

ZEISS LSM 880 Start-up/Shut-down Procedure

Starting up the LSM 880 system

1. Sign in on the login sheets.
 2. Switch on main power switch (#1), wait 3 seconds.
 3. Switch on the systems/PC (#2), **wait 2 seconds**.
 4. Switch on the components switch (#3).
- Never switch on #2 and #3 at the same time.

Starting up the ZEN software

1. Log into your user account.
2. Open the ZEN confocal icon.
3. When the startup window appears, select Start System. Once the system check is completed, you are ready to start using the confocal microscope.

Procedures after you complete your session

1. Copy your data to designated hard-drive.
2. Clean the objectives and put away water objectives if you use. Turn the system to 10x objective.
3. Sign out in the login sheets (leave comments if there is any issues, email Dennis and Fang)
4. Exit out of ZEN software. Check the calendar.
 - a. If there is a user in one hour, on the Laser indication box that appears, put **Argon Laser to the standby** mode; and leave everything on.
 - b. If not, follow the following shut-down steps.

Shutting down ZEN and the LSM 880 system

1. On the Laser indication box, turn the **Argon Laser to the off** position (in the software, not the actual key in the laser box).
3. After Zen is closed, shut down the PC (do not force close Zen, allow it to close).
4. Wait for the Argon laser to cool. You will hear the fan stop running, this usually takes ~5 minutes.
6. Switch off the Components switch (#3).
7. Switch off the systems/PC switch (#2).
8. Switch off the Main Power switch (#1).

The system needs to **remain off for 30 minutes** before restarting.

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880 User Guide Overview

- Starting up the system
- Turning on the lasers
- Viewing your sample
- Setting up a configuration
- Taking an image
- Saving your file

Starting up the system

1. Follow the directions for Powering up the 880, this is located on the wall behind the 880 monitor

2. Double click on the Zen icon



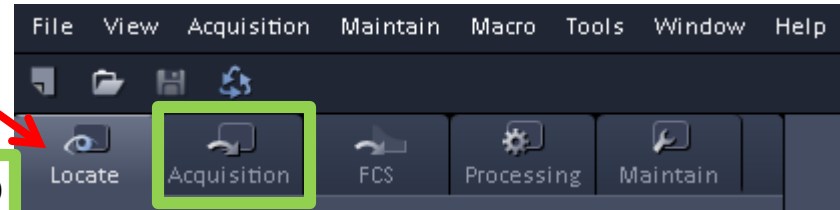
3. Click on the **Start System** tab



Turning on the lasers

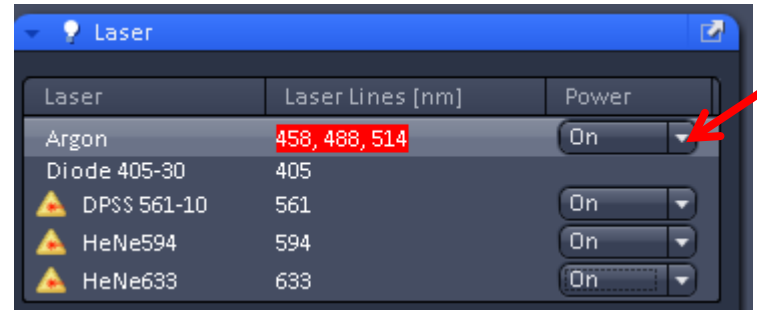
1. The system starts with the locate tab open

2. To turn on the lasers click the **acquisition tab**



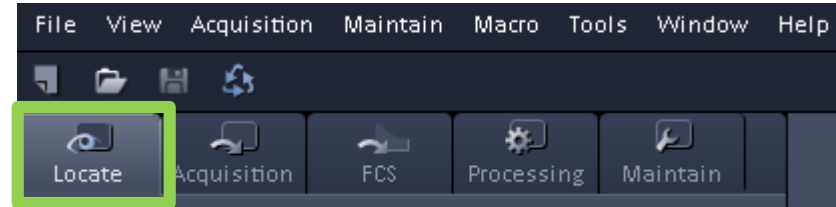
3. Use the pulldown tab to turn on the lasers

Note: the 488 laser takes about 5 minutes to warm up.



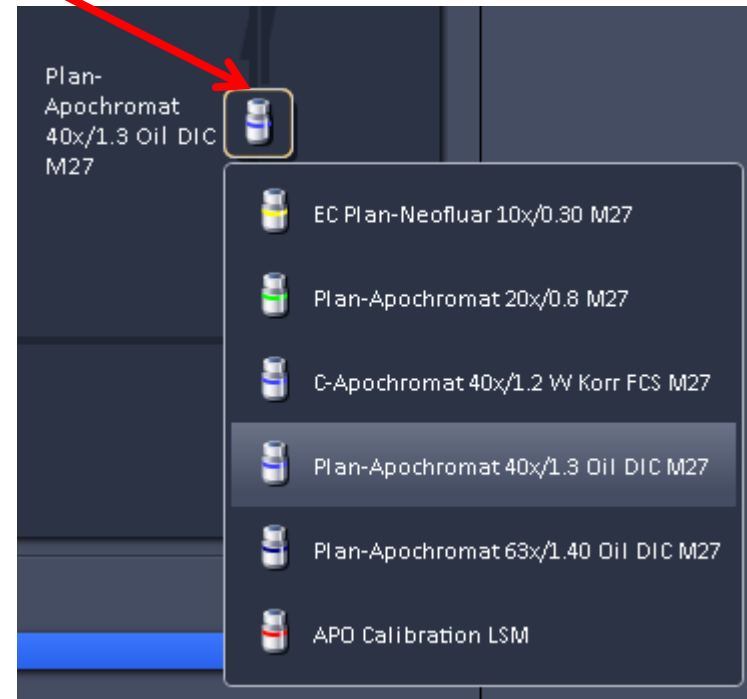
Viewing your sample

1. To view your sample through the eyepiece click the **locate** tab
2. Choose your objective by clicking on the objective button, this will bring up a list of objectives currently on the microscope.



