

LSM 880 Confocal - Operation Instructions

Turning the system ON: Locations have tape with numbers.

Uncover the microscope and turn things on in the following order:

1. **FluorArc (HBO) power supply.**
2. **Main Switch.**
3. **Systems/PC**, then wait until it is fully booted.
4. **Computer** and wait until fully booted.
5. **Components switch.**

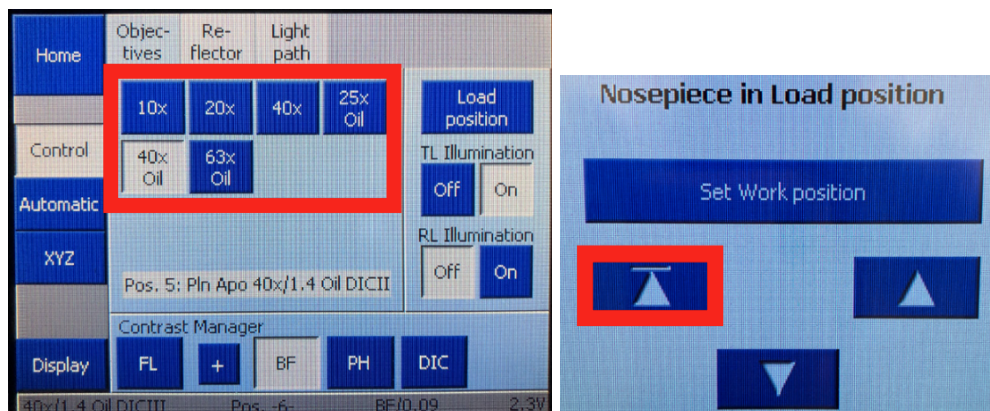
Login: click on **LSM USER**, no password.

Computer/Software:

1. Go to the **D Drive** and make a folder for yourself in the **UVA folder** if you do not have one already. You will store images here. No databases are required in this software. The images will be .ism format. You will have to download the ZEN browser to open these files onto your personal computer. You can also export images in any format desired. However, you should still save your raw images as .czi files regardless.
2. Click on **ZEN Black** icon, then click **Start System**

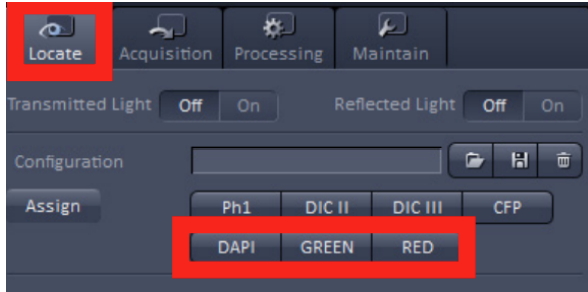
Mount your sample

1. If using an air objective, put on the sample, coverslip side towards the objective.
2. If using an oil objective, use the "**Load Position**" button on the touchpad to lower the objective before you place a sample in the holder. Then raise the objective with the triangle and line button on the touchpad. If the oil does not touch the objective, raise it with the focus knob until it does.



Getting Started and Image Acquisition

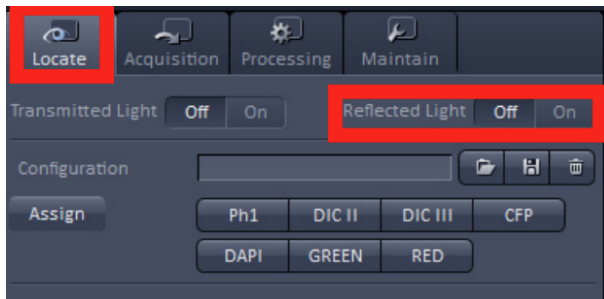
1. Click on **Locate** → **Ocular** → turn on **Transmitted Light**. Select the desired dye(s) you'd like to visualize. People typically go for DAPI first.



2. Look through the **eyepiece** to find your field of interest. You can use the **joystick** to move the stage around to locate your sample. Speed of the joystick can be toggled using the button on top of the stick as shown below.



