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:

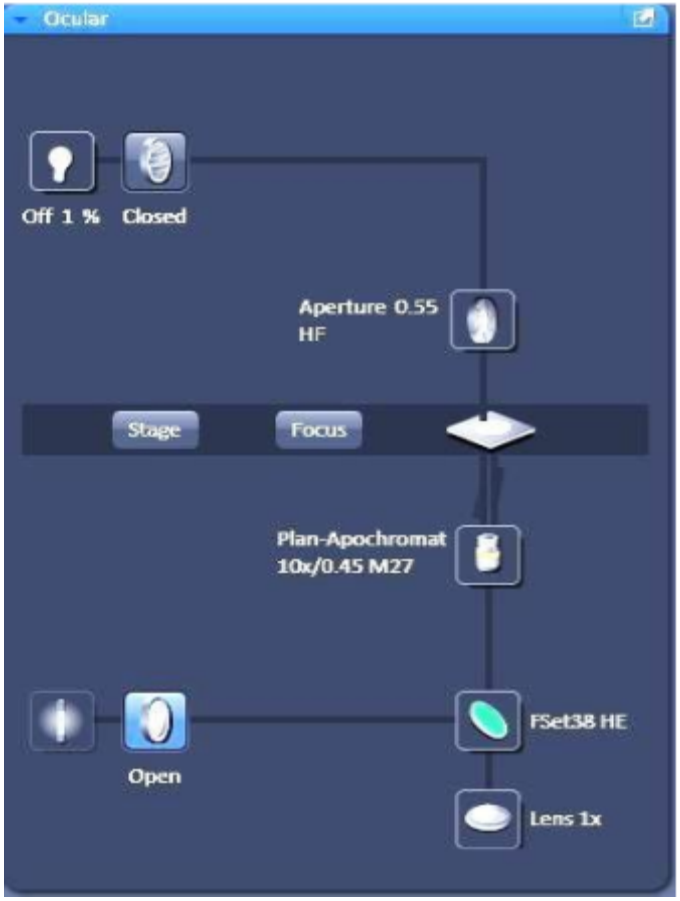
1. Main System Power
   - **Make sure Key on right hand side is in the horizontal position.**
   - 1A. Flip **MAIN SWITCH** to **ON** position
   - 1B. Flip **SYSTEM/PC** switch to **ON** position
     i. Wait for the Definite Focus module to display **OFF**
   - 1C. Flip **COMPONENTS** switch to **ON** 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
   - If all other hardware is on but the X-Cite light off, you can turn it on without restarting the whole system
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.*

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

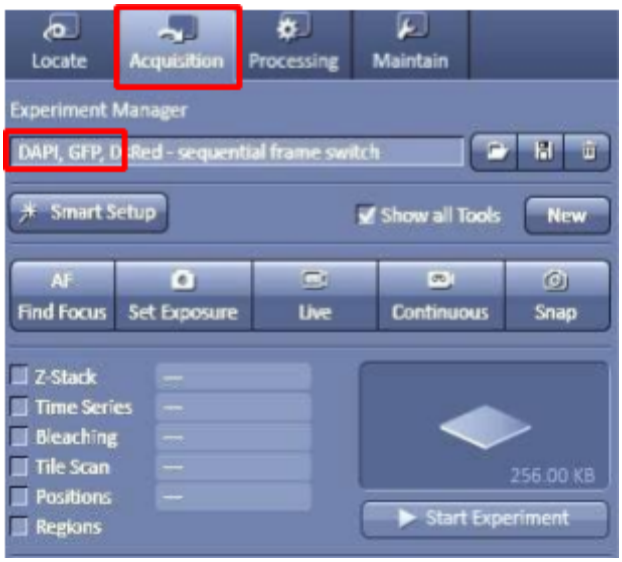
1. In Zen, go to the **LOCATE** tab
2. Turn on Transmitted and Reflected Light
3. Choose configuration:
   a. **BRIGHTFIELD**
   b. **DIC II** – For use with dry objectives
      i. 10x, 20x, 63x dry
      ii. Requires polarizers
   c. **DIC III** – for use with wet objectives
      i. 40x oil, 40x water, 63x oil
      ii. Requires polarizers
   d. **DAPI** – blue filter
   e. **GFP** – green filter
   f. **DsRED** – red filter
4. Focus on sample



