# Quick Start for Zeiss LSM 780 Confocal Microscope

1. Starting the LSM 780 Confocal Microscope

- Wait 30 seconds before turning on each switch. Turn on Main Switch (1) then turn on System PC (2) switch and components switch (3). X-Cite will turn on with Main switch (turn X-cite power off doing overnight expts)

- If you need Lasos Argon laser (458nm, 488nm, 514nm) switch (black box) turn key (4) to position 1 (90° clockwise) if you will use it and flip switch up to "Run" not "idle"(5) . If the system is left on for you check the Argon laser is in "run" mode. When done imaging switch to "idle" before turning off.

- Wait for the touchpad to finish initializing, then turn on the computer (6) and log in to "OBC" account.
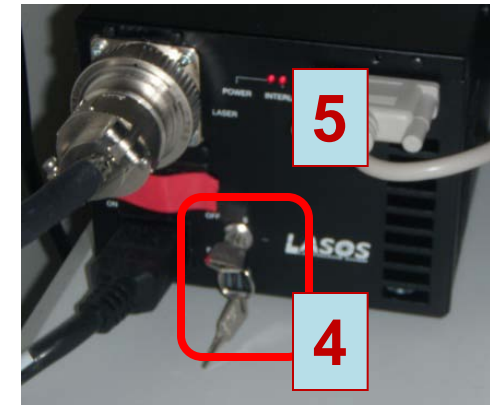
2. Starting the LSM 780 Software

- Wait 2minutes after logging in before opening ZEN 2010 on the desktop , choose "Start System" to begin software initialization.

- Select "Boot Status" to make sure all the systems boot up properly.

- *IF real time error appears, open REAL TIME PC under table, and push "reset" button"
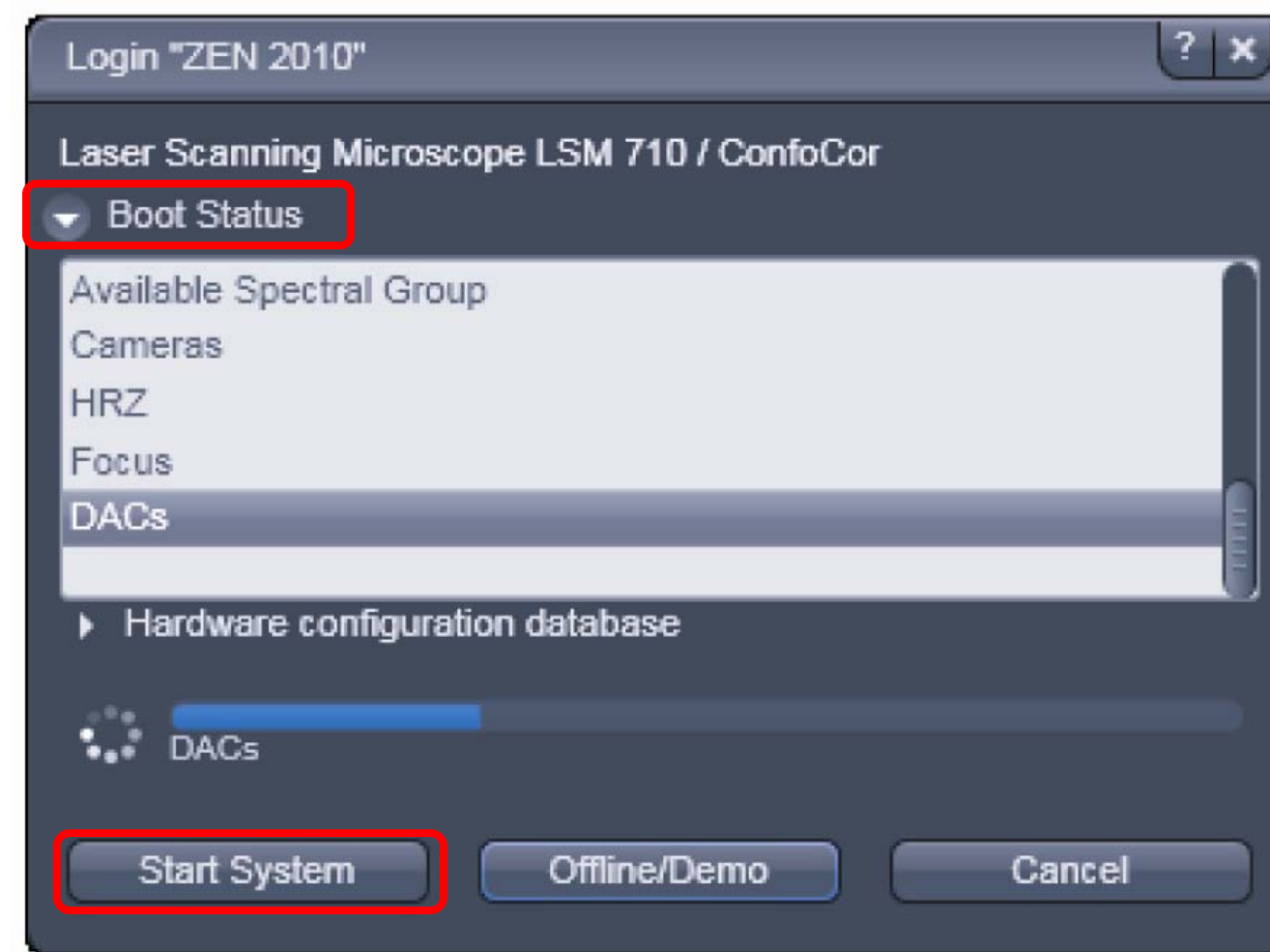
3. Turning on the Lasers

