Quick Guide for Zeiss 710 Laser Scanning Confocal
MGH Cancer Center

For any questions or concerns, please contact:

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Power Up

Turn on equipment in the following order:

1. Main System Power
   - **Make sure Key on right hand side in in the horizontal position.**
   - 1A. Flip **MAIN SWTICH** to **ON** position
   - 1B. Flip **SYSTEM/PC** switch to **ON** position
     i. Wait for the Definite Focus module to display **OFF**
   - 1C. Flip **COMPONENTS** switch to **ON** position
     i. Wait for components to initialize
2. Argon Laser Black Box Power Supply
   - Turn the key to the right (clockwise)
3. Argon Laser Remote Controller
   - Flip switch to **RUN** position
4. X-Cite widefield fluorescence light source
   - Flip switch to **ON** position
   - Write down the bulb hours on the sign in sheet
5. Turn on PC
   - Login as **LSM USER**
   - Wait for ~3 min for RTC to establish connection with computer
6. Zen software
   - Double click ZEN black icon in center of desktop **(Not Zen blue)**
   - When startup screen appears, click on the **BOOT STATUS** drop down menu
   - Click **START SYSTEM**
     i. If the screen stalls at 2% or an error about the Real Time Controllers pops up. Press the RTC reset button on the box under the confocal for 5 seconds. Wait 30 seconds. If it fails to continue, close the software, press the button again, and then restart the ZEN software. If that fails, close Zen software, then power off the RTC. Wait 30 seconds. Power the RTC back on. Wait 1-2 minutes. Re-launch Zen.

When startup screen appears, click on **BOOT STATUS** then **START SYSTEM**

Loading your sample

1. Push microscope head back. Do NOT push on gray HAL100 light box.
2. On Touchpad, choose objective. View using **10X** first, then progress to higher magnification
3. Tap **LOAD POSITION** (Drops down stage)
4. Load slide onto stage coverslip down (inverted microscope)
5. Bring microscope head back into position
6. On Touchpad, tap **SET WORK POSITION**

Finding your sample

1. In Zen, go to the **LOCATE** tab

2. Choose configuration:

   a. **BRIGHTFIELD**

   b. **DIC II** – For use with dry objectives
      i. 10x, 20x, 63x dry
      ii. Requires polarizers

   c. **DIC III** – for use with wet objectives
      i. 40x oil, 40x water, 63x oil
      ii. Requires polarizers

   d. **DAPI** – blue filter

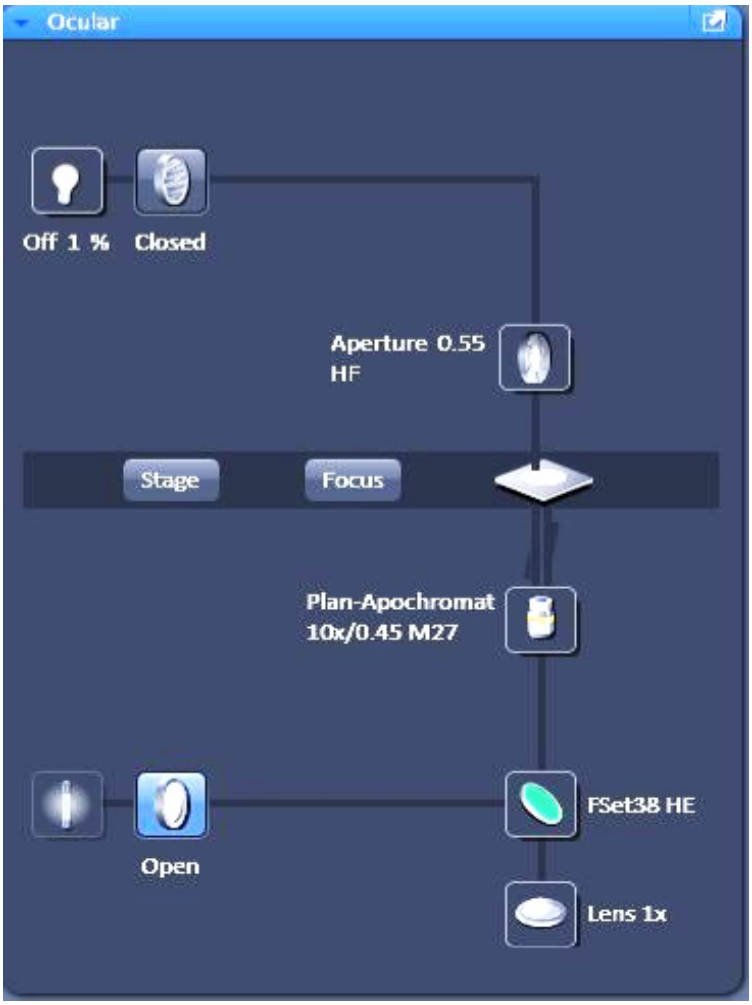
   e. **GFP** – green filter

   f. **DsRED** – red filter

3. Focus on sample

Too bright? Lower bright field transmission in ocular tab or using large black dial on XCite light box.

