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710 User Guide Overview

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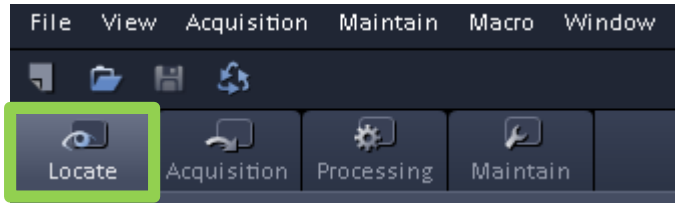
Starting up the system

1. Follow the directions for Powering up the 710, this is located to the left of the 710 monitor

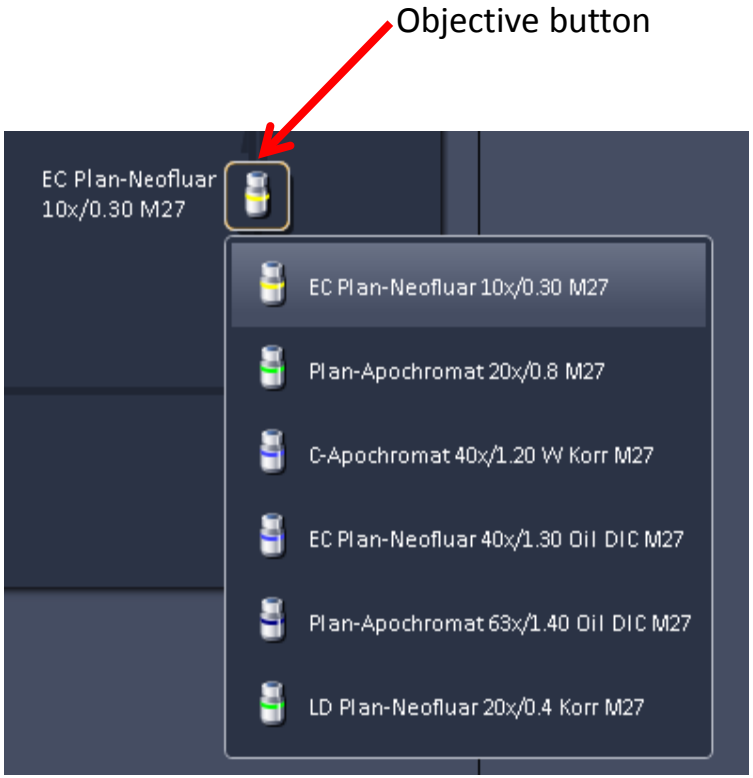
2. Double click on the Zen icon

3. Click on the Start System tab

Viewing your sample



1. When Zen opens you will be under the Locate tab, this is where you can view your sample through the eyepiece.



2. First, choose your objective by clicking on the objective button, this will bring up a list of objectives currently on the microscope. Select the objective you would like to use

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

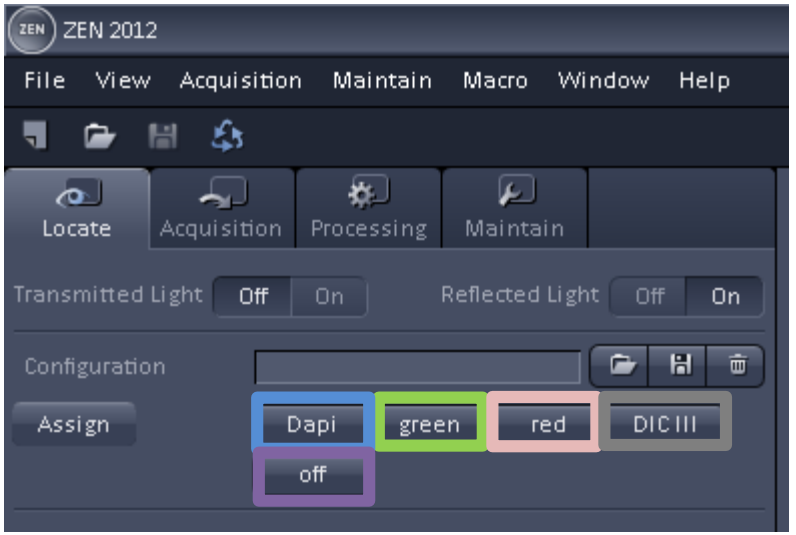
3. After you choose your objective make sure to clean it with lens cleaner
4. Set your sample on the stage, please remember to add oil or water as appropriate

Viewing your sample

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

Blue Green Red

If you would like to see white light select DIC III



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.

