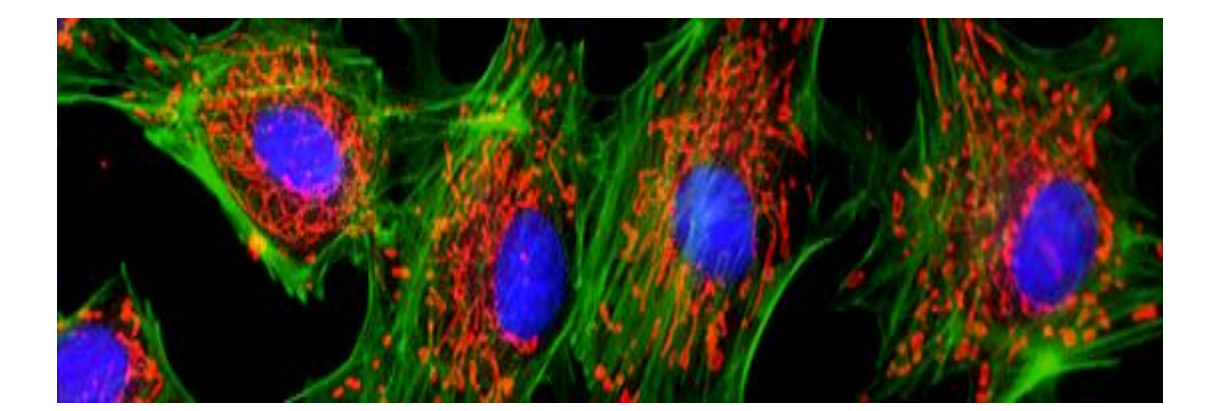# Quick Start for Zeiss LSM 710 Confocal Microscope

1. Starting the LSM 710 Confocal Microscope

1. First turn on the Main Switch, followed by the System/PC. Turn the computer ON wait till it starts loading (letter s on the screen) Then turn Components ON