You may also choose your objective by using the touch screen next to the monitor – Touch “Home” then “Microscope” and click on the “Objectives” Tab.

3. After you choose your objective make sure to clean it with lens cleaner
4. Set your sample on the stage, please remember to add oil or water as appropriate



Viewing your sample

1. To view your sample through the eyepiece select what color you would like to use.

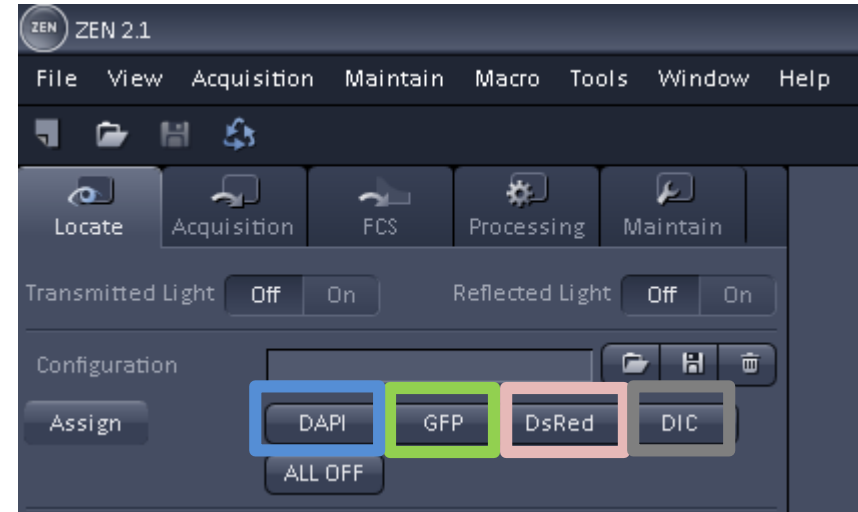
Blue Green Red

If you would like to see white light select DIC

2. Use the focus wheels on the side of the Microscope, or on the side of the touch screen to bring your sample in focus.

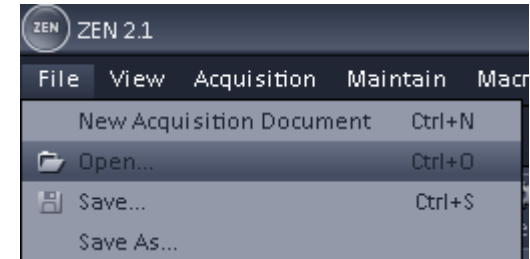
Towards yourself moves the objective down
Away from yourself moves the objective up

3. Use the joystick next to the microscope to move your sample.

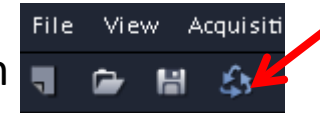


Setting up a configuration

1. To load a configuration from a previous experiment, go to file, open, then choose the experiment you would like to reuse

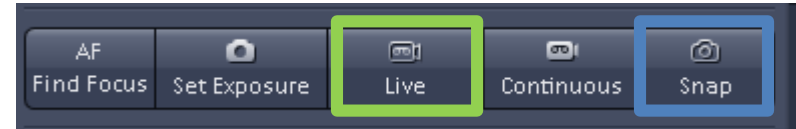


2. Click the reuse button either on the top left of the screen or at the bottom of your image



Taking an image

1. To view your sample click on the **Live** button.

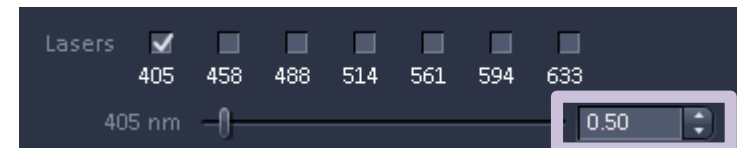
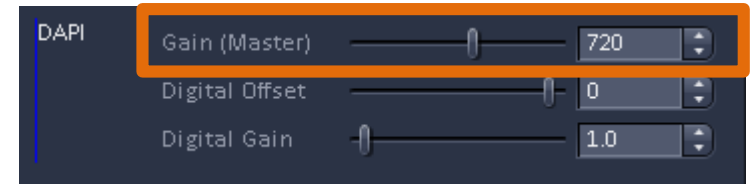


2. You may need to adjust to focus by using the focus wheels on the microscope or on the side of the touch screen.

3. To change the intensity of your sample go to the **channels** tab and click on the **track** you want to adjust. You can increase or decrease your intensity by adjusting the **Gain(Master)** It is recommended you stay between 600-800



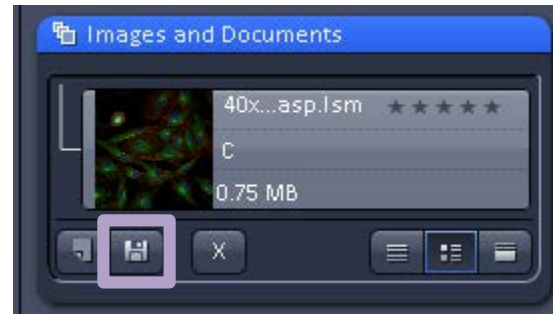
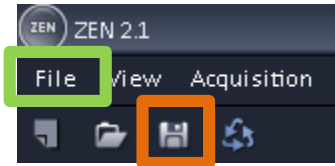
4. You may also need to adjust the **laser power**. **Generally keep the laser power between 1-5%** **More laser power = yield more signal = more photo bleaching of your fluorophores.**



4. Once you have your image in focus and at the intensity you like, click the **Snap** button to acquire your data quality scan.