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

5. 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

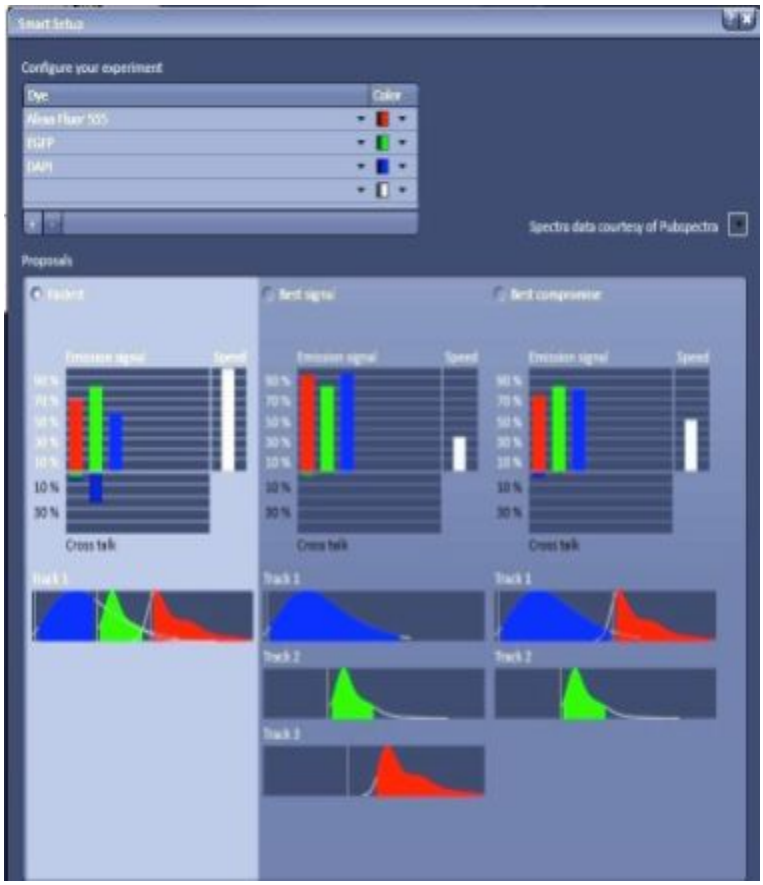
Setting confocal configuration



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

2. In **EXPERIMENT MANGER**, choose **SMART SETUP**

3. In the Smart Setup popup window, under **CONFIGURE YOUR EXPERIMENT**, choose dyes / fluorophores and image display colors
    a. Add and delete dyes / fluorophores using the + / - buttons.

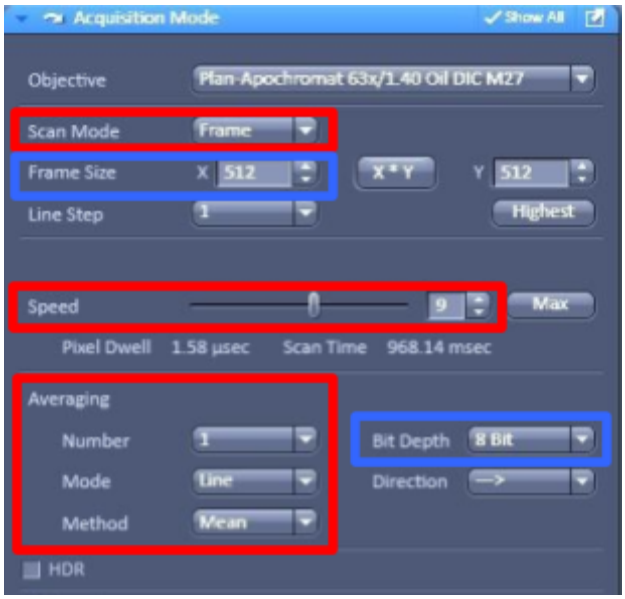


4. Choose imaging option
    a. **FASTEST**:
        i. Advantage: faster image acquisition
        ii. Disadvantage: potential cross-talk (spectral overlap) between channels.
    b. **BEST SIGNAL:**
        i. Advantage: Only one detector and one laser are switched on at any one time. This reduces crosstalk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

NOTE: The tracks displayed show which lasers will be turned on at the same time. The tracks will go in sequence.