4. Adjust Kohler illumination, if needed:
   a. Close top aperture
   b. Focus with condenser head until a clear hexagon is formed
   c. Center the hexagon by adjusting the centering screws
   d. Re-open top aperture

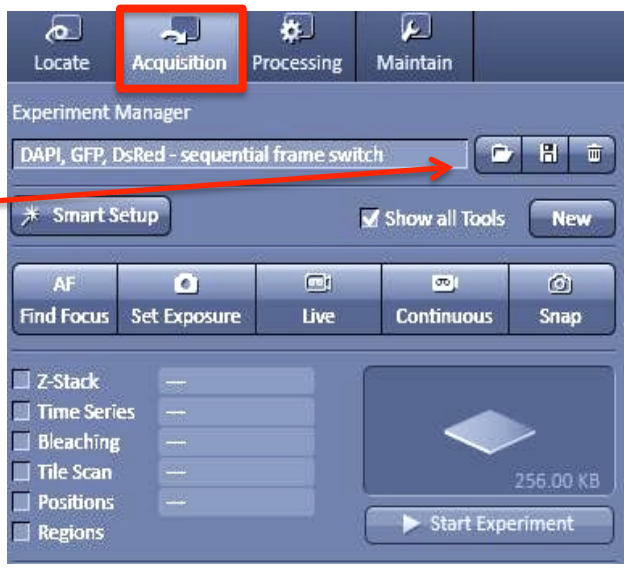


Setting confocal configuration

1. Click **ACQUISITION** tab to enter confocal mode

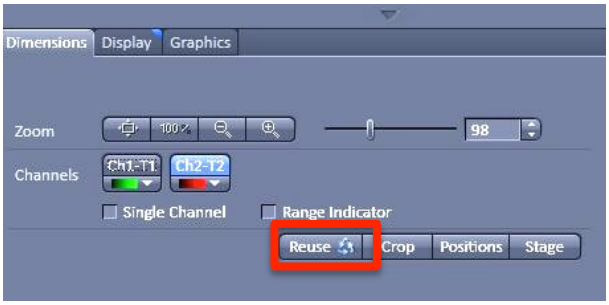
NOTE: There are three different ways to setup a scan:

   a. Load saved configuration *(These can be changed, so check before use.)*
   b. Load template image (File / Open), then click **REUSE**
   c. Smart Setup (see instructions below)