Saving your file

1. After you have taken your image you may save your file by using one of the following options:
 - clicking on **File**, then Save
 - clicking on the **disk icon** on the top left
 - clicking on the **disk icon** on the right, under images and documents

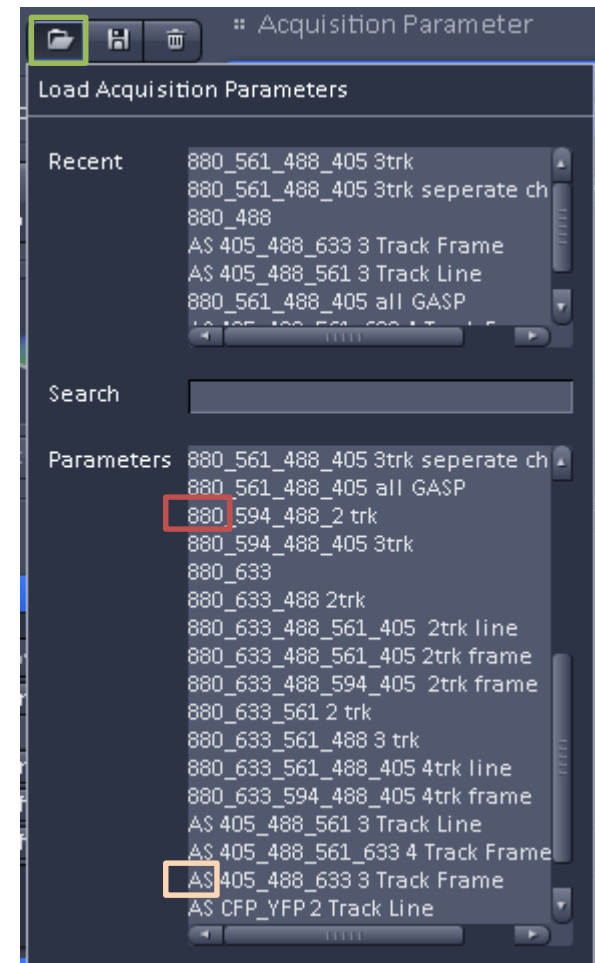


Setting up configurations on your own

1. Click on button that looks like an open file folder it will bring up a menu called Load Acquisition Parameters
2. Choose what laser lines you would like to use to excite your sample. Then click on the setup that contains those lasers.

Note: Examples of commonly used dyes and the laser lines used to excite them are listed on the next page.

Note: The files that start 880 are for the regular confocal and the ones that start AS are for the Airyscan



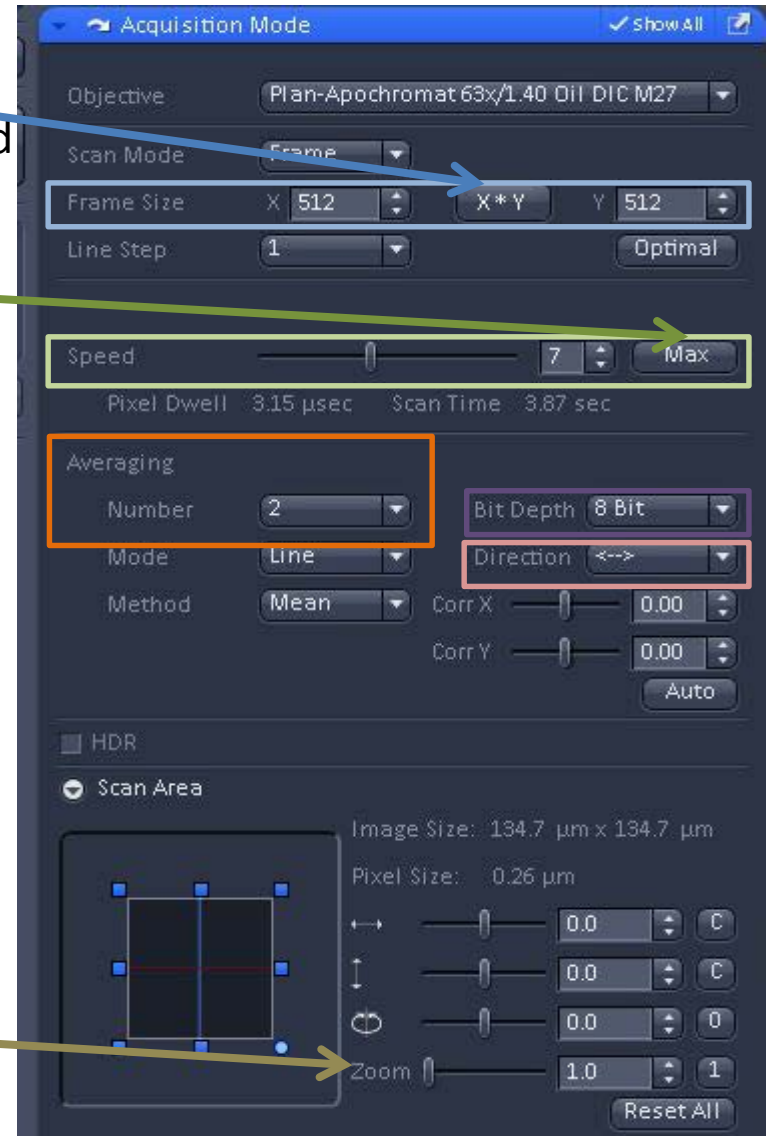
What laser should I use?