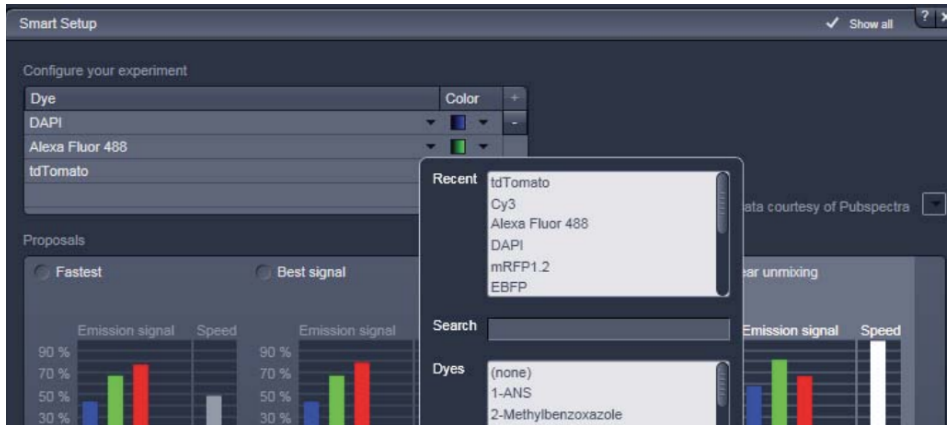
3. You can look at other fluorescence channels by changing settings under the **Locate** tab.
4. After finding your field of view using the eyepiece, you can close the shutter for the illuminating light by using the **Reflected Light** button in the software or the **RL** button the right side of the microscope stand.



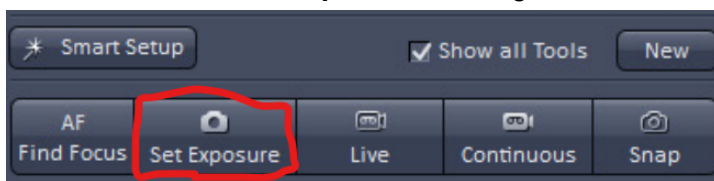
5. Select the **Acquisition** tab and click the **Smart Setup** button. The **Smart Setup** window should pop up.



6. In the **Configure Your Experiment** section, choose your dye(s) - click on the bar and the drop down arrow. Then go down to the next bar for each dye until all dyes have been added. Then, click on the **Best signal** choice. then press **Apply**.



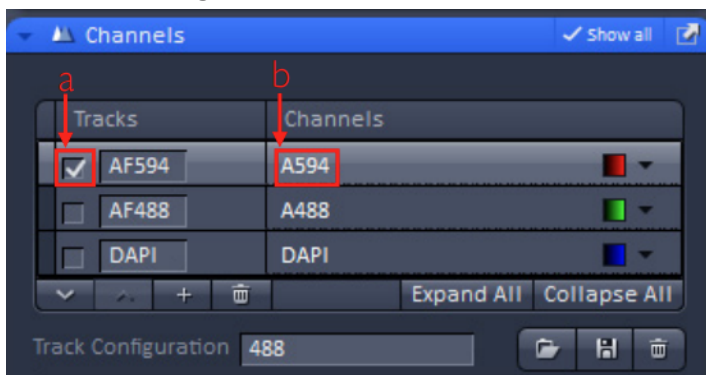
7. Click on **Auto Exposure** - a rough start to visualizing your sample.



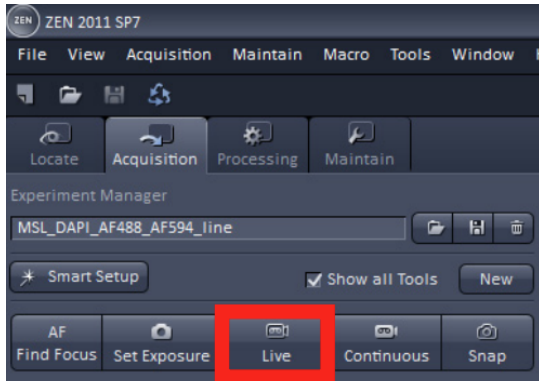
8. Go to the **Channels** window and deselect channels' checkboxes until only one is active. Channels with the checkmark will be scanned by the confocal.