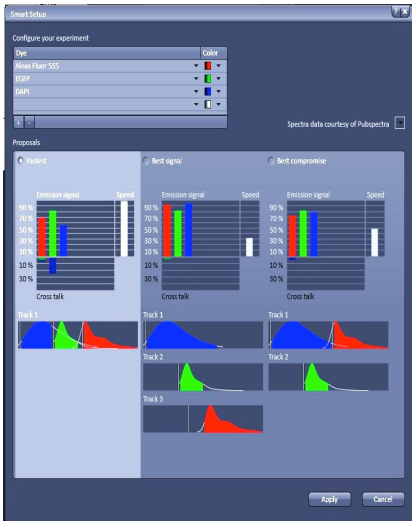


a. Load Configuration

b. Re-use template image settings



c. Smart Setup



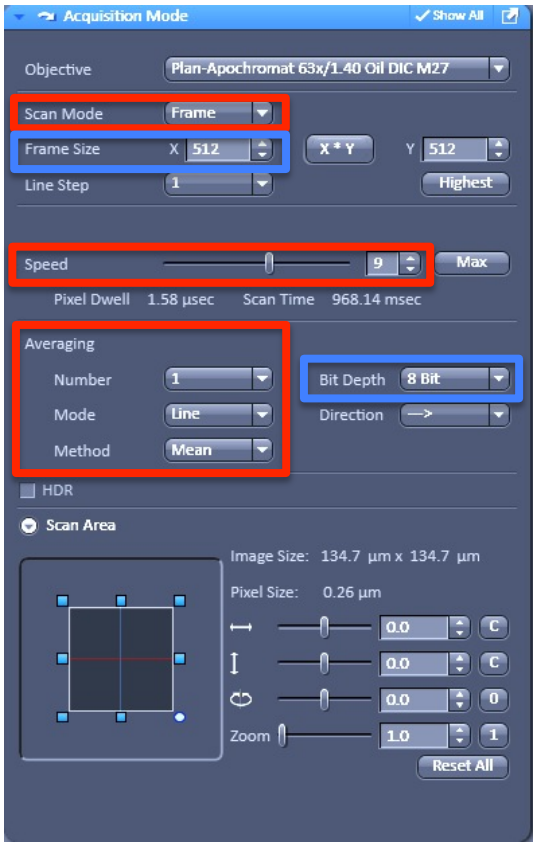
Smart setup

1. In the **EXPERIMENT MANGER** panel, choose **SMART SETUP**
   a. Configure your experiment. Choose dye / fluorophore and image display color
   b. Add additional dyes / fluorophores using + button. You can also delete dyes using the – buttons

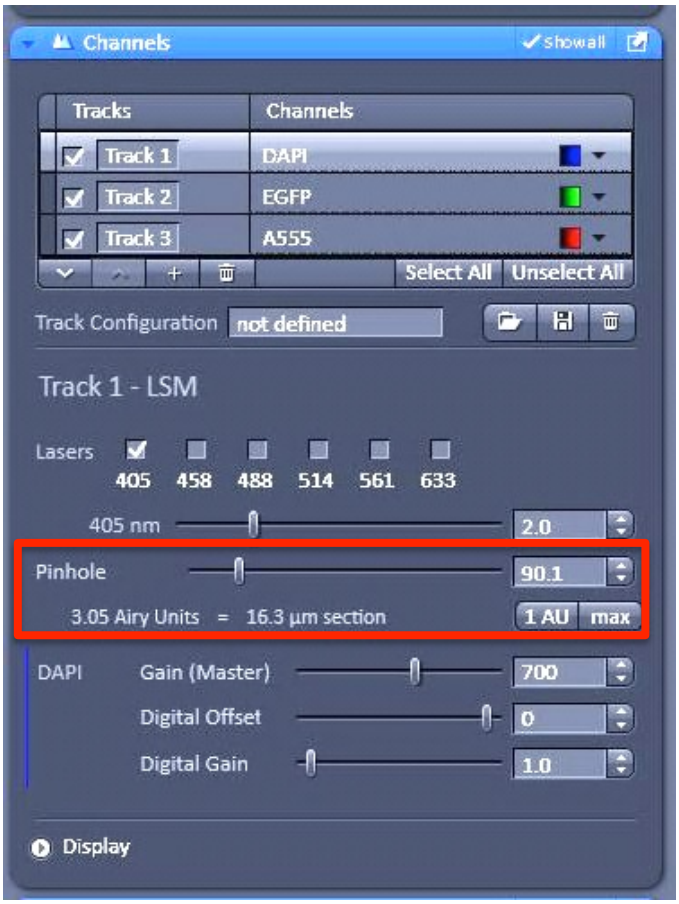
2. Choose imaging option
   a. **FASTEST**:
      i. Advantage: faster image acquisition
      ii. Disadvantage: potential cross-talk (spectral overlap) between channels.
   b. **BEST SIGNAL:**
      i. Advantage: Only one detector and one laser are switched on at any one time. This reduces crosstalk
      ii. Disadvantage: slower image acquisition (each color track imaged separately)
   c. **BEST COMPROMISE** - between both speed and best signal

3. Click **APPLY**

Image capture settings



1. In the **ACQUISITION MODE** panel, set the following parameters:
    a. **SCAN MODE** = **FRAME**
    b. **FRAME SIZE** (i.e. pixel density) = 1**024x1024**
    c. **SCAN SPEED** should be set between **7-9**
        i. NOTE: faster scanning gives smaller time interval between images, but lower signal-to-noise ratio. Slower scanning gives longer time intervals, but higher signal-to-noise ratio
    d. AVERAGING = 1
        i. NOTE: if you have a dim sample with white noise, you may want to consider averaging at least 4 frames. Note that adding frames adds to the total scan time.
    e. **BIT DEPTH = 12** for publication images (or 16 bit for high dynamic range images)
        i. NOTE: increasing bit depth increases number of gray scale levels (2^n)



2. Set **PINHOLE** to desired spatial resolution

*NOTE: For best resolution, click **1 AU***

NOTE: There is an inherent tradeoff between spatial resolution and image brightness. If your sample is dim and high resolution is not critical, you may want to set pinhole larger

3. If you want to acquire bright field images, highlight the channel with the shortest wavelength (usually blue), and them select **T-PMT** (bottom right hand corner of **LIGHT PATH** tool tab)