5. 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)

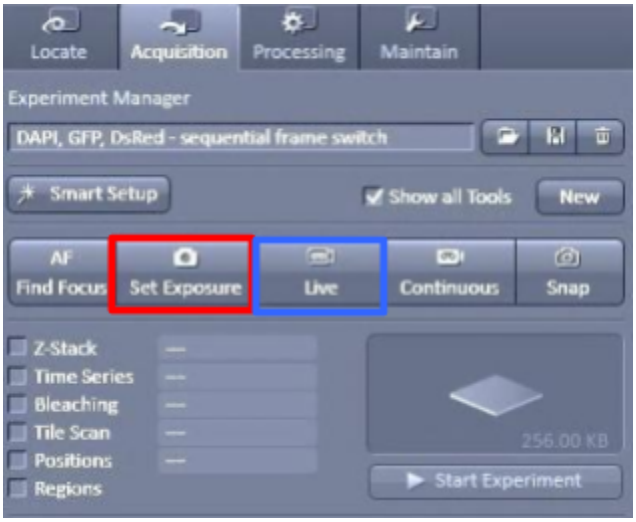
2. In the **CHANNELS** panel, set **PINHOLE** to desired spatial resolution. For best resolution, click **1 AU**

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. To acquire bright field images, highlight channel with shortest wavelength (usually blue), and enable **T-PMT** (bottom right hand corner of **LIGHT PATH** panel)

Setting the detector gain

1. In **EXPERIMENT MANAGER**, click **SET EXPOSURE**
    a. *System will find optimal gain setting for each color channel*



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

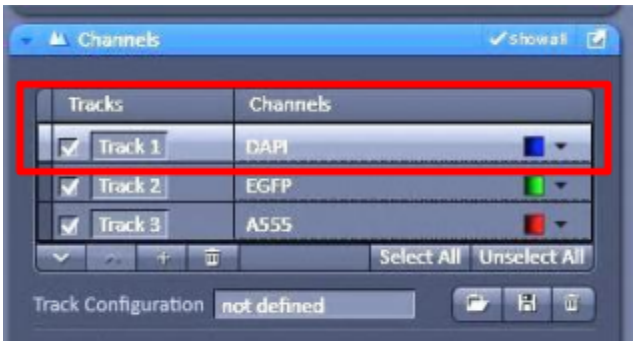
*NOTE: **LIVE** continuously scans sample at low resolution to allow quick adjustments. **SNAP** scans sample with image capture settings above*

3. Adjust focus, if needed

4. Click **STOP**
    *a. (next to **SET EXPOSURE**)*
    *b. **LIVE** button switches to **STOP** upon being clicked)*

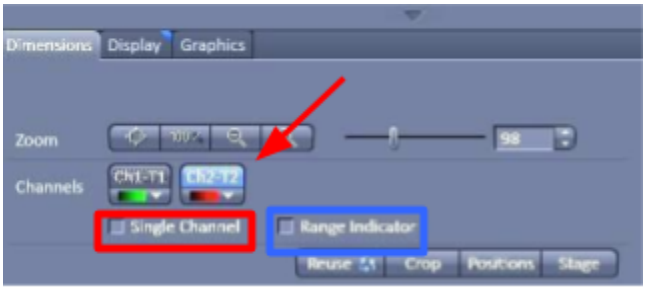
5. In the **CHANNELS** panel, click **TRACK 1**
    a. Desired track will be highlighted in light gray. Other tracks can be unchecked

NOTE: All channels in the track will be highlighted in light gray. You will set the gain for each channel in the track before moving to another track.