2. Turn the key 90° clockwise on the Argon laser (458nm, 488nm, 514nm) if you will be using it.

3. Turn the switch on the Lasos controller module from standby to run.

4. Turn X-Cite light source for fluorescent ON and set the power level to one.
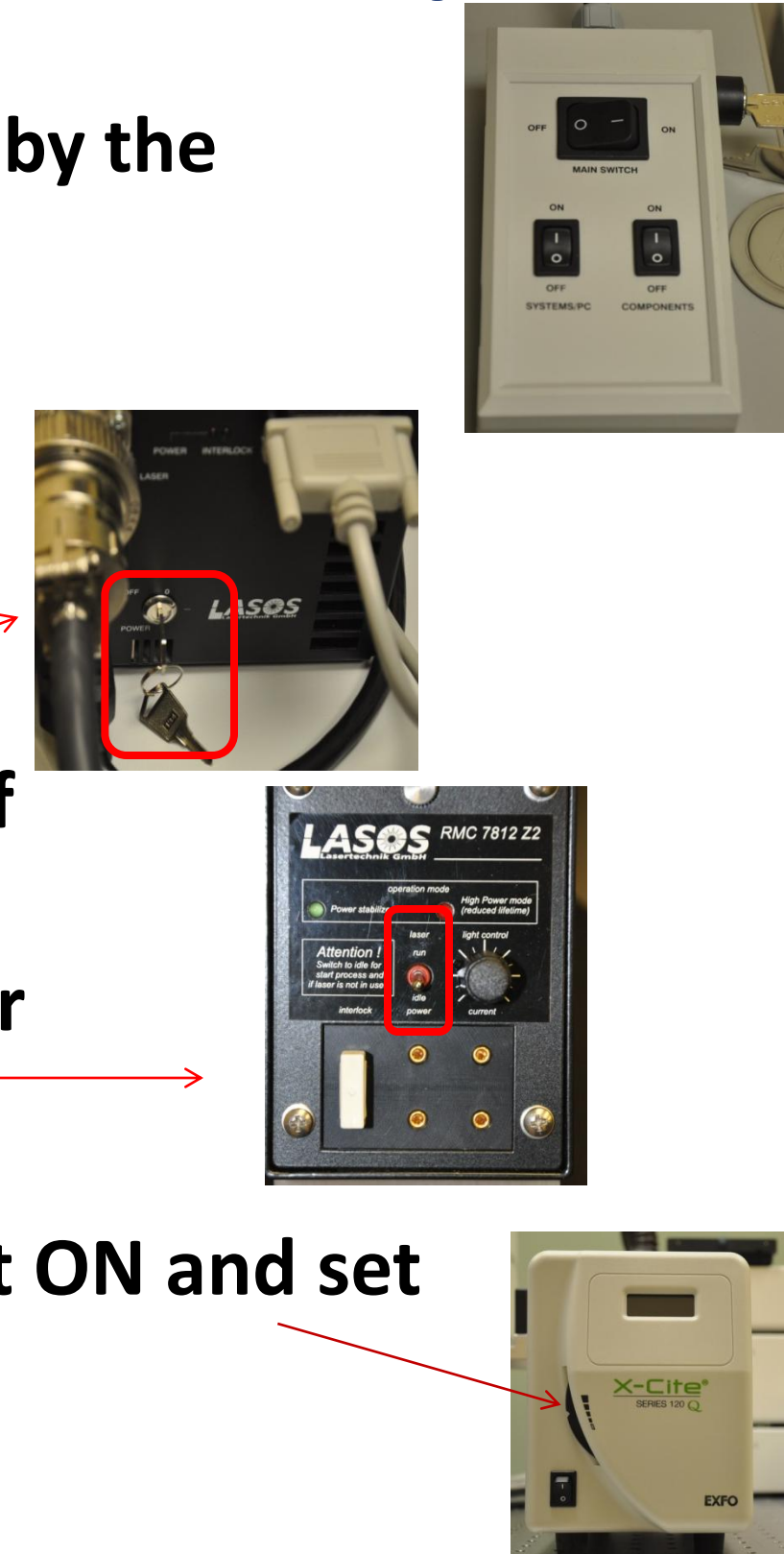
## 2. Starting the LSM 710 Software

1. Login: LSMUser
2. Password: qblood
3. Double click the ZEN 2009 icon found on desktop.
4. Click once on "Start System" to begin software initialization.

3. Turning on the Lasers

- **If you need the Argon laser (458, 488, 514):** the laser should have already been turned on when starting up the system by turning the key on the unit.

- **If you need the 561nm laser:** turn the power to "On" through the ZEN software in the Laser window under Setup Manager.

- **If you need the 405nm, 594 or 633nm laser:** these lasers will turn on automatically with the system.

- If you need the 2-photon laser: the Chameleon must be turned on by turning the key on the power module.
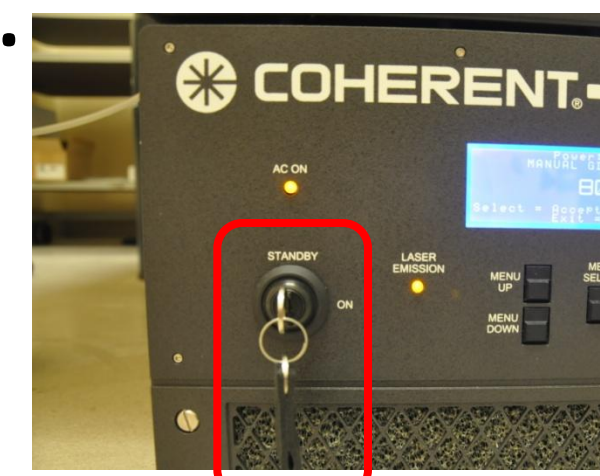
4. Finding Your Specimen