Setting the detector gain

1. Click **SET EXPOSURE**
   a. *(System will find optimal gain setting for each color channel)*

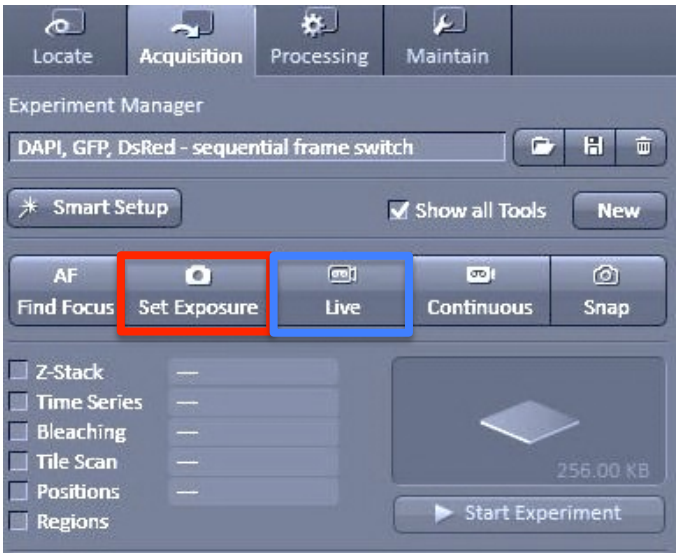
2. Click **LIVE**
   a. (next to **SET EXPOSURE**)
   *b. NOTE: live continuously scans sample at low resolution to allow quick adjustments. The **SNAP** image will be captured with Image capture settings above*

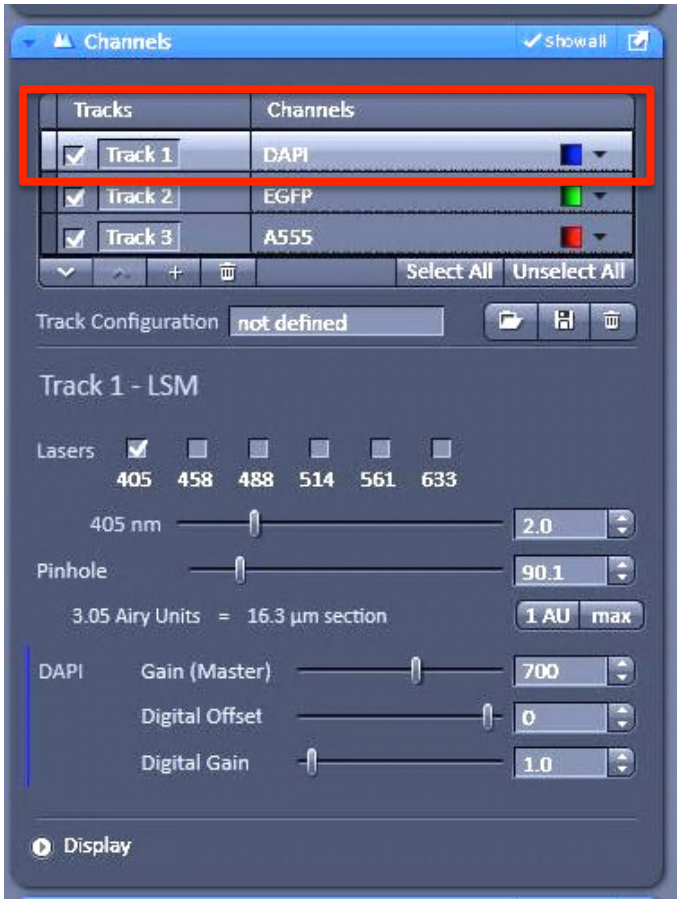
3. Adjust focus, if needed

4. Click **STOP**
   a. (next to **SET EXPOSURE**)

5. Click **TRACK1**
   a. Desired track will be highlighted in light gray. Other color channels can be unchecked

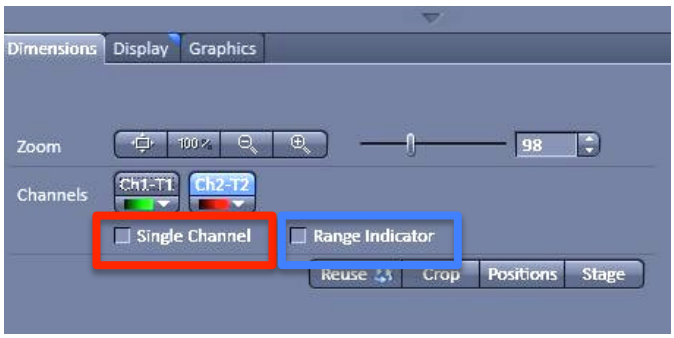
6. Click **LIVE**
   a. (next to **SET EXPOSURE**)