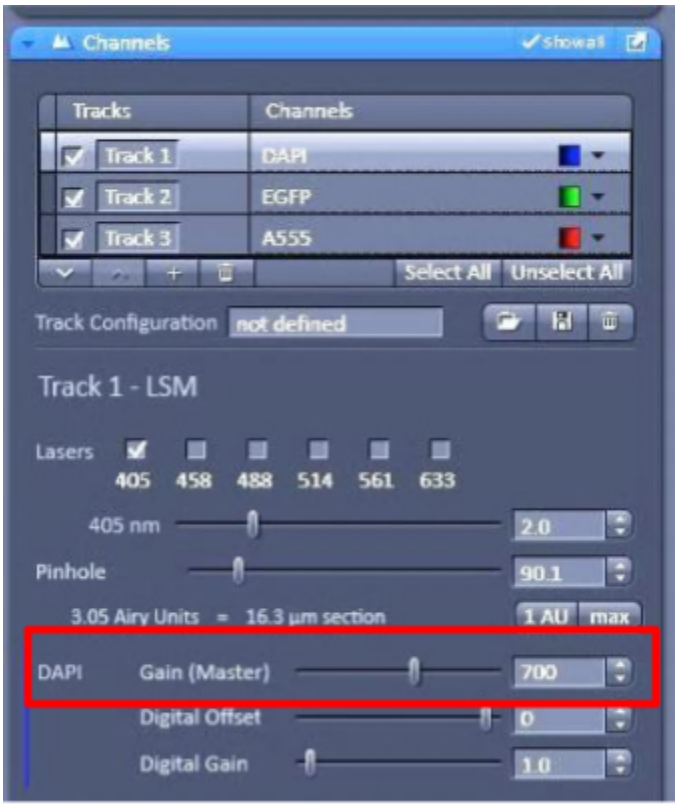


6. Under **EXPERIMENT MANAGER,** click **LIVE**
    *a. (next to **SET EXPOSURE**)*

7. In the **DIMENSIONS** tab in the image viewing window, enable **SINGLE CHANNEL** and **RANGE INDICATOR**
    a. *Active channel will be highlighted blue*
    b. ***Red** shows pixels that are saturated*
    c. ***Black** shows pixels that are underexposed*
    d. ***Blue** shows pixels that have optimal exposure*
    e. Aim for an image that has few red pixels



8. In the **CHANNELS** panel, next to the appropriate channel name, adjust **GAIN (MASTER).**
    a. *A good initial setting is ~650*
    b. *The maximum gain is 1200*
    c. ***Aim for an image that has few red pixels***



NOTE: All channels in the track will be available for gain adjustment. Make sure you adjust the gain for the appropriate channel.

9. View the next channel in the track
    a. (**IMAGE VIEWER,** under the **DIMENSIONS** tab, click desired channel)
    b. You may need to enable **RANGE INDICATOR** again

10. Adjust appropriate **GAIN (MASTER)** in the **CHANNELS** panel.

11. In **EXPERIMENT MANAGER,** click **STOP**
    a. *(next to **SET EXPOSURE**)*

12. View the next track (in the **CHANNELS** panel, click on **TRACK 2**)

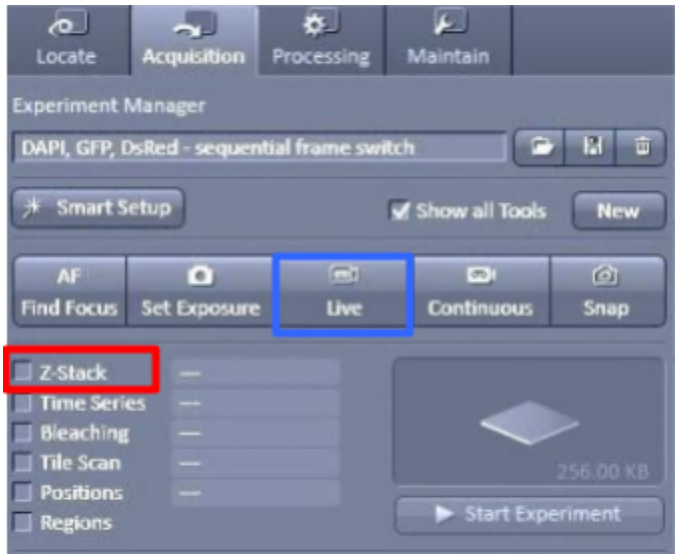
13. Adjust **GAIN (MASTER)** for the channels in **TRACK 2**