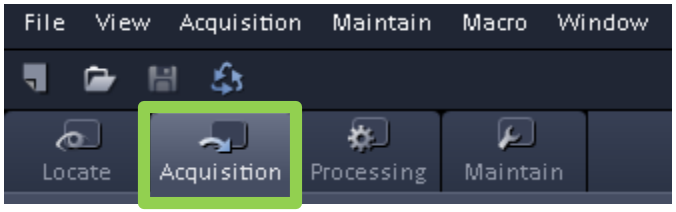
Towards yourself moves the objective down
Away from yourself moves the objective up

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

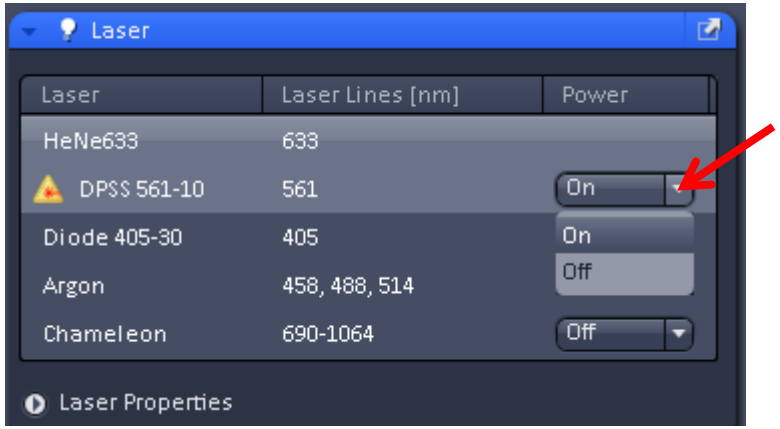
4. Click OFF when you are done viewing your sample through the eyepiece.

Turning on the lasers

1. To turn on the lasers click the acquisition tab

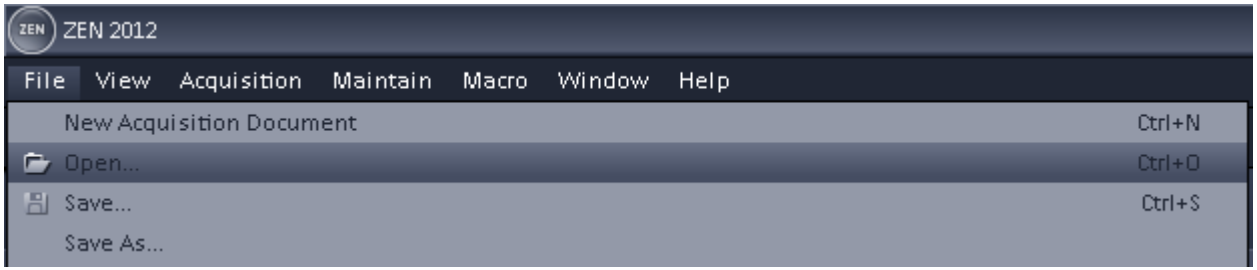


2. Use the pulldown tab to turn on the lasers

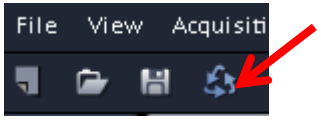


Setting up a configuration

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



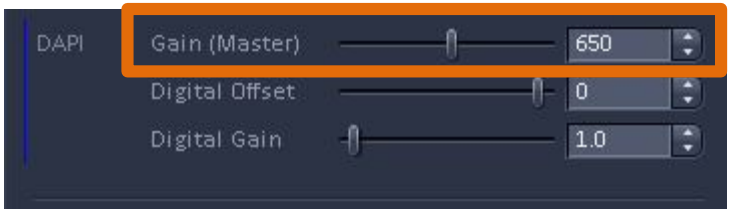
3. Please contact Jeff, Agnes or Erica if you need to set up a new configuration.

Taking an image

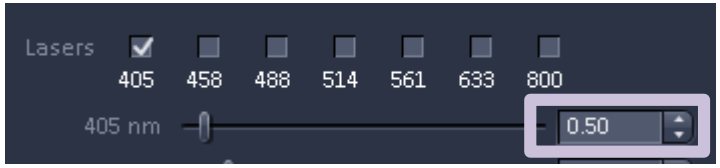
1. To view your sample click on the Live button.