7. Enable **SINGLE CHANNEL**
   a. *(Active channel will be highlighted blue)*

8. Enable **RANGE INDICATOR**
   a. ***Red** shows pixels that are saturated*
   b. ***Black** are pixels that are underexposed*
   c. ***Blue** are pixels that have optimal exposure*
   d. Aim for an image that is has few red pixels



9. Adjust **GAIN (MASTER)**
    a. A good initial setting is ~650
    b. The maximum gain is 1200

*** *Aim for an image that is has few red pixels* ***

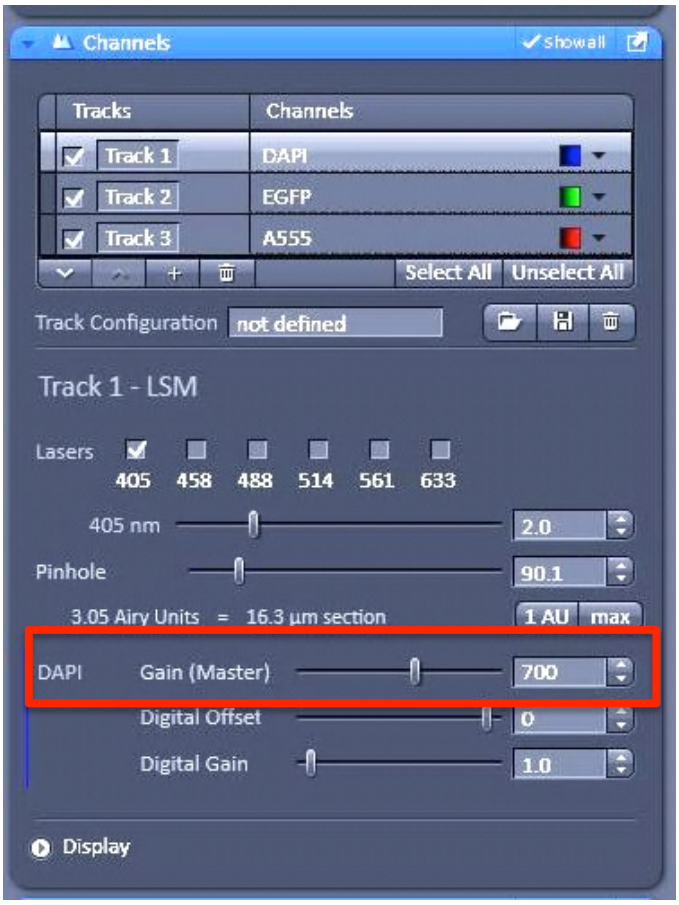
10. Click **STOP** in **EXPERIMENT MANAGER**
    a. (next to **SET EXPOSURE**)

11. To view another track (i.e. color channel), click on **TRACK2** in the **CHANNELS** panel.

12. Adjust **GAIN (MASTER)** for **TRACK2**

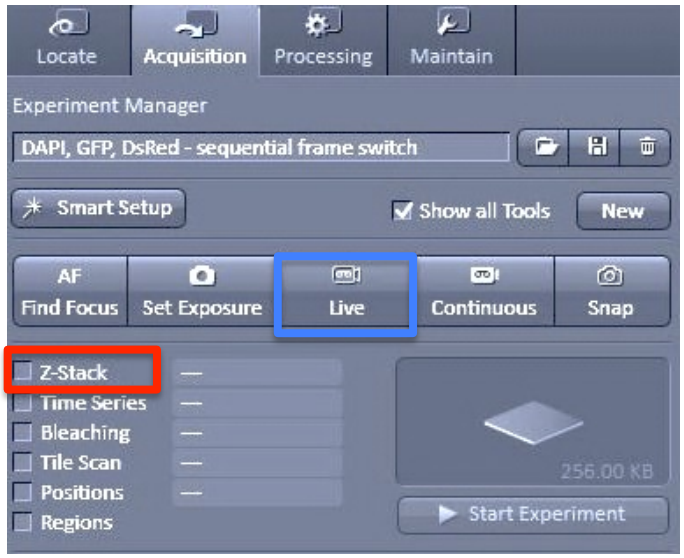
13. Repeat until all color channels give good images

