is critical.

1. Power switches 1 and 3 on.

2. If computer is off, turn it on.
   If the computer is already on, make sure it is logged out.
3. The computer must be on before you turn on switch 4. (This is a critical wait step.)
4. Log in to computer with account **LSM user**
5. *Optional:* Turn on environmental chamber and stage insert.
   * All doors/panels closed on incubator box. Have all stage inserts, oil, etc in incubator to be at proper temperature. *To minimize focus drift, this chamber should be on for two hours before you start imaging.*
   On Zeiss touchscreen:
   * Settings > Incubation, H Dev, optional H Insert P
6. iLab Kiosk
   Log in to your iLab account and start the Kiosk.
7. Run Zen Black software (icon should be at center of screen).
8. Click "Start System."
   While the system is in startup mode, do not touch any microscope controls.
9. If you need the 488 nm laser, immediately go into the laser window in the acquisition tab and turn it on. (You may turn on the other lasers you need too.)
10. Make sure the oil or other immersion medium you are using matches the lens. Using the wrong oil will make images look bad.
11. When in Acquisition tab, open an image with the settings you need and click Reuse button.
12. Locate tab to look by eye.
    Acquisition tab to take pictures by confocal.

General Confocal Best practices:

- The pinhole is what makes the confocal a confocal. Set at 1AU (which means 1 Airy unit) and click the 1AU button each time you change lenses. 0.5 to 1.0 AU ok.
  If you are opening it for imaging fixed samples, you should use a widefield fluorescence scope instead.
  Except in special case of live cell imaging where you understand that images are not confocal, this is NOT AN ACCEPTABLE WAY TO MAKE IMAGES BRIGHTER. You won't hurt the instrument, but when you write your methods, you won't be accurately describing your microscopy as "confocal".
- Offset. Always use at 0 or 1.
  Other numbers are wrong.
- Digital gain. The preset is 1. Leave it there.
- Use the Range Indicator button to make sure you have no saturated pixels. If you see red pixels, you need to turn down the Gain or Laser.
  Turning gain down will reduce noise. Less noise means you can scan faster. (More here.)

DO NOT SATURATE THE IMAGES. The Zeiss 880 has detectors that may be permanently damaged by repeated saturation.

Tiling or mosaics:
Zoom set at 1X to 1.25X. If you zoom out, the edges of each tile will be darker than the center.
With this microscope, it is best to have 10% overlap and online stitching turned on.
If you want to save each original tile, turn off online stitching and images need to be stitched in the Process tab with "fuse tiles" turned on.

Saving Files

All files should be stored in Drive D:.

Files left on the desktop, drive C, Pictures folder, etc will be deleted.

Use CZI format. These files can be opened directly into image analysis software. These files retain instrument settings, channel integrity, bit depth, and spatial scale that may be necessary for image analysis.

If you save files as TIF or other formats, the integrity of color channels may be lost and you will have no metadata regarding instrument settings and spatial scale.

NEVER use JPG.

Move data to your lab's shared server space. Instructions:
http://microscopynotes.com/how-to-connect-to-server.html