9. You can change each channels' settings by clicking on its row (will appear highlighted white). Make sure to adjust settings for channels one at a time with the following:

- a. Pinhole diameter = **1AU button**
- b. **gain(master)** = 750

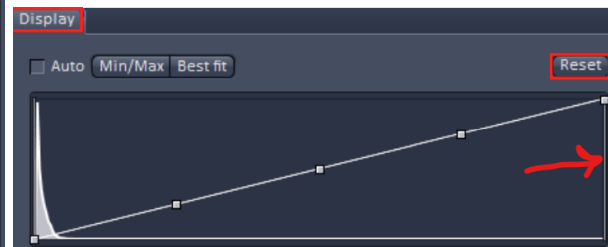
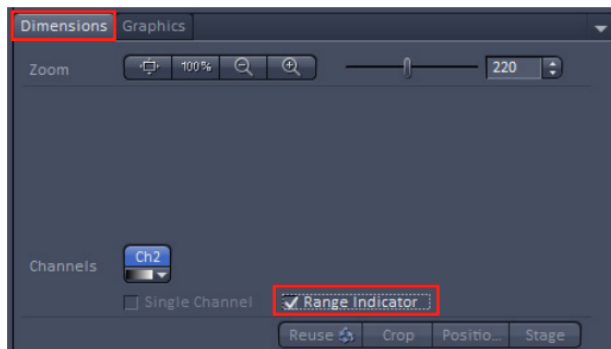


10. Click **Live** - continuous fast scanning - and focus as required. The **Live** button will turn into a **Stop** button that you can press to end live imaging. **NOTE:** it is important to focus on the brightest area of the sample even if it is oversaturated. This will be adjusted for later on.

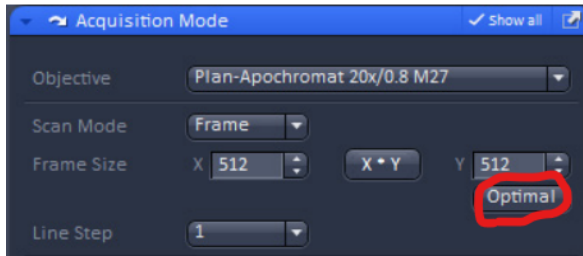


11. Engage **Range Indicator** in the **dimensions** tab found under the image. This will indicate pixels that have a zero intensity as blue, pixels that are saturated as red, and everything in between on a grayscale.

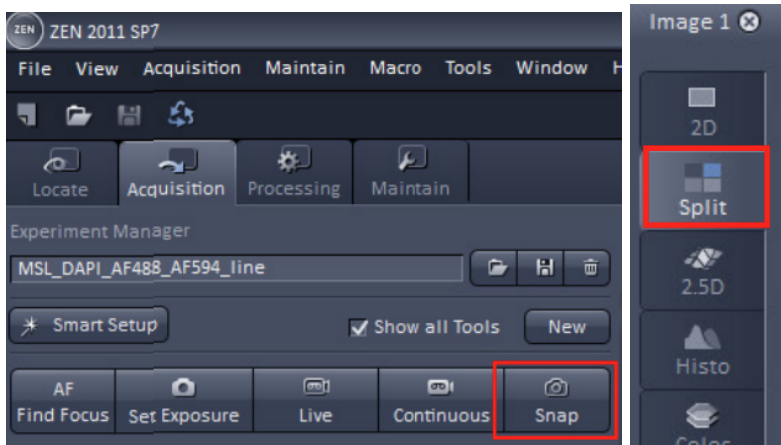
- a. Click on the small rectangular **Channel color** tab under the image - enables the **Range Indicator box** under the image. Do the following for each channel:
 - i. Adjust the **vertical bar** in the graph shown under the **Display** tab to better visualize your sample.
 - ii. Exit **Range Indicator** by unchecking its box.



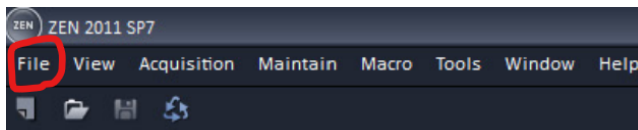
- b. Adjust **gain(master)** and **Laser power** until there are minimal red and blue dots, respectively. Consider the following while doing so:
 - i. Pixel intensities
 - ii. Bleaching: if you want to lower bleaching, increase **gain(master)** and lower **laser power**.
 - iii. Image quality: if you want to maximize image quality, lower **gain(master)** and increase **laser power**.
- c. To acquire a good image, select the Acquisition bar and click the **Optimal** button. This sets the resolution and scan speed. If you want to average, you have to set the number of averages manually.