14. Click **STOP** in **EXPERIMENT MANAGER**
    a. (next to **SET EXPOSURE**)

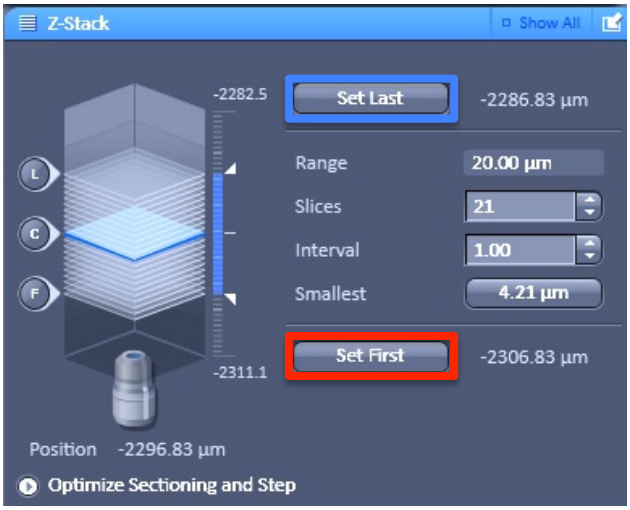


Acquiring a z-stack

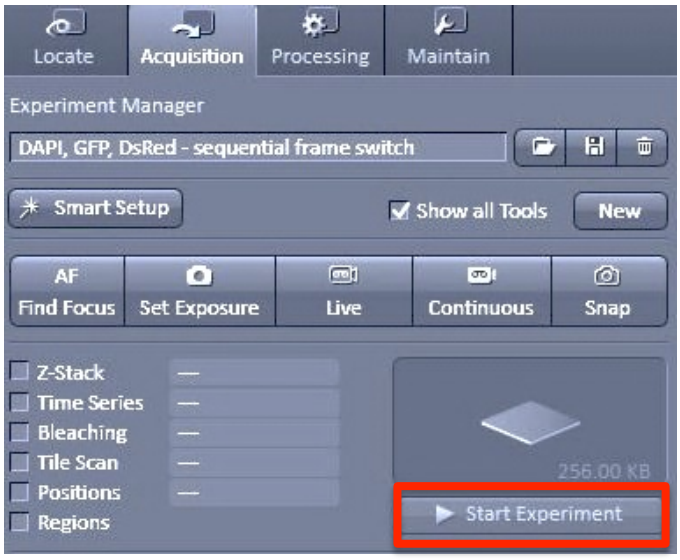
1. Check the **Z-STACK** box
2. Click **LIVE**



3. Focus (see the blue plane on the screen) manually on the plane closest to the objective, then click **SET FIRST**
4. Focus (see the blue plane on the screen) manually on the plane furthest to the objective, then click **SET LAST**
5. Click **STOP** to stop scanning
6. Select either **SMALLEST** slice interval or select the number of **SLICES** desired