- If you need the 2-photon laser: the Mai Tai must be turned on from the "MaiTai" external computer located next to META 1. Instructions are posted next to MaiTai computer, if you are unfamiliar with this, please ask Adeela for assistance.

- When setting up your configurations under "smart setup" or "reuse" software will automatically prompt you to turn on lasers.

4. Using Smart Setup – Configuring Settings

- Click on "Smart Setup" or load saved configuration.

- Click the arrow under "Dye" to open a drop-down window with a list of common dyes and fluorophores in the database and appropriate select dyes.

- You will be given three choices of configurations – Fastest (single track), Best Signal (multi-track), and Best Compromise. Choose the configuration that best fits your sample. Note: Best Signal will take longest to acquire, but will cut down on potential bleed-through between channels.

- Click "Apply". The software will automatically configure the parameters to appropriate settings for the dyes chosen.
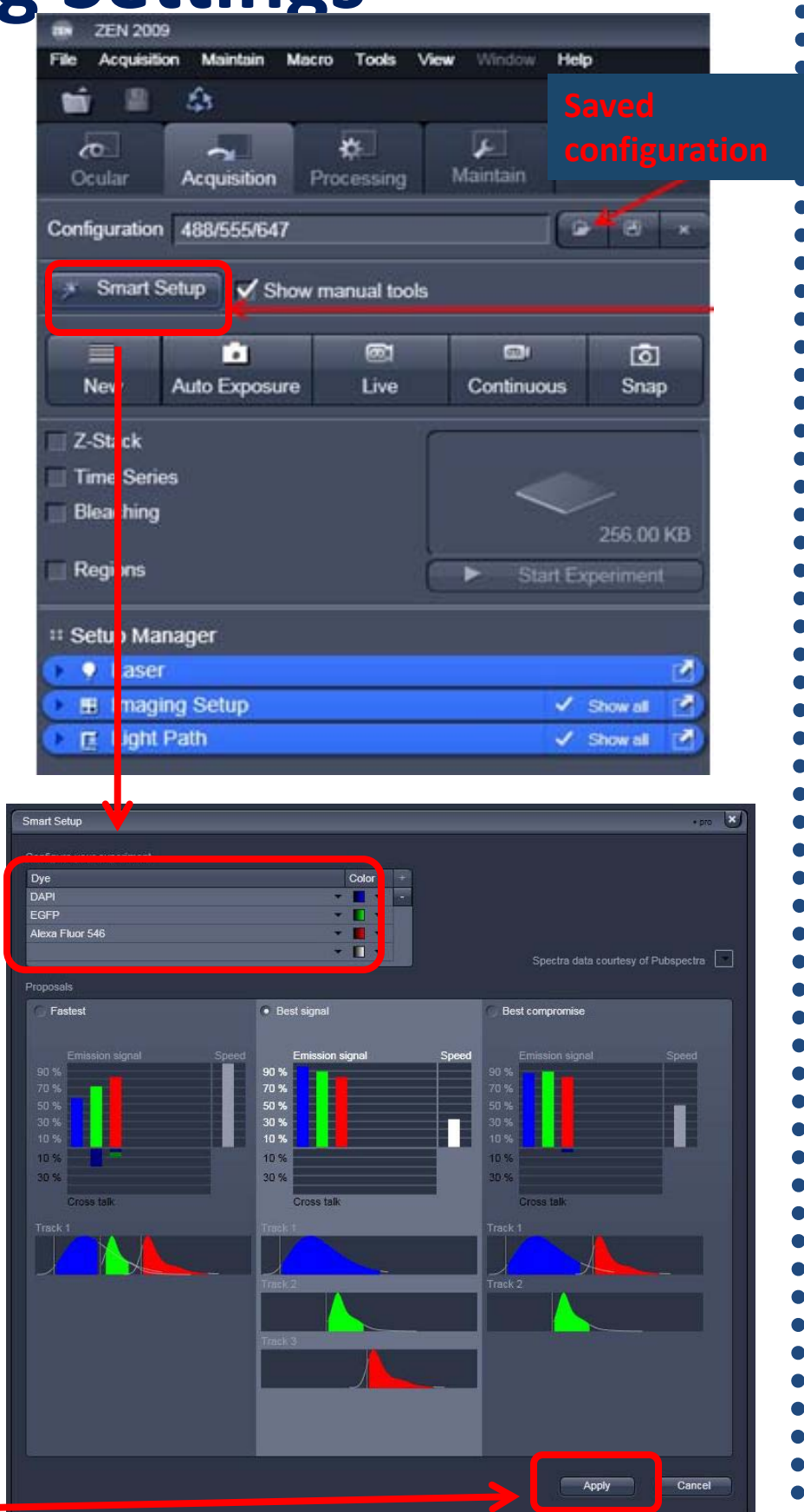
5. Finding Your Specimen