1. Load your sample "upside down" onto the stage of the microscope.

2. Under "Ocular" tab in the top left corner of ZEN click on "online" and "Brightfield" so you can see your sample.

3. In the "Light Path" window under the "Ocular" tab, click on the objective icon to select desired objective. Apply oil or nanopure water if needed. **Be careful & read the touchpad.! Do not put oil onto an air or water objective!!**

4. Turn on the transmitted light by clicking the "Brightfield" configuration button to apply the correct settings. Make sure to have a filter (blue, green, red) in the light path.
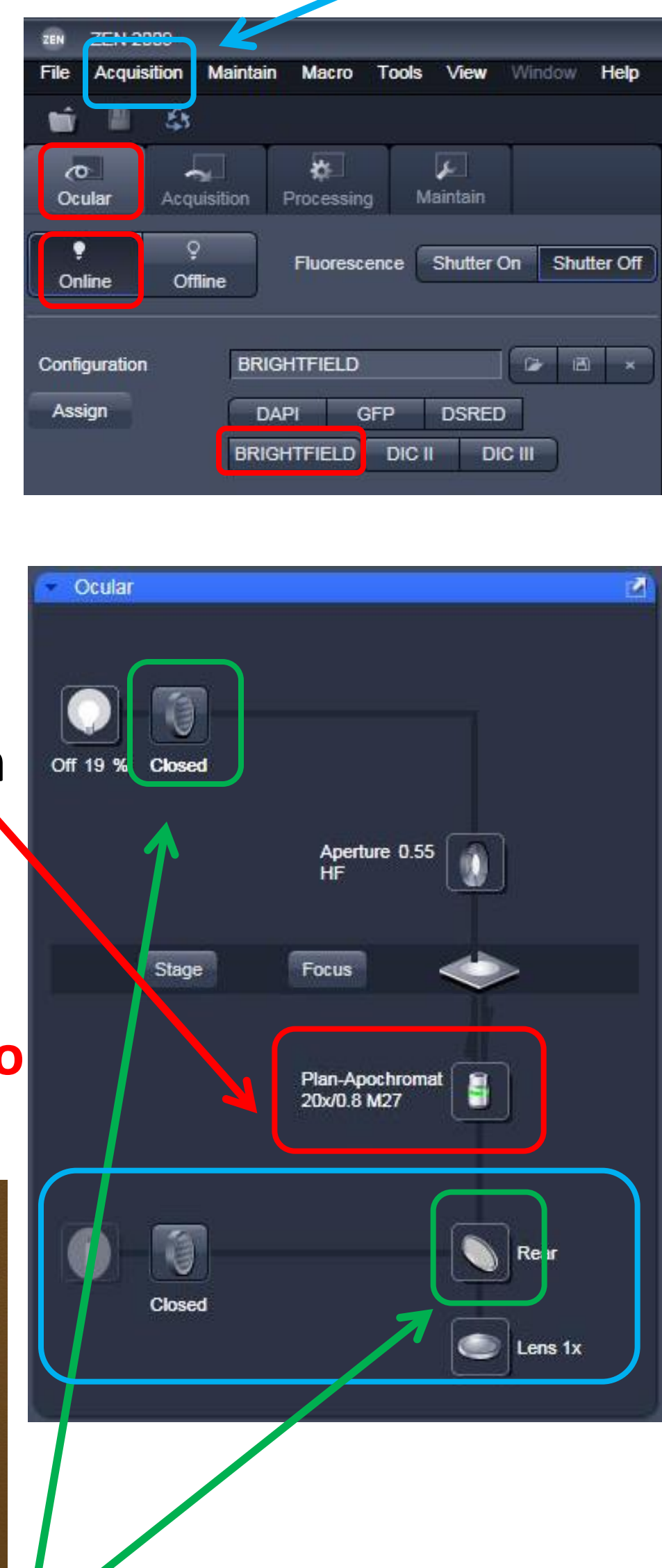
5. To see the fluorescent image through the eyepiece, select a filter for viewing the fluorescence (blue, green, red) the shutter will automatically turn on.