- d. Click **Snap** to take an image. Multiple channels can be imaged by having their bar checkmarked. Images proceed to pile up on the right side of the image panel. You can view each and all of the channels by clicking the **Split View** option on the left of the image panel.



- e. Save images as desired. Remember to save .czi files on the computer first!
- i. Go to **Files** → **Save as** → name the file and save file type as **.czi**. Make sure this is in the **UVA folder's D drive** under your name.
 - ii. Then, you can also save your images as jpeg or tifs: **File** → **Export** → **tif** or **jpeg**.



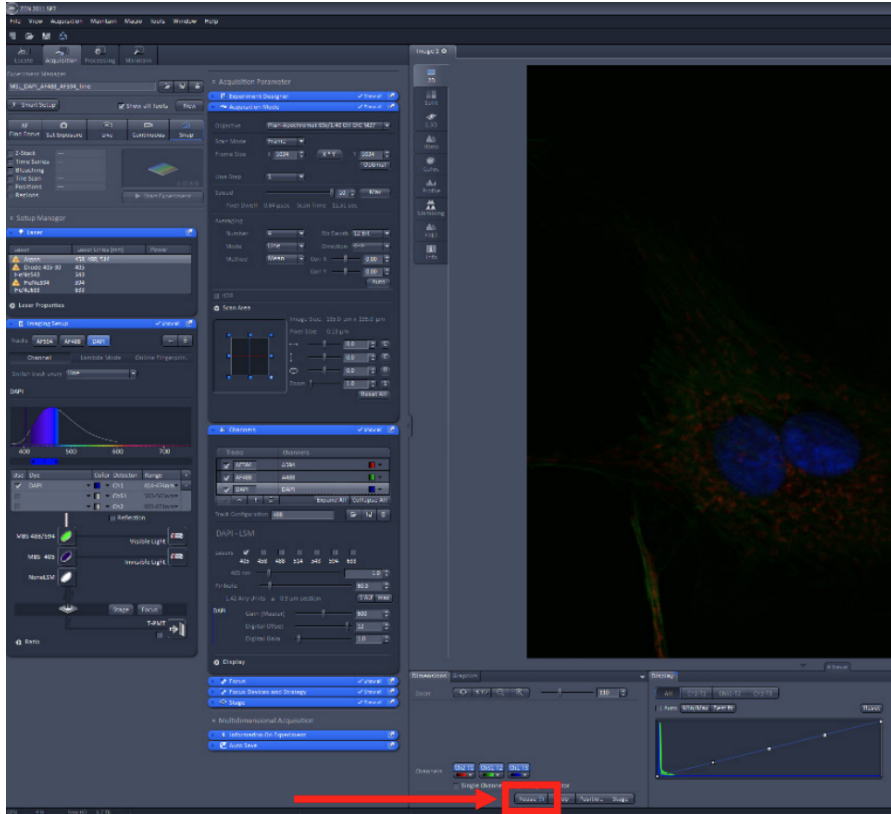
Ending your session

1. Lower the objective: On the **touchpad**, click **Load Position** and **Set Work position**.
2. Make sure all your desired images are saved as the proper file type in addition to the **.czi** in a folder under your name in **D Drive**. Clean the objective lens with some Sparkling **solution** if you used oil immersion.
3. Turn off everything following the labeled numbers backwards.
 - a. **Components** switch.
 - b. **Computer** and wait until fully booted.
 - c. **Systems/PC**, then wait until it is fully booted.
 - d. **Main Switch**.
 - e. **FluorArc (HBO) power supply**.
4. Cover the microscope.