- Go to the Ocular tab (1), click "online" (2) and choose the filter for viewing (DAPI, GFP, dsRed).

- In the "Light Path" window under the "Ocular" tab, click on the objective icon (3) to select desired objective, or select from touch pad. Apply oil or nanopure water if needed. Be careful not to put oil onto an air or water objective!!

- Turn on the fluorescence light by clicking the "On" (4) button to apply the correct settings. To view using transmitted light turn "On" light (5)
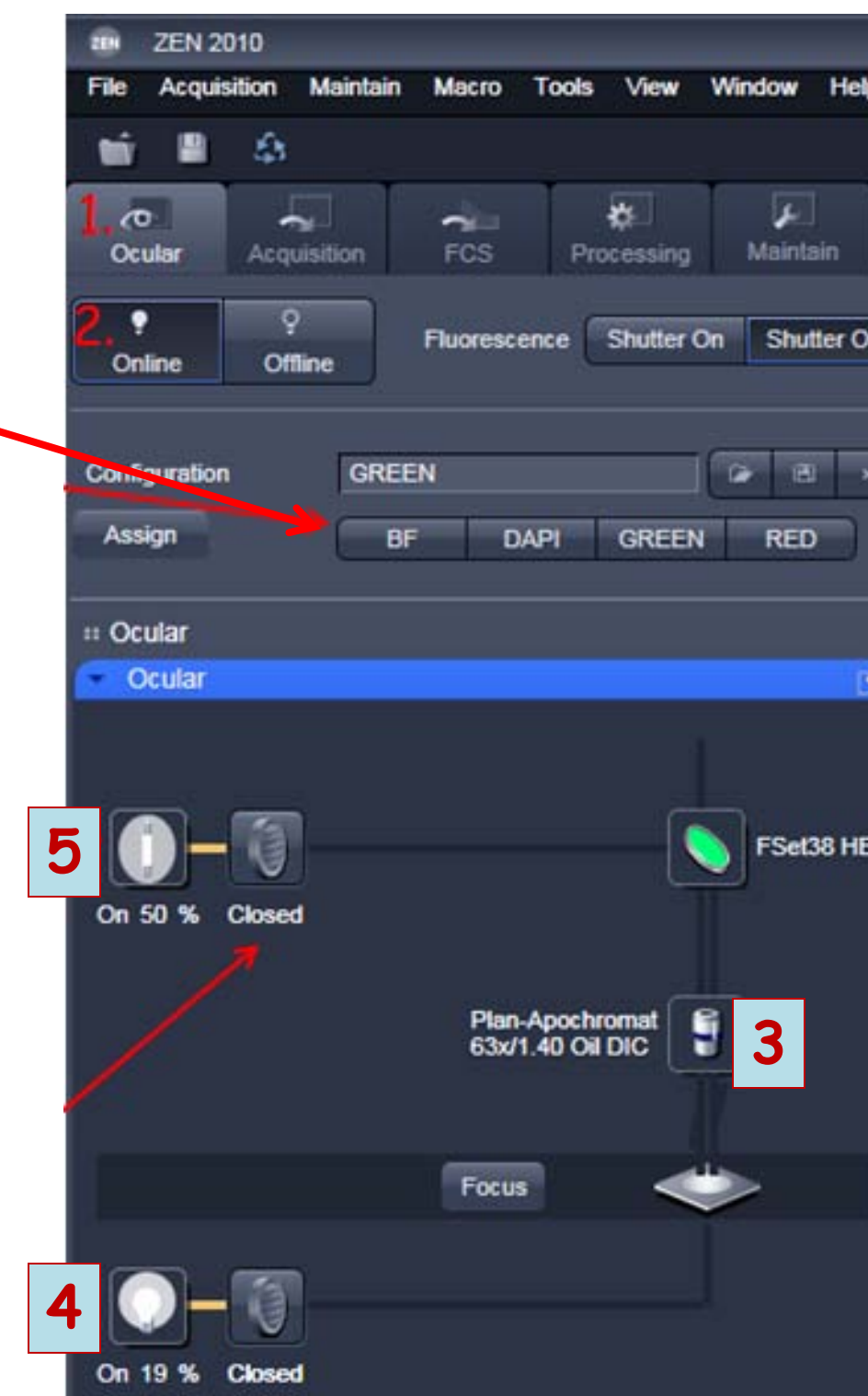
6. Acquiring a Preliminary Image

- Open the Channels window and select a channel to optimize. (If doing multi-track, it is easiest optimize one track at a time)

- Set "Pinhole" to 1 Airy unit (click 1 AU button) for your most important channel. Match the optical slice thickness of all other channels by adjusting the pinholes. For 2-photon, select "Max" pinhole