Here are some examples of commonly used dyes and the laser lines used to excite them:

Laser	Dye
405	Dapi, Alexa 405
458	CFP
488	GFP, FITC, Alexa 488
514	YFP
561	Alexa 543, Alexa 568, Alexa 594, Rhodamine, Mitotracker red
594	Alexa 594
633	CY5, Alexa 633, Alexa 647

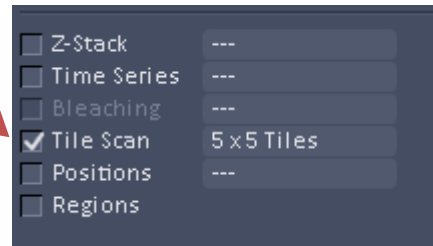
Acquisition mode – Recommended settings

1. A good **Frame Size** to use is **1024 x 1024**, if you need to change your Frame Size click on the X*Y button
2. **Speed** should be set about **7 or 8**, if you just want to do a quick scan you can increase the speed by clicking on Max
3. For **Averaging** 2 or 4 are a good numbers to use
4. **Bit Depth** should be 8 bit
5. Direction: you can scan faster by using **bidirectional** scanning <-->
6. If you would like to see more detail in an area you can increase the zoom



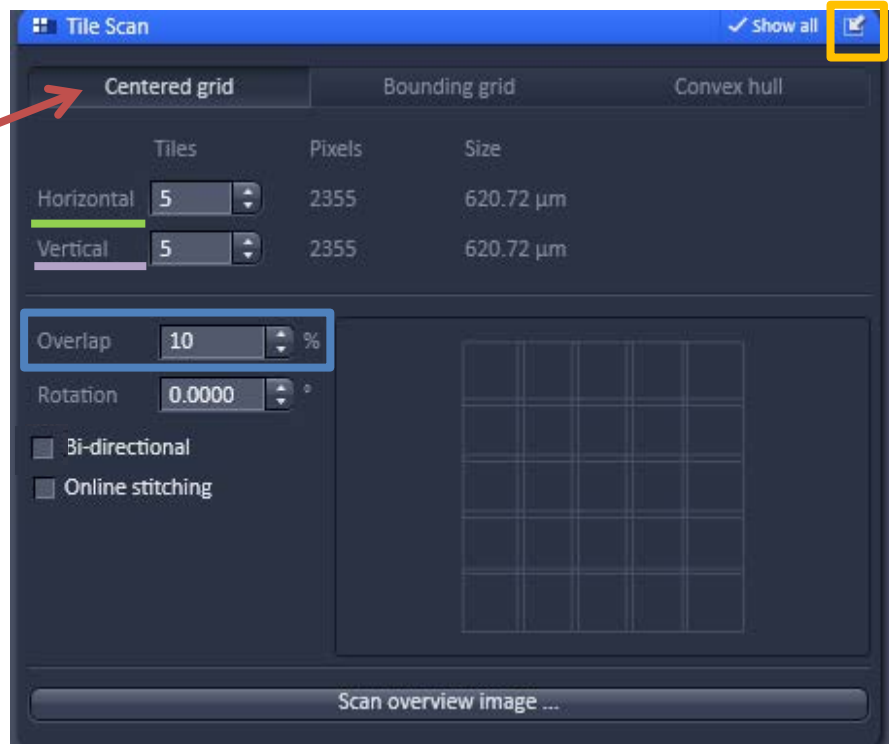
Tile Scans

1. To do a tile scan, first click Tile Scan, a Tile Scan box should appear on the right if it doesn't scroll down on the left until you see it listed under Multidimensional Acquisition.



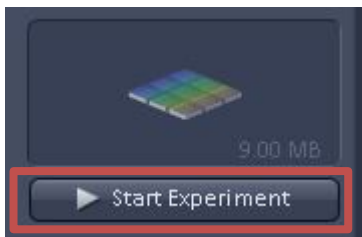
2. If you would like to move the tile scan box, click the **undock** button

3. Under the Centered grid tab choose the number of Horizontal and Vertical tiles you would like to use.



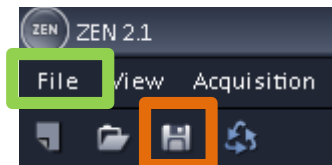
4. Enter an **overlap of 10%**

5. Click the **start experiment tab**



Stitching a Tile Scan

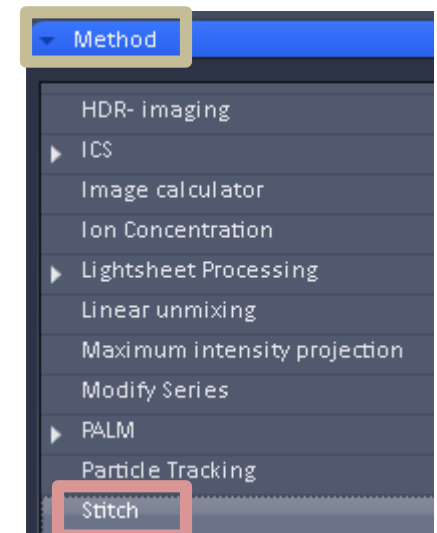
1. After you take your image save your file
 - click on **File** then Save or
 - click on the **disk icon** on the top left



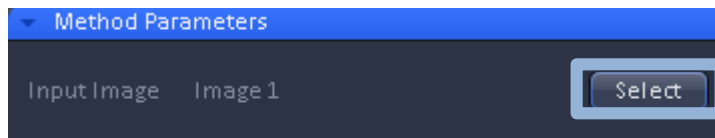
2. Click on the **Processing** tab



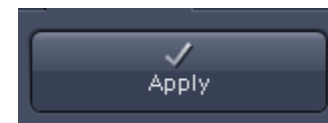
3. Under **Method** click on **Stitch**



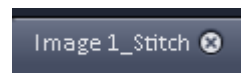
4. Click on the image you would like to stitch and then under Method Parameters click on **Select**