6. Move your sample to the correct location and focus on your specimen. After you are done click "Shutter Off"

5. Using Smart Setup – Configuring Settings

1. Under Acquisition Tab. Click on "Smart Setup."

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

3. 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.**

4. Click "Apply". The software will automatically configure the parameters to appropriate settings for the dyes chosen.
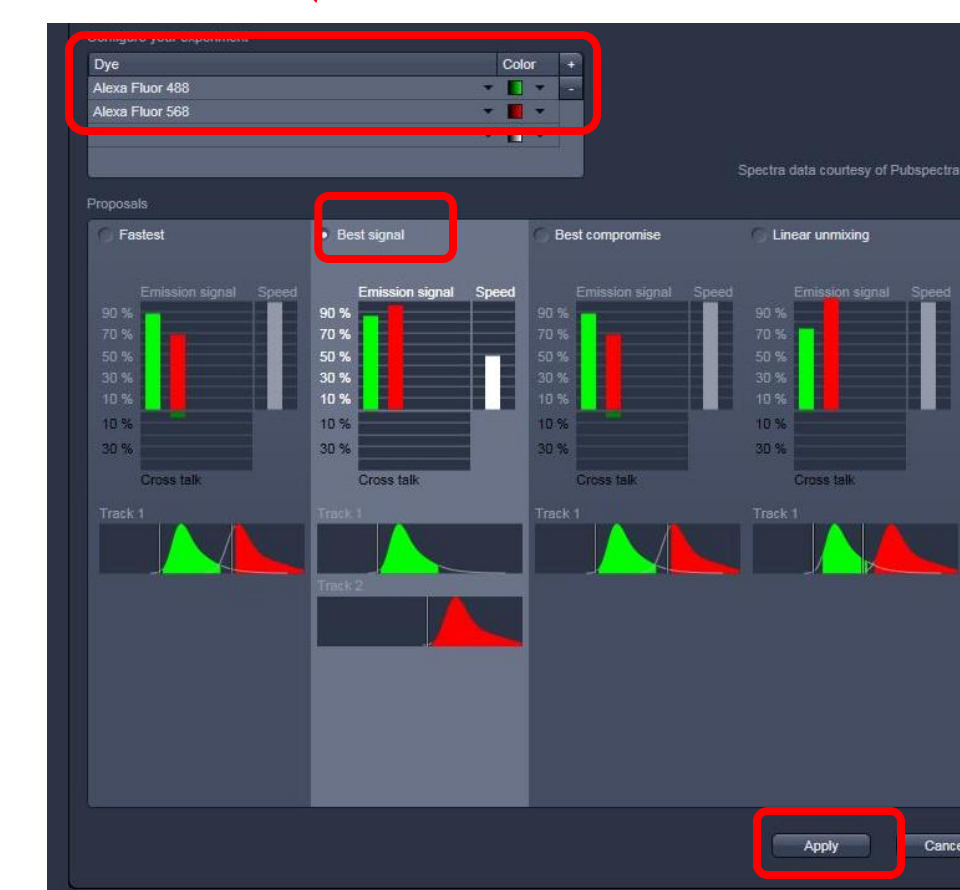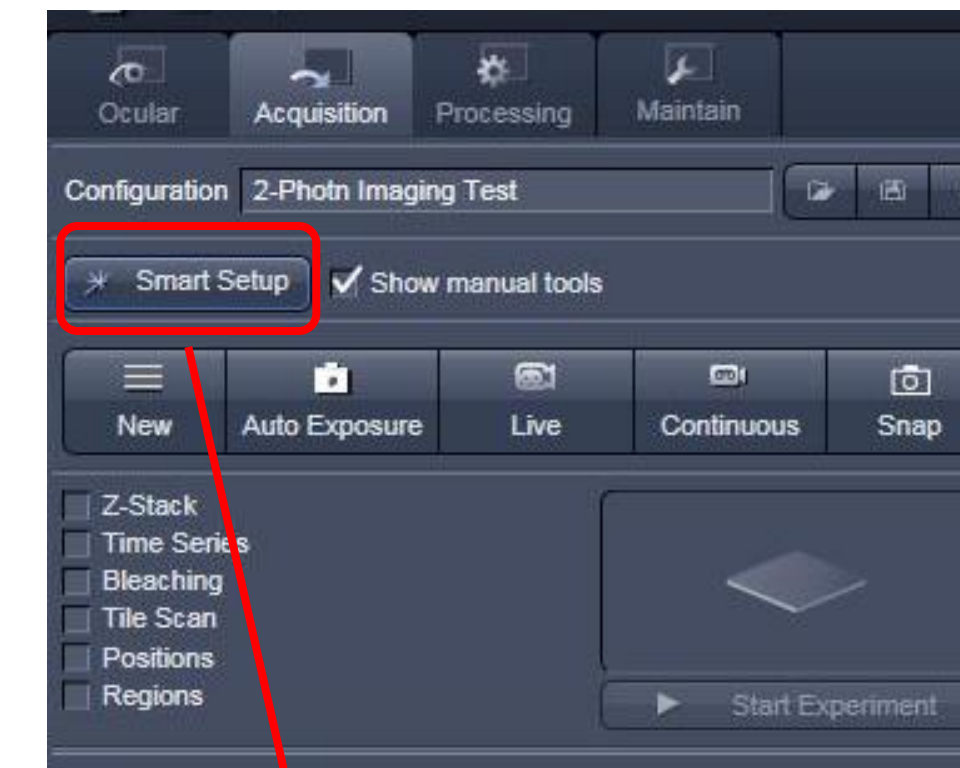
6. Acquiring a Preliminary Image