- Use either "Live" (fast scan) or "Continuous" buttons to start the scanning procedure to acquire an image.
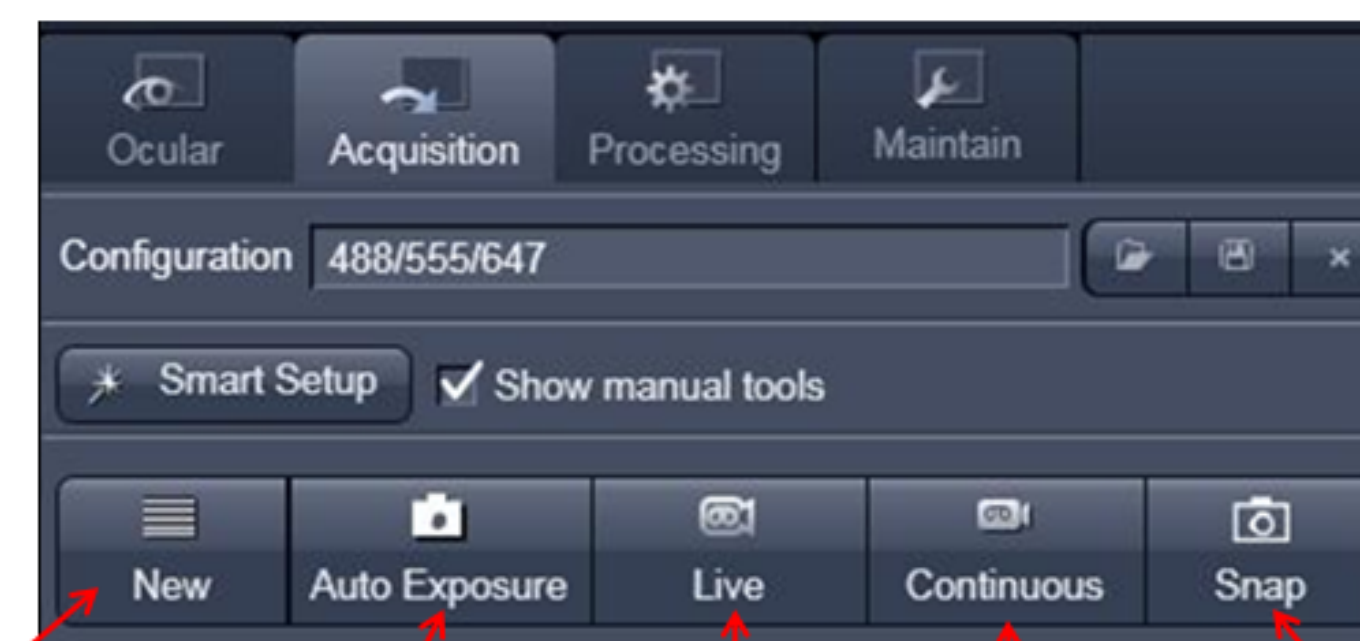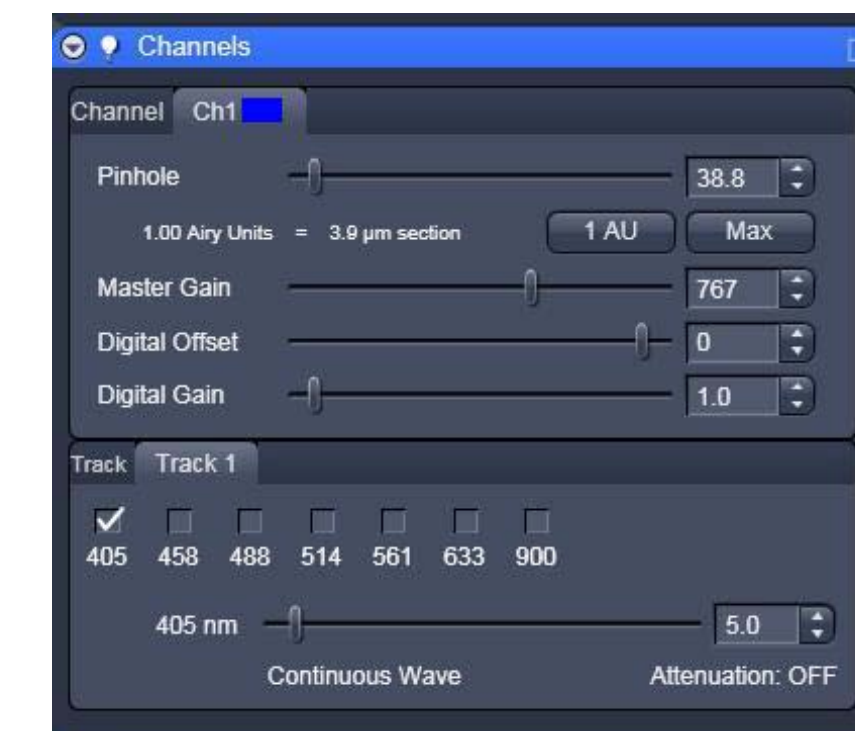
Create a new image tab — Auto-gain adjustment — Constant scan at 1 fps, 512px – a preview mode for adjusting — Scan continuously with the averaging and scan parameters you have set — Take a single 2D image with whatever settings you have specified

- To optimize the brightness of the image, adjust laser power and change the "Master Gain" (voltage across PMT)

- Adjust "Digital Offset" (detector black level, default is 0) and "Digital Gain" (digital multiplier, default is 1)