5. Next click on Apply, this will stitch your image for you

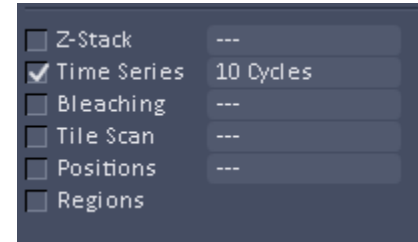


6. Finally save your file, it will have the original image name with an “_Stitch” at the end.



Time Series

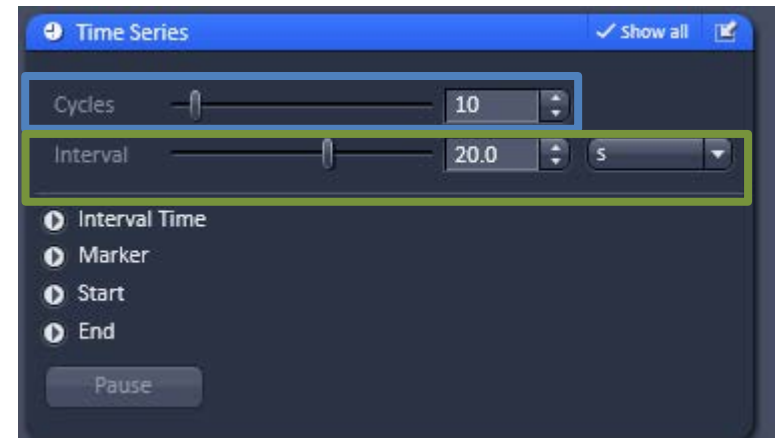
1. To do a time series, first click on Time Series.



2. The Time Series tab will appear under the Multidimensional Acquisitions on the left side. To move the Time Series box, click the **undock** tab

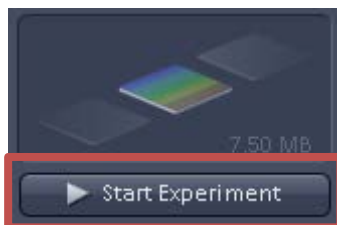


3. Set the number of **Cycles** for how many images you would like to take.



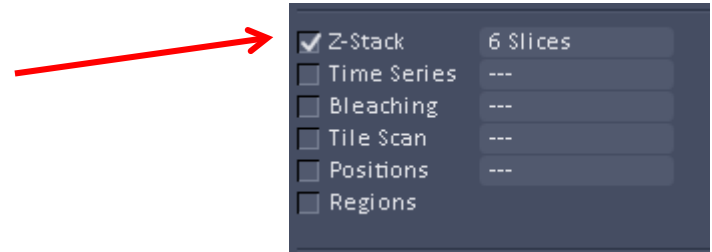
4. Set the **Interval** for how often you would like to take an image.

5. Click **Start Experiment**



Z-Stacks

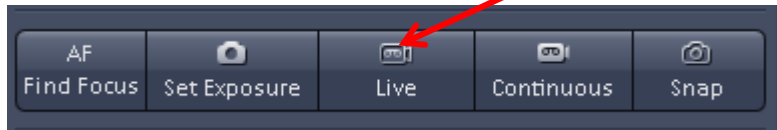
1. To make a z-stack first click on Z-Stack, a Z-Stack box should appear on the right if it doesn't scroll down on the left until you see it.



2. If you would like to move the Z-stack box, click the **undock** button



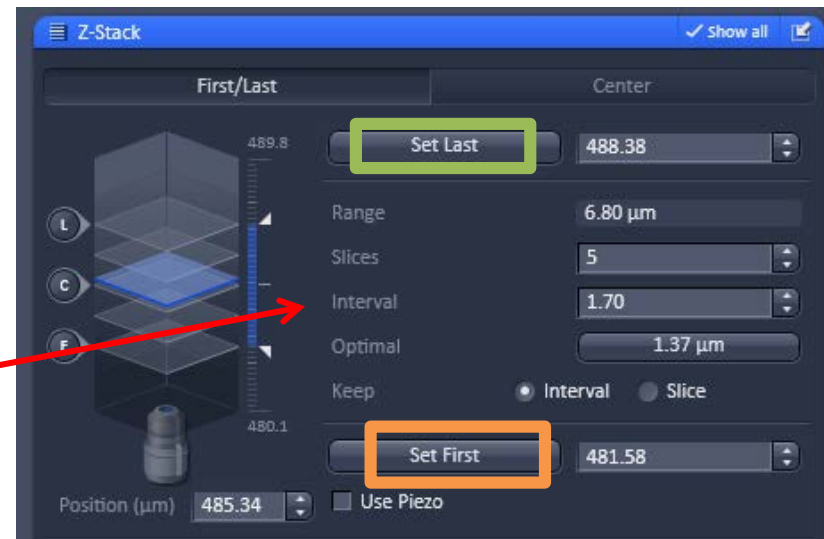
3. Click on live



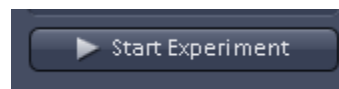
4. Find the bottom of the sample, roll the focus wheel towards your body, then click **Set First**

5. Find the top of the sample roll the focus wheel away from your body, then click **Set Last**

6. Set your interval and check your number of slices **note if you are doing 3D you must oversample, check with us for your interval

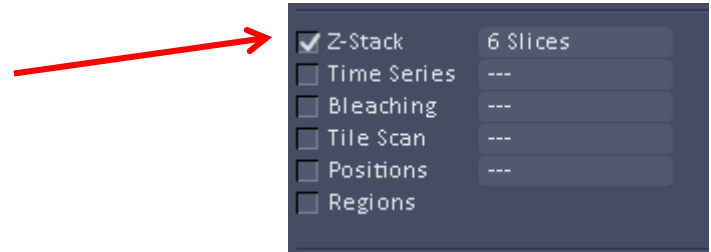


7. Click on start experiment



Z-Stacks using Center

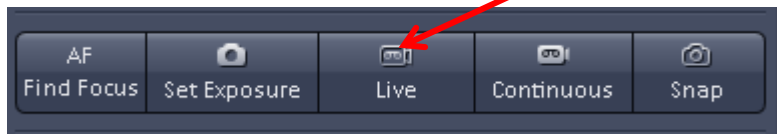
1. To make a z-stack first click on Z-Stack, a Z-Stack box should appear on the right if it doesn't scroll down on the left until you see it.