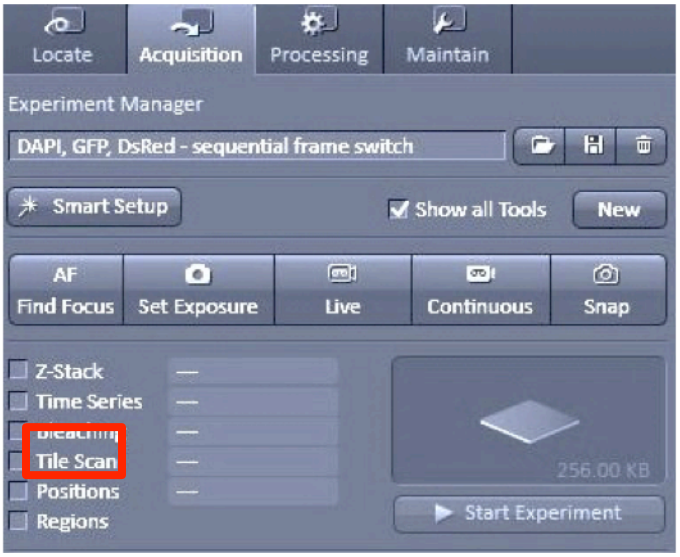


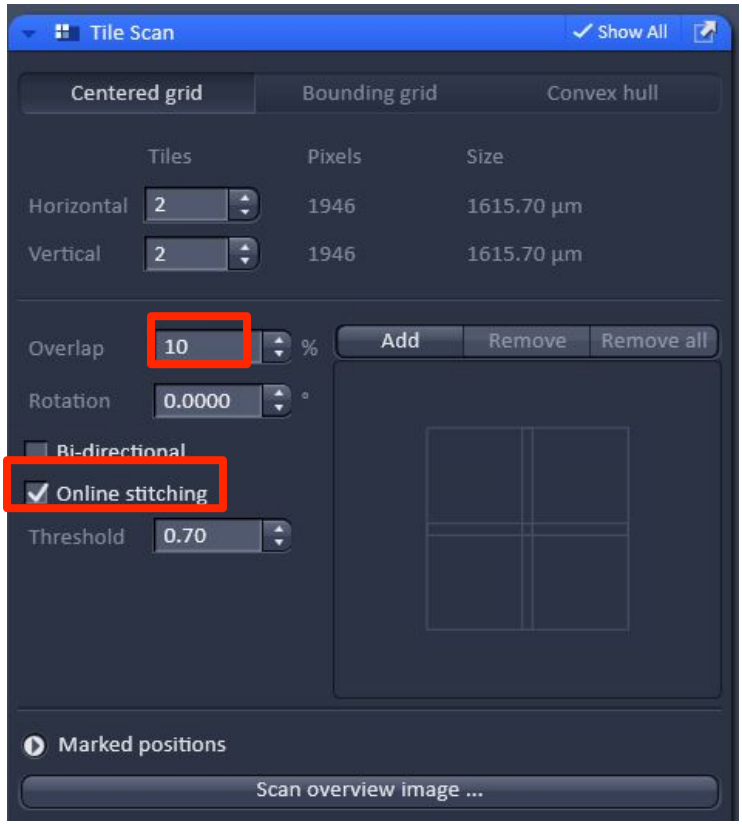
7. Click **START EXPERIMENT** to begin acquiring z-stack



Tile scan

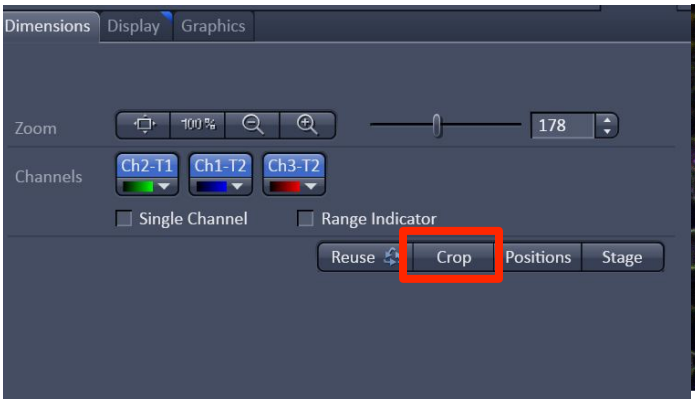
1. Check the **TILE SCAN** box
2. Find the **TILE SCAN** panel
3. Modify number of tiles
4. Choose overlap percentage (10%)
5. Enable **ONLINE STITCHING**
6. Click **START EXPERIMENT**

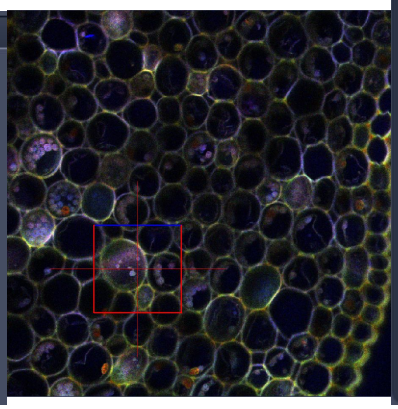


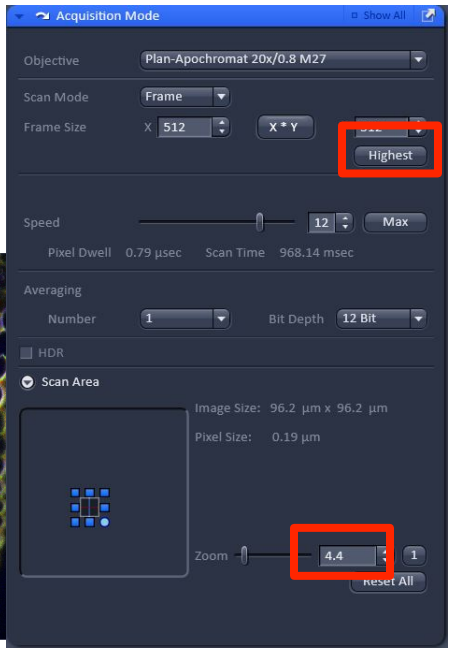


Crop

1. Click on **CROP**
2. Adjust the zoom area on your image
3. Go **LIVE** and then **STOP**
4. In **ACQUISITION MODE**, and click on **OPTIMAL** Frame size to get best resolution