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 600-700



4. You may also need to adjust the laser power. Please talk to Jeff, Agnes or Erica if you have questions about selecting the appropriate level for the laser intensity.



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

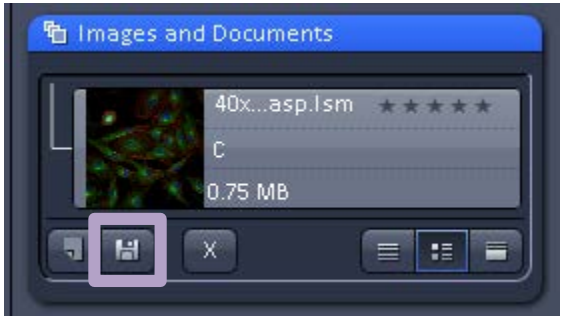
Saving your file

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

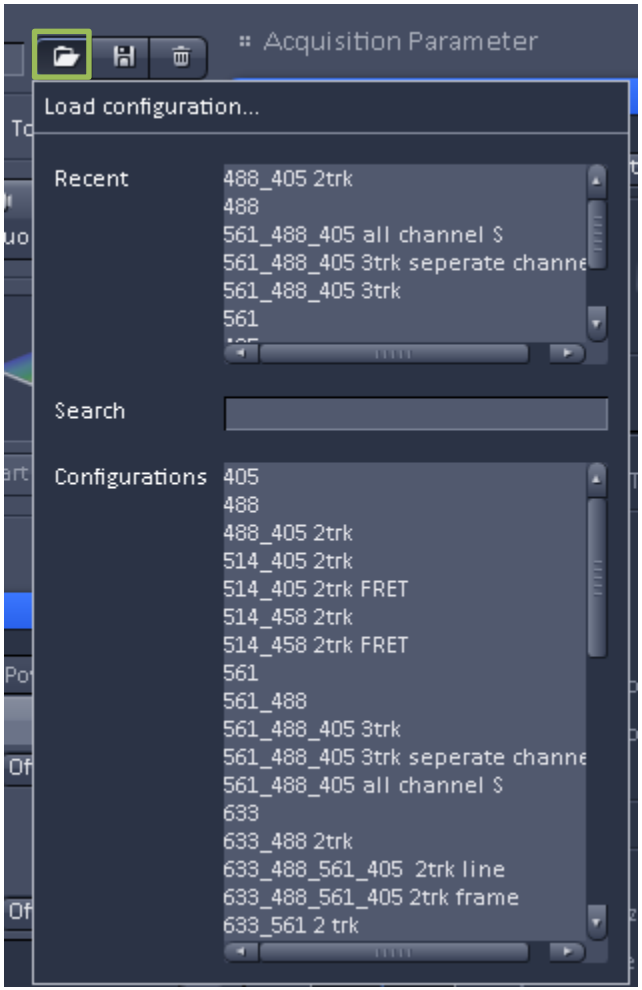


Setting up configurations on your own

1. Click on button that looks like an open file folder
   it will bring up a menu called
   Load configuration

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.



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
633	CY5, Alexa 633, Alexa 647, DRAQ5
800	Please talk to Jeff, Agnes or Erica