1. Under the Acquisition tab go to the "Channels" window. Select all channel to optimize.

2. 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, leave the pinhole open all the way.

3. Click "Auto Exposure" this will automatically pre-adjust the detector and gain by the Zen 2009

4. Now if doing multi-track, its easiest to turn off the other tracks and only optimize ONE at a time

5. Use one of the "Live" or "Continuous" buttons to start the scanning procedure to acquire an image.

6. Under the "Dimension" Tab in the middle of the screen, Click on the Range indicator (color box) and this will show how much saturation (red) or background (blue) your image has.

7. Alternatively, change the "Gain Master" to increase or decrease the detector sensitivity (Adjust for saturation). and "Digital Offset" for background

8. Adjust focus as needed.

9. Adjust "Digital Offset" (default is 0) and "Digital Gain" (default is 1) to increase contrast in the image or minimize background signal.
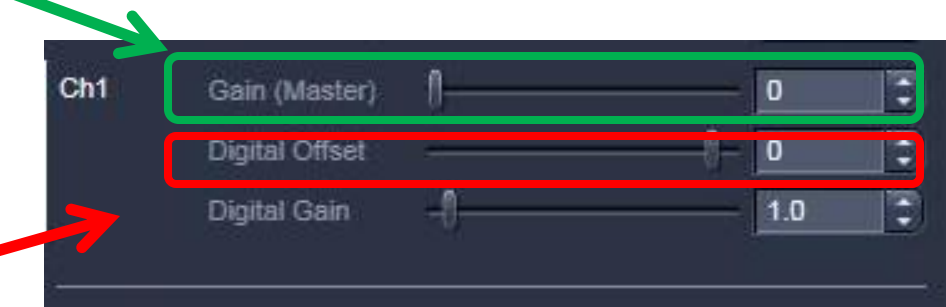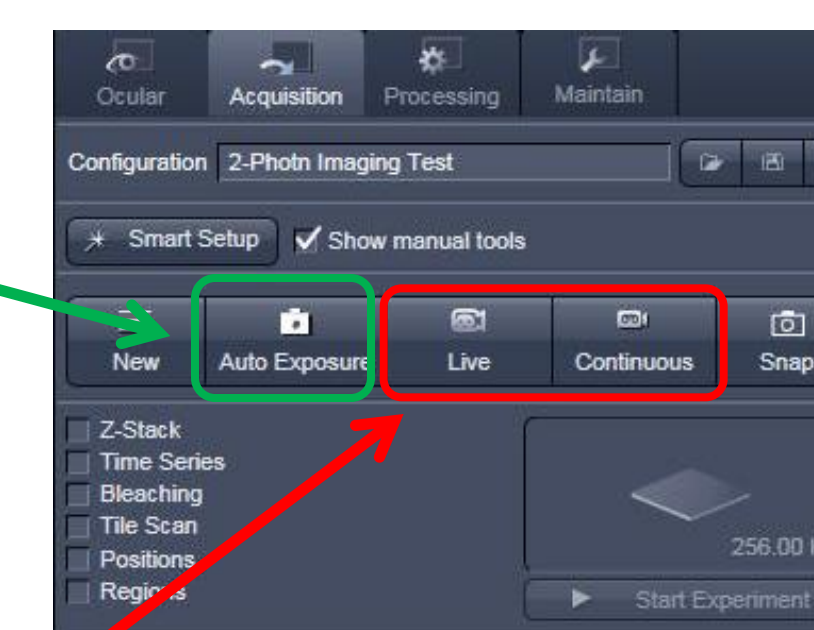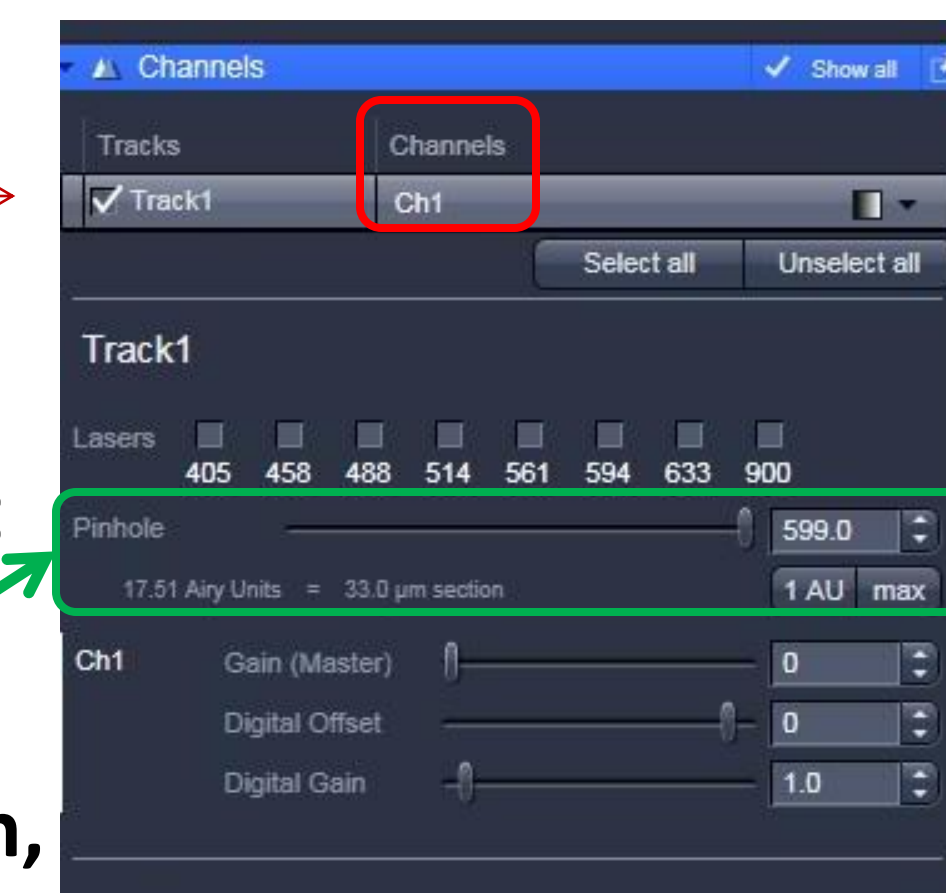
7. Acquiring Your Final Image (Single XY Plane)