2. If you would like to move the Z-stack box, click the **undock** button

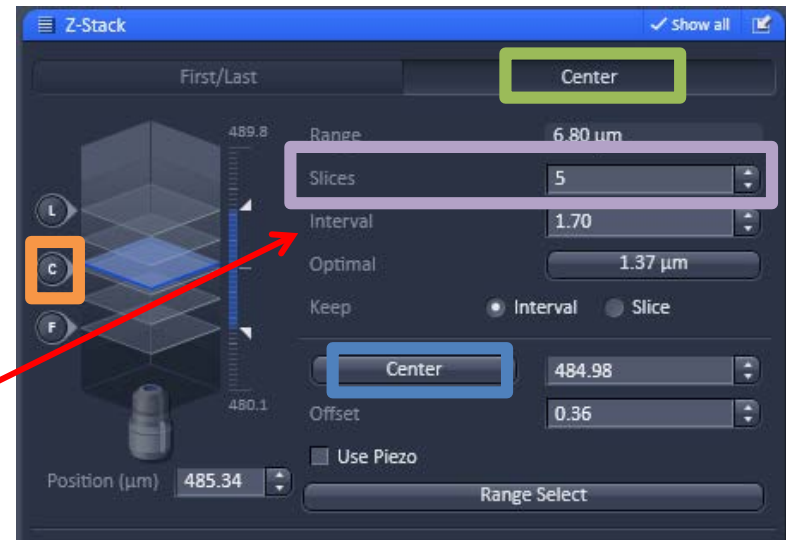


3. Click on live

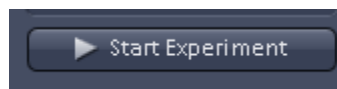


4. Click on **Center**, find the center of the sample you would like to image. Click on the **C** to take a Snap of the center image to make sure you are in the right spot. Click **Center** to confirm this is the slice in the middle of the stack.

6. Set your interval and check your number of **slices**
Make sure you have an odd number of slices
**note if you are doing 3D you must oversample, check with us for your interval

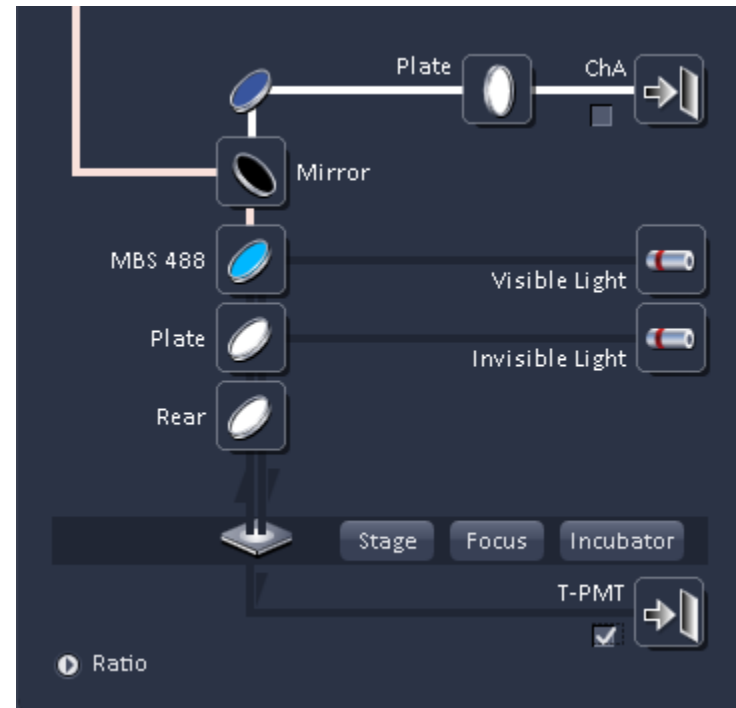


7. Click on start experiment



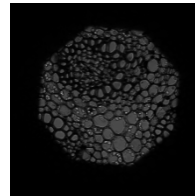
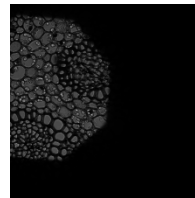
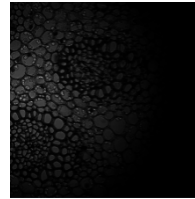
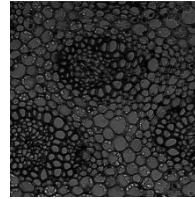
Adding a DIC Transmitted Light Image

1. If you would like to add a DIC transmitted light image click the T-PMT while you are under the Acquisition tab.
2. To have the best DIC image check your Kohler illumination, check out the Kohler illumination cheat sheet on the next page.