Extra tools to know

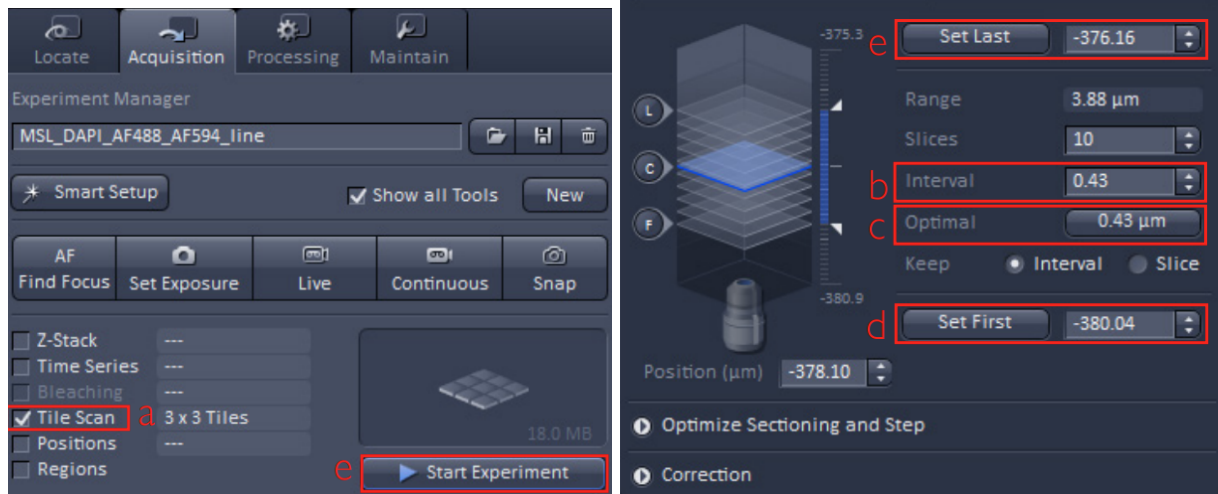
Loading settings from previous session

If you already acquired an image with the settings you want saved from a previous session, open that image and click the **Reuse** button (looks like a recycling symbol). All of the settings except the objective will be loaded.



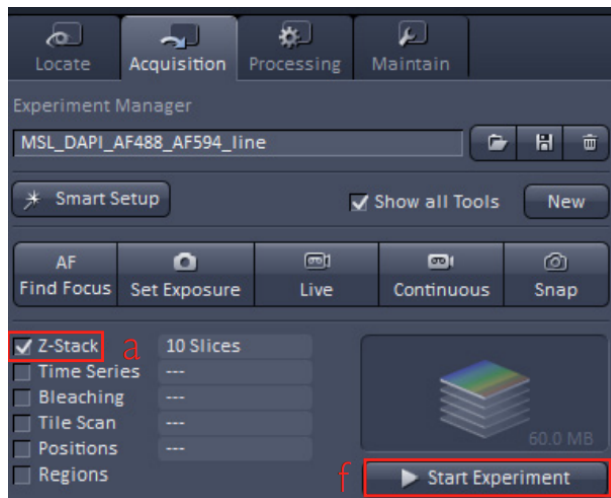
Tile scanning

1. Click on the **Tile Scan** checkbox in the top left corner of the Zen interface to activate the **Tile Scan** window.
2. Click on **Centered Grid**.
3. Put in the **number of tiles** desired in the horizontal and vertical directions. The mosaic of tiles will be centered on the current position.
4. Set **Overlap** to 10%, leave rotation at 0, click on **Bidirectional**, click on **Online stitching** and leave **Threshold** at 0.70.
5. Press **Start Experiment** button to acquire the **Tile Scan**.



Z-sectioning

1. Go to the **Acquisition tab** and check mark the **Z-Stack** option. Then, scroll down to the Z-Stack window on the right column.



2. Select **ONE** channel and start **Live**.
3. Move the objective up until the image is faint or becomes invisible - Click on **Set First**.
4. Move the objective down until the image is faint or becomes invisible - Click on **Set Last**.
5. Hit **Stop** and do not touch the focus knob.
6. Look at **Distance covered** and **set # of slices** (depending on interval between slices).
7. Go back to the **Channels** window and select all channels (checkmark all of them).
8. Go up to the **Acquire Mode** window and select **Frame Size**, **Scan Speed**, etc.
9. Click **Start Experiment** to start collecting the Z-stack images.
10. You can display the images in the stack by selecting the **Processing** tab, then **Maximum Intensity Projection**, **Select** (allows a preview), then **Apply**.