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

1. Go to the "Acquisition Mode" window.

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

3. Recommended: reduce noise by slowing the scan speed and averaging a number of lines.
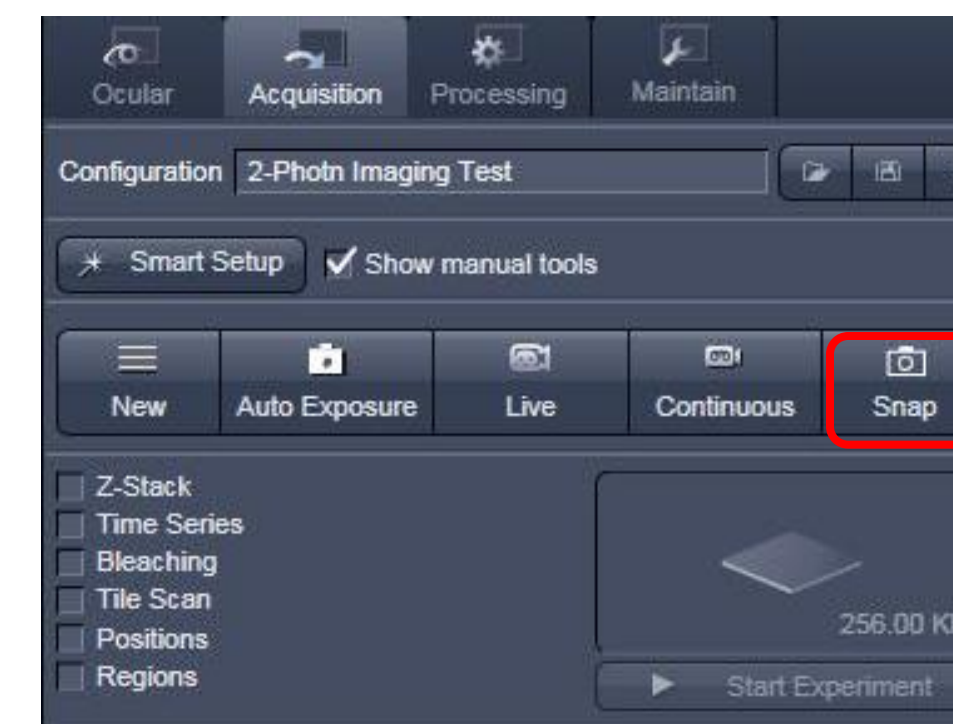
   For publication, you could try the following parameters:

   Frame size: 1024 x 1024
   Scan speed (pixel dwell time) : 7
   Bit depth: 16 Bit
   Averaging Number: 4

4. Turn all your tracks ON

5. Click on the "Snap" button in the Scan control window to acquire your final image.

5. Save your image in your own folder on the LSM710-WORKSTATION D: Drive (File → Save As). 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.

8. Shutting the System Down

Please first check the online calendar to see if there is another user coming in up to an hour after you, and call to confirm they are still 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 slides, turn the argon laser to standby and power the x-cite lamp to zero, but leave everything else ON!!**

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

1. Move the objective to the empty position

2. Turn the Argon laser to standby and the key off (90° counter-clockwise) if it is on. **WAIT FOR 10 MINUTES FOR THE LASER TO COOL BEFORE PROCEEDING TO STEP 6! Feel the fan on the back of the LASOS box. It needs to be off.**

3. Backup your data.

4. Turn off the 561nm laser in the laser control window (if turned on).

5. Exit the ZEN software.

6. Choose "Shut Down" from the Start Menu to shut down the computer.

7. Turn off the X-cite lamp

8. After the Argon laser is cooled, turn off the two component switches, followed by the Main Switch.

9. Clean any oil or water slides that were used but **DO NOT** clean OBJECTIVES! log your usage on the sign-in sheet and note any problems encountered. Thank you!