Acquisition mode – Recommended settings

1. A good Frame Size to use is 512x 512, if you need to change your Frame Size click on the X*Y button

2. Speed should be set about 7, if you just want to do a quick scan you can increase the speed by clicking on Max

3. For Averaging 4 is a good number to use

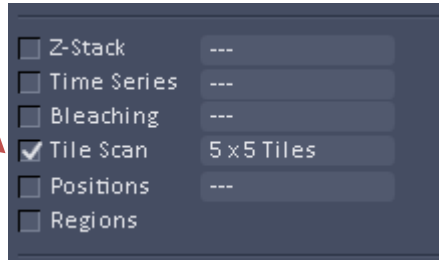
4. Bit Depth should be 8 bit

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

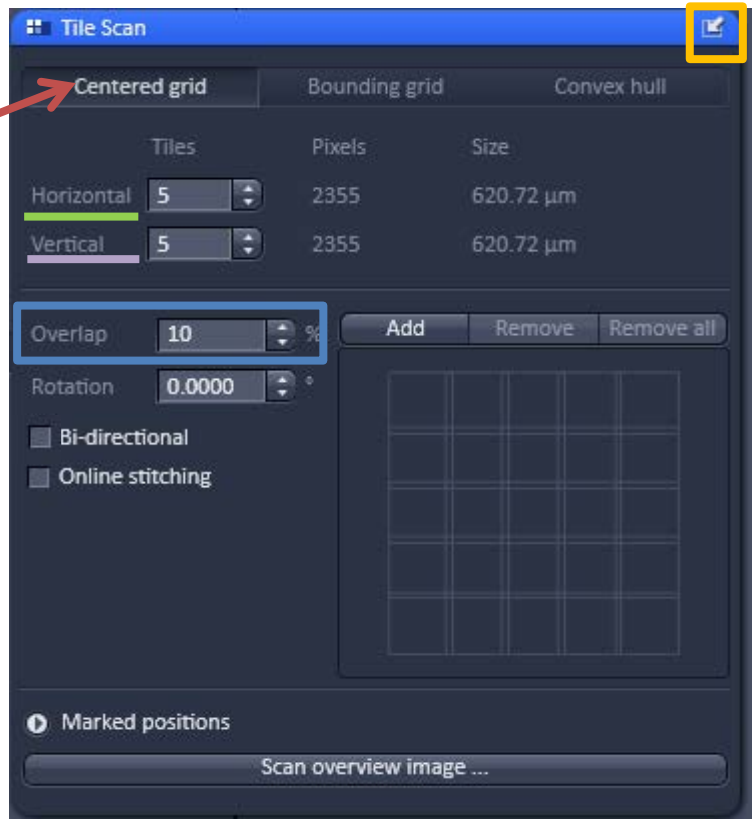
Tile Scans

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.



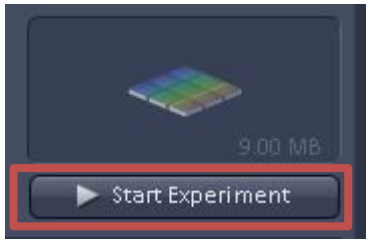
2. If you would like to move the tile scan box, click the undock button

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



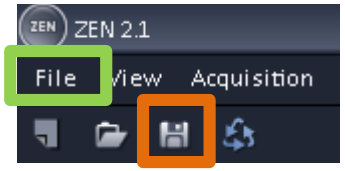
4. Enter an overlap of 10%

5. Click the start experiment tab

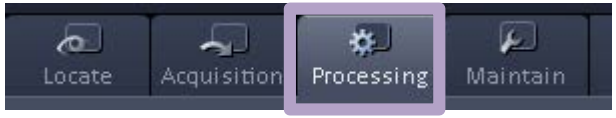


Stitching a Tile Scan

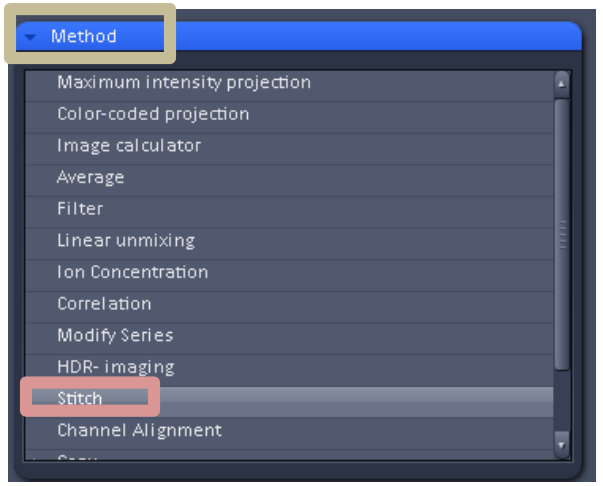
1. After you take your image save your file
   - click on File then Save or
   - click on the disk icon on the top left



2. Click on the Processing tab

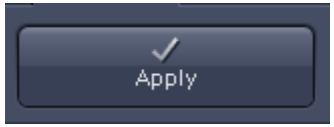


4. Click on the image you would like to stitch
   and then under Method Parameters click on Select



5. Under Method click on Stitch

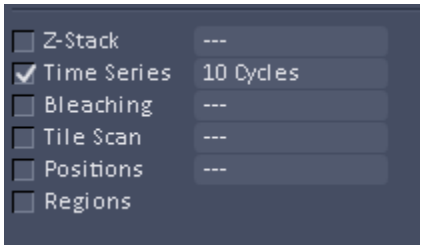
5. Next click on Apply, this will stitch your image for you