- To optimize your image, select "range indicator" (grey arrow) Red = maximum saturation, blue = minimum saturation (zero).
- Adjust Master Gain from 450-950 or laser power to have only a few red pixels.
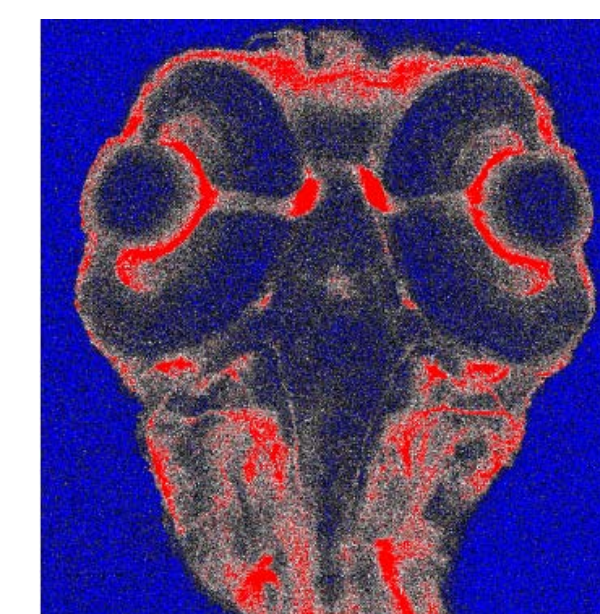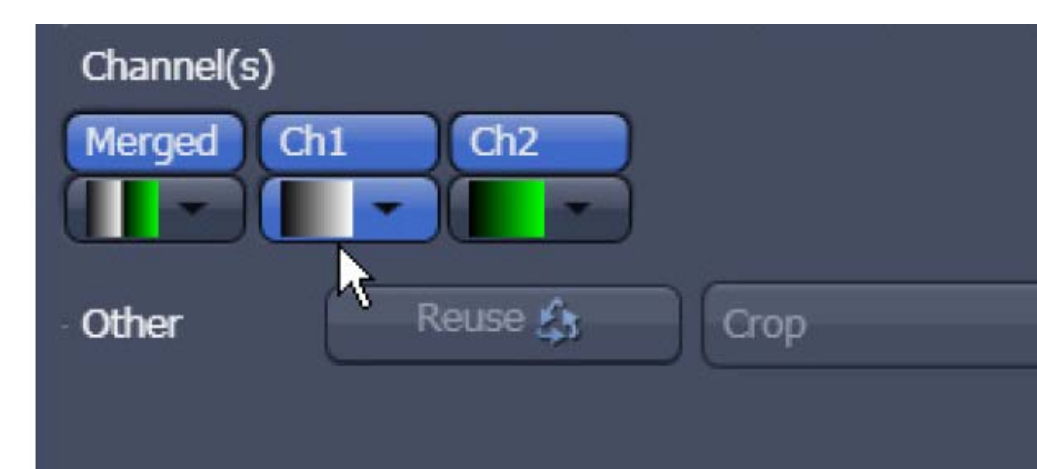
7. Acquiring Your Final Image (Single XY Plane)

Once you are satisfied that each channel's detector is set optimally to the range of your image, you can create your final image.

- Go to the "Acquisition Mode" window.

- Choose a frame size resolution (number of pixels per frame).

- Recommended: reduce noise by slowing the scan speed and averaging a number of lines.

  For example, you could try :
  Frame size: 1024 x 102
  Scan speed (pixel dwell time) : 8
  Bit depth: 12 Bit
  Averaging Number: 4

- Click on the "Single" button in the Scan control window to acquire your final image. (Make sure all tracks are turned on)

- Save your image in your own folder on the OBC server. It is recommended that you save as LSM 5 (.lsm) file type, which will have all of the Zeiss setting parameters, even if you eventually want to export to TIFF, etc.

- If using "auto save" function save images onto D drive and then move to your O

- Z-stack, Time series, bleaching, Tile scan, Regions, etc can be selected in menu under "ocular" tab. Once activate adjust experiment parameters and click "start experiment" .

8. Shutting the System Down

Check online calendar to see if another user coming in up to an hour after you, and CALL TO CONFIRM THEY ARE COMING (especially if it is near the end of the day). If there is another user coming in, close all files, exit the ZEN program, and clean used oil/water objectives but leave everything else ON!!

If there is not another user coming in after you, follow the complete shutdown procedure:

1. Clean any oil or water objectives that were used. Log your time onto sign in sheet and include any problems.
2. Switch to 10x objective
3. Exit the ZEN software.
4. Choose shut down from Start Menu
5. Flip Lasos switch down to "idle", turn key to off (90° counter-clockwise) WAIT FOR 5 MINUTES FOR THE LASER TO COOL BEFORE PROCEEDING TO STEP 4!
6. After the Argon laser is cooled, turn off the two component switches (3 and 2), followed by the Main Switch (1).