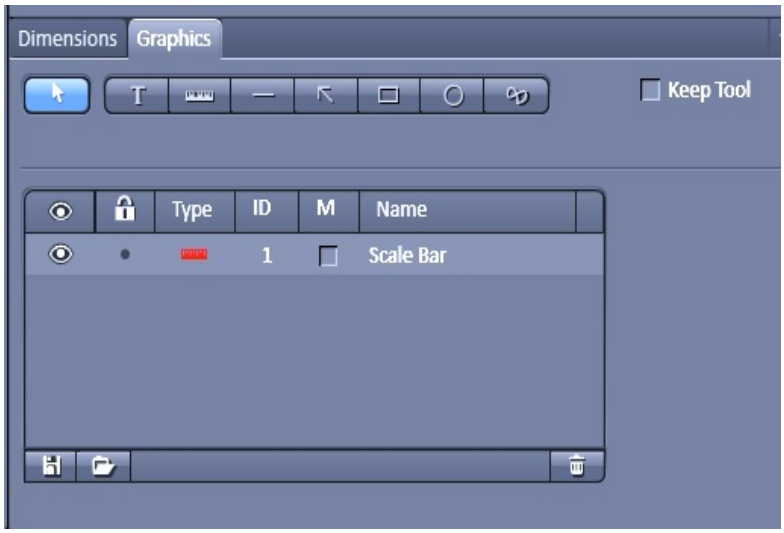




Last update: 8/25/2017 CL

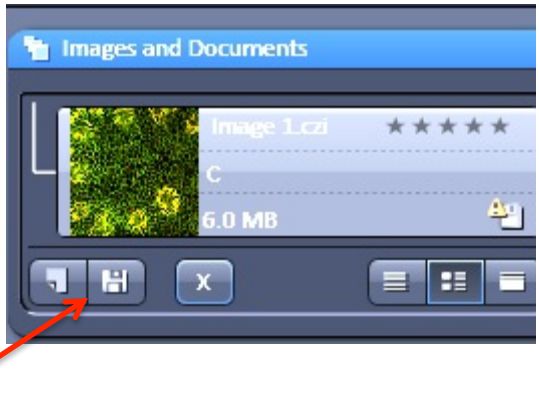
Graphics / Overlays

1. Add in:
    a. Text
    b. Scale bar
    c. Lines
    d. Arrows
    e. Boxes
    f. Circles
    g. Free draw



Saving your images

1. Click disk icon to save
2. Save to Data / LAB PI NAME / YOUR NAME if saving locally
3. Please do not store data permanently on computer. It is not backed up and data folders are periodically deleted
4. Images can be opened using FIJI (ImageJ)
5. Images can be exported as TIFF from the FILE / EXPORT menu



Finishing up

Turn off equipment in following order:

6. Close **ZEN** software
5. Shut down PC
4. Turn off X-Cite light source
3. Argon Laser Remote controller: Flip switch to OFF Position
2. Argon Laser Black Box Power Supply: Turn key to left (counter-clockwise)
1. Main system power:
    1C. Flip COMPONENTS switch to OFF position
    1B. Flip SYSTEM / PC switch to OFF position

    ****** WAIT 5 MINUTES TO LET LASER COOL ******
    (you will hear fan shut off when laser is cool enough)

    1A. Flip MAIN SWITCH to OFF position

1. Clean work area
2. Clean any immersion objectives that you used with lens paper
    DO NOT USE KIMWIPES!
3. Sign out
4. Put dust cover over microscope

ZEN lite for Windows

Download ZEN lite, your free copy of the powerful ZEN imaging software. Use this free microscope software to try out key features of the ZEN core package or simply install it as a viewer for your CZI files.

Use ZEN lite to:

- Control Axiocam microscope cameras
- Create microscope images and simple video sequences
- Measure distances interactively
- Manage images/videos and export them into various formats
- View meta information relating to CZI image files
- Use simple report functions

Expand ZEN lite with selected modules: create impressive multichannel and time lapse images of your specimens or use extended measurement functions.

To download go to:

http://www.zeiss.com/microscopy/en_us/products/microscope-software/zen-lite.html

FIJI (is just imageJ)

ImageJ is an open source Java image processing program inspired by NIH Image. It runs on any computer with a Java 1.8 or later virtual machine. Downloadable distributions are available for Windows, Mac OS X and Linux. ImageJ has a strong, established user base, with thousands of plugins and macros for performing a wide variety of tasks.

To download go to:

https://imagej.net/Fiji/Downloads