14. Repeat steps 7-13 for each track until all color channels give good images

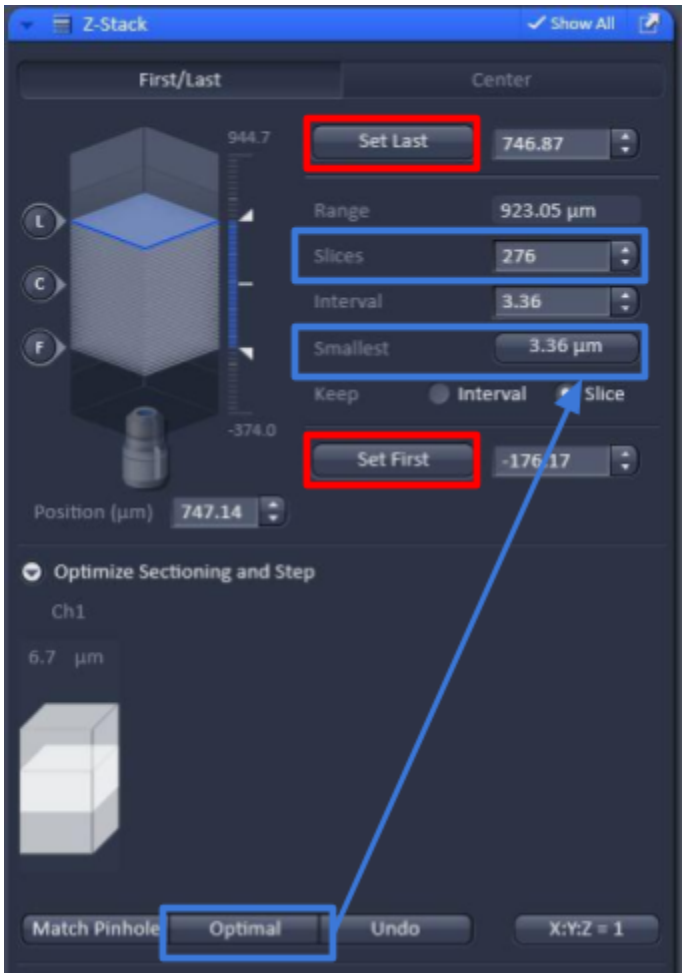
15. Click **STOP** in **EXPERIMENT MANAGER**
    a. *(next to **SET EXPOSURE**)*

Acquiring a z-stack

1. In **EXPERIMENT MANAGER**, enable **Z-STACK**
2. Click **LIVE**
3. Go to the **Z-STACK** panel



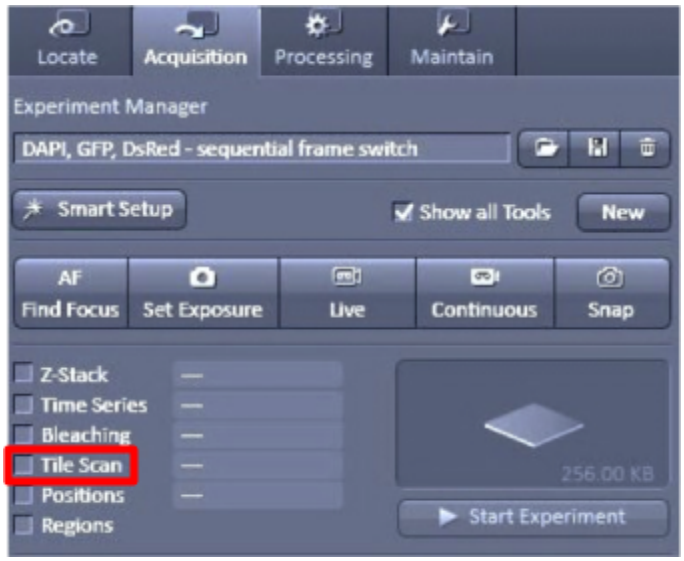
4. Focus (see the blue plane in the diagram move) manually on the plane closest to the objective, then click **SET FIRST**
5. Focus (see the blue plane in the diagram move) manually on the plane furthest to the objective, then click **SET LAST**
6. Click **STOP** to stop live scanning
7. Select **OPTIMAL** slice interval and click smallest interval button OR select number of **SLICES** desired
8. In **EXPERIMENT MANAGER**, click **START EXPERIMENT** to begin acquiring z-stack