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

Time Series

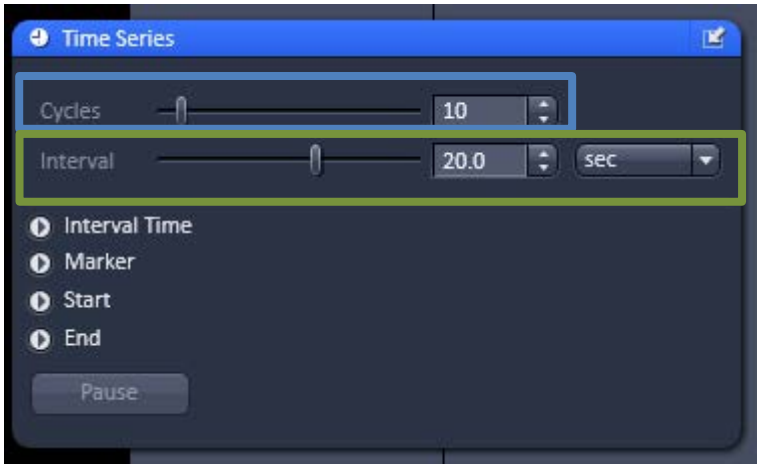
1. To do a time series, first click on Time Series.



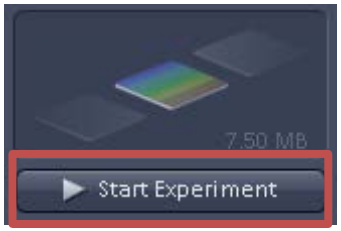
2. The Time Series tab will appear under the Multidimensional Acquisitions on the left side. To move the Time Series box , click the undock tab

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.

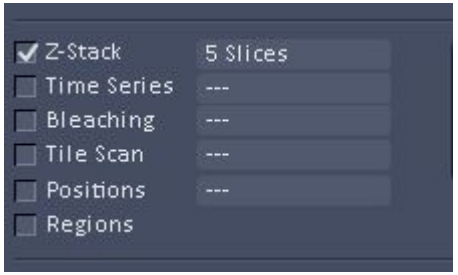


5. Click Start Experiment



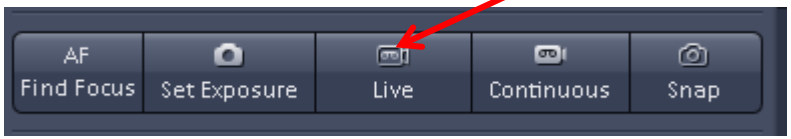
Z-Stacks

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

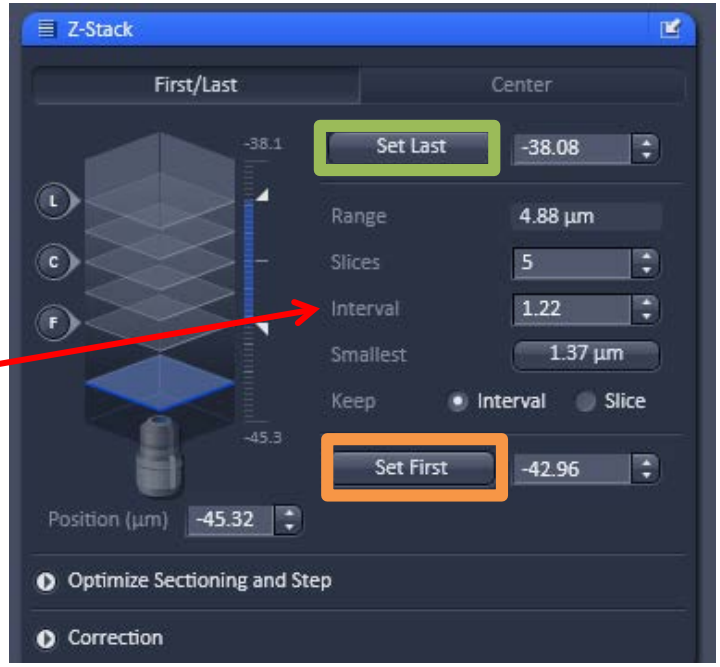
3. Click on live



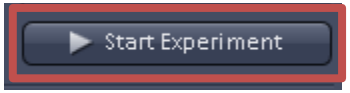
4. Find the bottom of the sample, roll the focus wheel towards your body, then click Set First