Kohler Illumination for the 880

1. Bring the sample into focus
2. Close the **field diaphragm** until you can see at least one edge
3. Adjust the **condenser height** until the Edges of the diaphragm image are crisp
4. Center the diaphragm image using the two **centering screws**
5. Open the field diaphragm, just until the image fills the field of view



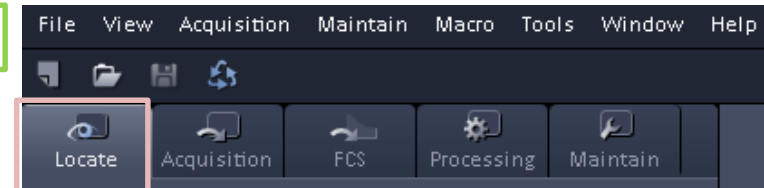
Incubation

1. Make sure to turn on the incubation and CO2 controls before you open Zen (there is one switch on the TempModuleS and one on the Heating Unit XL S)

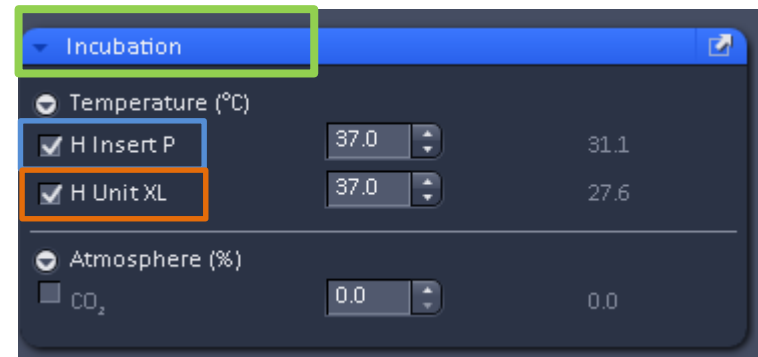
Note: The TempModuleS light will turn on when you switch it on, the light on the Heating Unit XLS will not turn on until the temperature is turned on in Zen

3. Under the **Locate tab** you will find the **Incubation tab**

4. Check the **H Insert P** if you would like to use the heated insert, check the **H Unit XL** if you would like to use the incubation chamber.



Note: Once you check the **H Unit XL** the bottom light on the Heating Unit XL S will turn on and the middle light will blink yellow telling you the heating unit is warming up

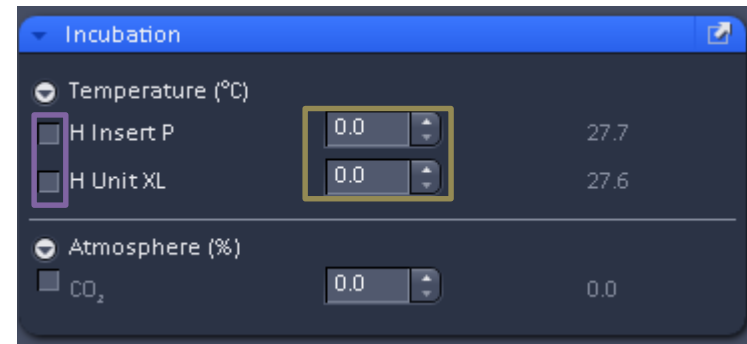
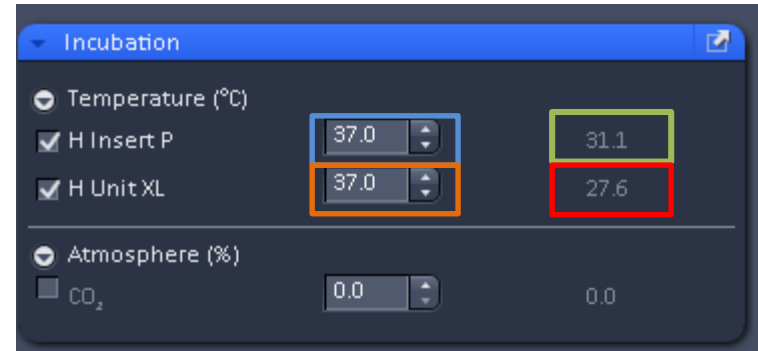


Incubation

5. Type in the temperature you would like to use for your incubation, for either the **insert**, or the **incubation chamber**
6. The numbers on the right hand side are the actual temperature for the **insert** and the **incubation chamber**
7. After you have completed your experiment **uncheck** the H Insert P and H Unit XL and set the **temperature to zero.**

NOTE: After you have unchecked the H Unit XL make sure the lights on the Heating Unit XL S are off then **Wait 15 Minutes** before you log out out of Zen. This will allow the heating unit to cool down.

8. After you log out of Zen, turn of f the TempModuleS and Heating Unit XL S

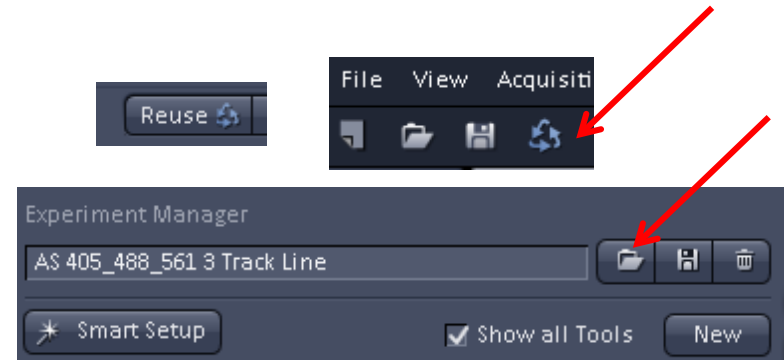


Calibration for Airyscan

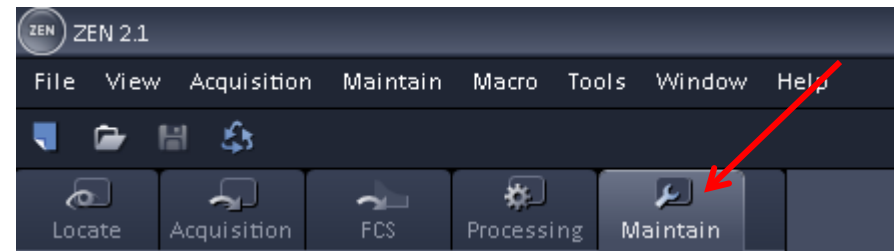
When you are setting up your calibration it is best to use a channel with bright fluorescence, one that will fill most of the scanned area, and is in the visible range. Remember in Airyscan you should not adjust the pinhole or the gain, you should only adjust the laser power.