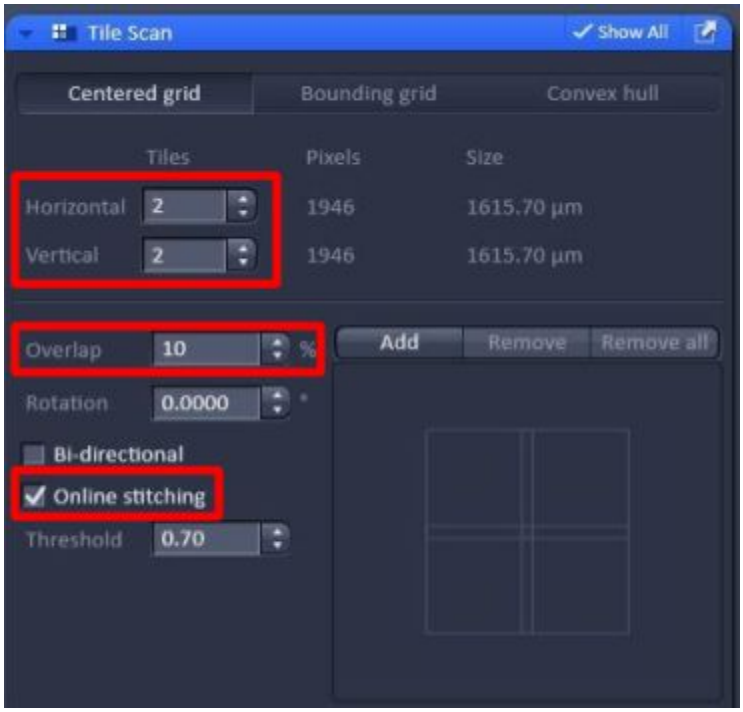
Last update: 10/18/2019 KX

Tile Scan

1. In **EXPERIMENT MANAGER**, enable **TILE SCAN**

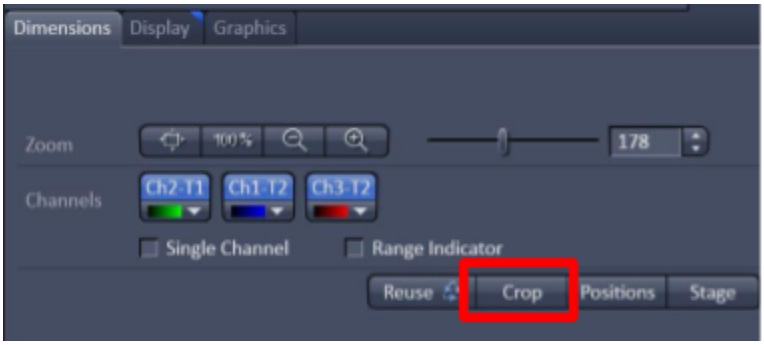


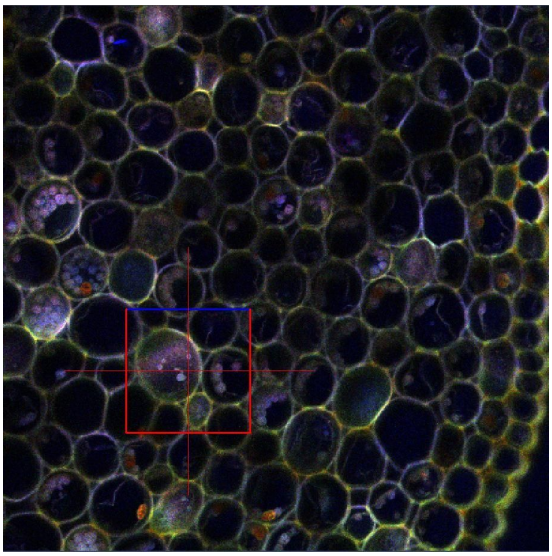
2. Find the **TILE SCAN** panel and click **CENTERED GRID** tab
3. Modify number of tiles
4. Choose overlap percentage (10%)
5. Enable **ONLINE STITCHING**
6. In **EXPERIMENT MANAGER,** click **START EXPERIMENT**