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

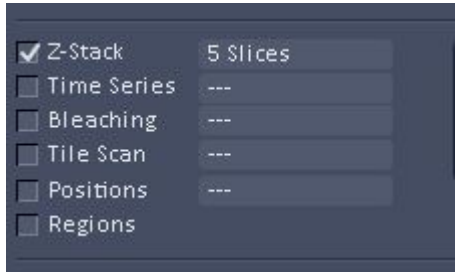


7. Click on start experiment



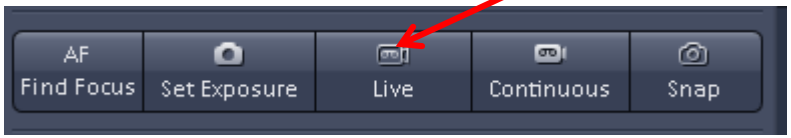
Z-Stacks using Center

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.



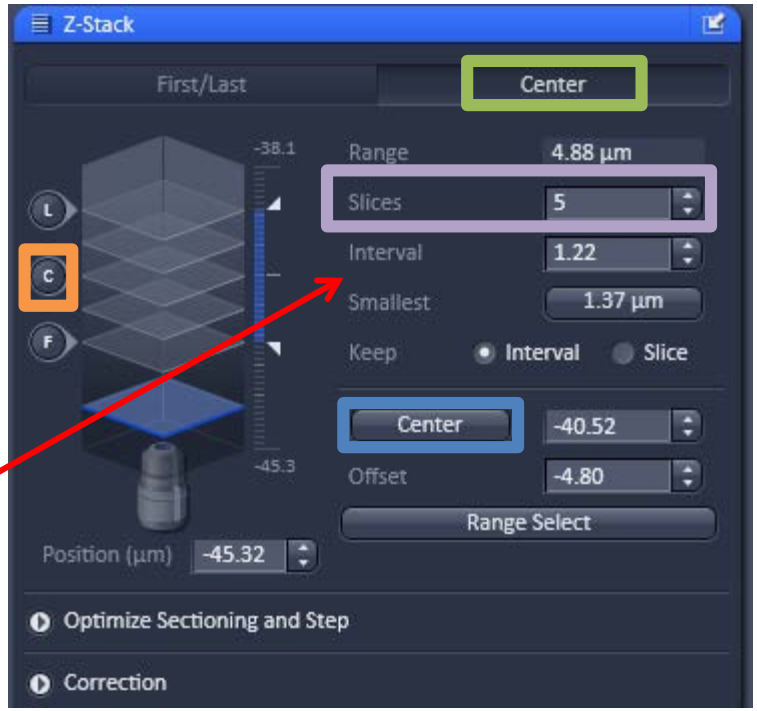
2. If you would like to move the Z-stack box, click the undock button

3. Click on live

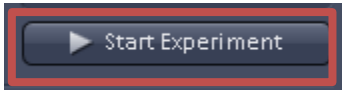


4. Click on Center then find the center of the sample you would like to image. Click on the C to take a Snap of the center image to make sure you are in the right spot. Click Center to confirm this is the slice in the middle of the stack.

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

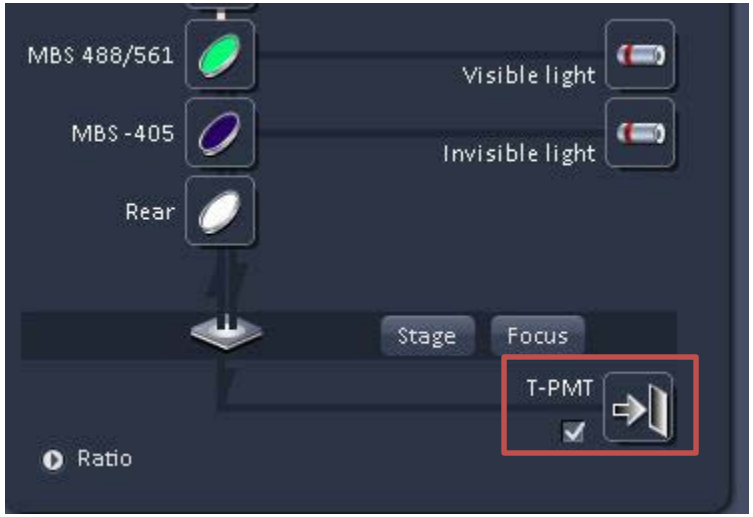


7. Click on start experiment



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, if you need help with this ask Jeff, Agnes or Erica or check out the Kohler illumination cheat sheet

Kohler Illumination for the 710

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