Note: If you feel your sample is not bright enough, or if you are afraid it may get bleached out, you may use a slide provided by the FMIC to do your calibration.

1. Open an Airyscan configuration, by reusing an old configuration or by opening a new one.
Note: The Airyscan configurations begin with AS

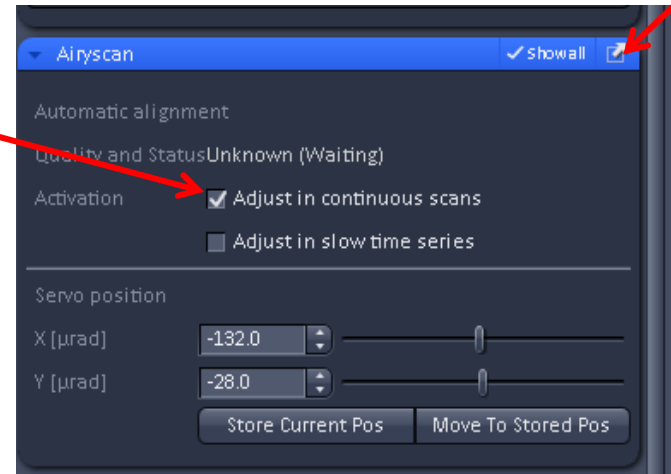


2. Click on the maintain tab

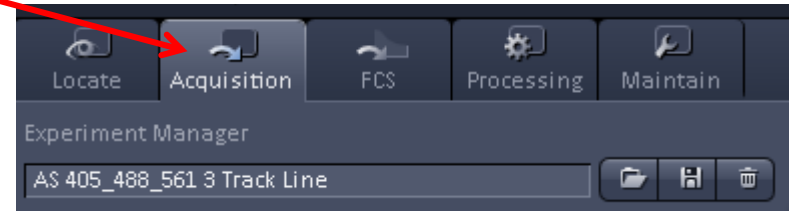


Calibration for Airyscan

3. Open Airyscan, undock and click adjust in continuous.



4. Click on the Acquisitions tab

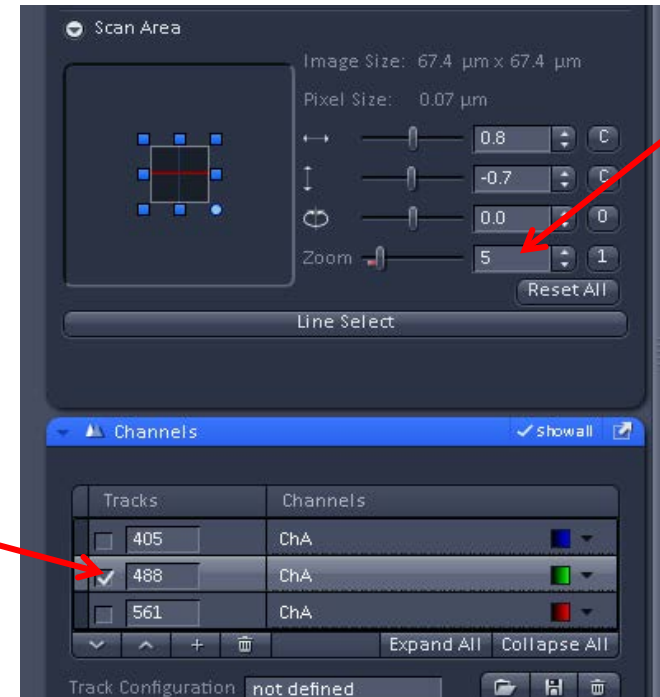


5. Change the frame size to 512 x 512

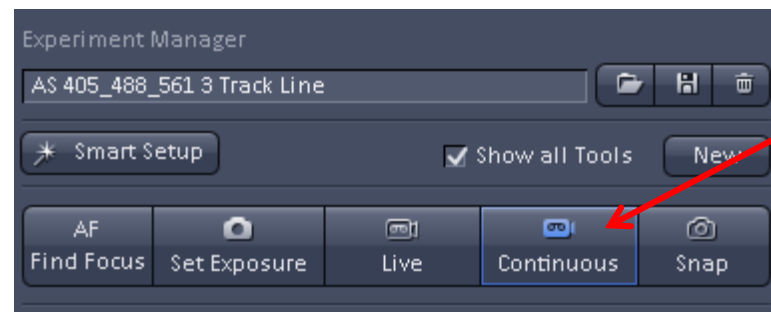


Calibration for Airyscan

6. Highlight only one channel and choose a zoom of 5. Remember, it is best to use a channel with bright fluorescence, one that will fill most of the scanned area, and is in the visible range.



7. Click on Continuous



Calibration for Airyscan

8. In the Airyscan box, look at the Quality and Status. It will begin with “Unknown (waiting)” in white, then go to “Bad (Waiting)” or “Bad (adjusting)” in red and when it is completed it will say “Good (Waiting)” in Green. Once your calibration is complete uncheck the Adjust in continuous scans.

Note: if it stays in “Unknown (waiting)” in white for a long time your sample may not be bright enough, or it may not be filling up enough of the scanned area. If this happens please use one of the slides provided by the FMIC.