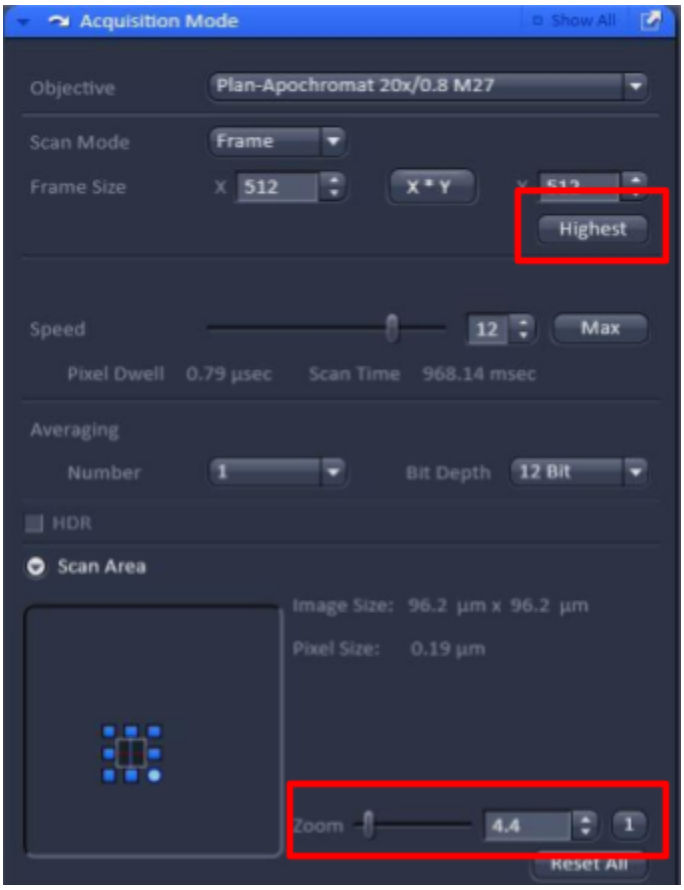


Crop

1. In the **DIMENSIONS** tab in the image viewing window, click **CROP**







2. In the **ACQUISITION MODE** panel, under **SCAN AREA**, adjust the **ZOOM** area on your image.

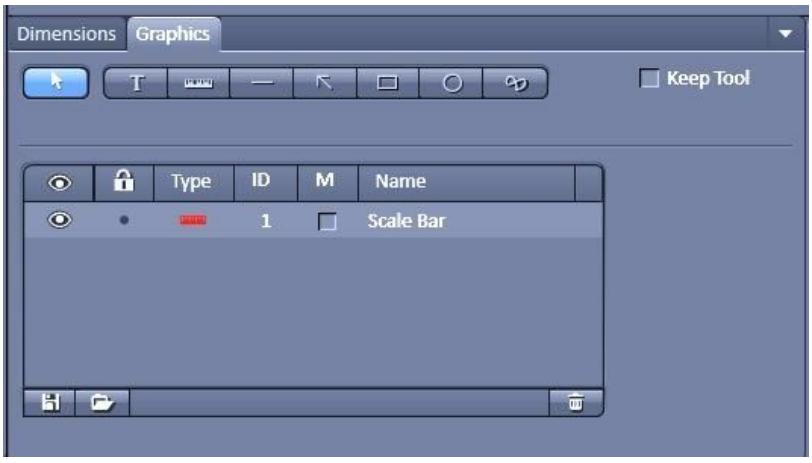
3. Click **HIGHEST** Frame Size to get best resolution

4. In **EXPERIMENT MANAGER**, click **LIVE** and then **STOP**

Graphics / Overlays

In the image viewing window, in the **GRAPHICS** tab, add in:

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



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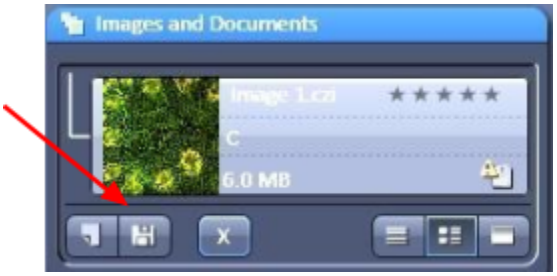
Saving and Opening Your Images



1. In the **IMAGES AND DOCUMENTS** window on the right side of the screen, click **DISK ICON** to save

2. Save to **Data / LAB PI NAME / YOUR NAME** if saving locally to the D drive

3. Please **do not store data permanently on computer**. It is not backed up and data folders are periodically deleted

4. Images are saved as **.czi** files, which can be opened in **ZEN LITE** (free version of ZEN) or **FIJI (ImageJ)**

5. You can export your images 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
    a. for each wipe, use a new part of the paper and wipe in one direction

****** DO NOT USE KIMWIPES! ******

3. Sign out
4. Put dust cover over microscope (slit on back)

Image Viewing Software

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