Zeiss AxioObserver with ApoTome
Zen software

User Guide

LSU Health-Shreveport

Research Core Facility (RCF)

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July 30th 2021

If the room lights are not on when you arrive to use the Axioobserver, **DO NOT TURN THEM ON**. Please be courteous of those using the other microscopes, since they may have light-sensitive slides, dishes, or plates on the microscope stages, which could be damaged by the room lights. Use a flashlight if needed.

Check whether the stage **holder** is properly in place. If not, please contact the Research Specialist. Make sure the **table is clean**. No food or water is allowed in the microscope room.

Before you start, please check the objective turret has the 10x objective in place. This is a safety precaution. If the 10x objective is not in place, please clean the current objective, drop the objective to the bottom, and switch it to the 10x objective. Please also inform the specialist that the objective is not positioned to 10x before you start.

Stage holder

START UP THE SYSTEM

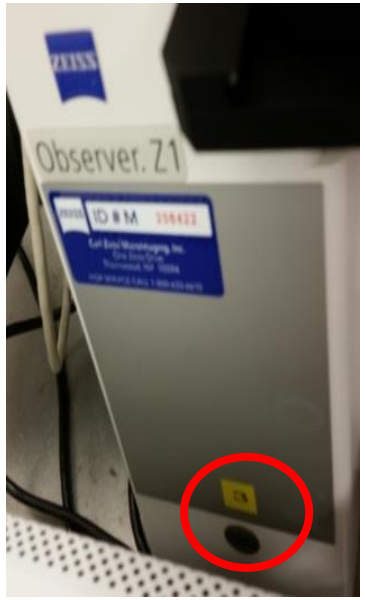
Please sign in on the login sheet. The format for the date is MM/DD/YYYY, eg. 05/25/2015. Write legibly for this is how the staff knows who has been using the microscope. Check in and Check out time is written in 24 hour format, e.g. 1pm is 13. The format for writing the bulb time is ###h ## m, eg. 100h 25m. (You will not be able to write the bulb time until you turn on the power supply.)

The computer should be on. Log in with your **LSUHSC ID and password**. Make sure the domain is set to **LSUMC-MASTER**, selected from the drop-down menu. If you only wish to access documents/files, then login and do not turn on the mercury lamp power supply or the other buttons. Please indicate this on the sign in sheet.

Please sign in on the login sheet. The format for the date is MM/DD/YYYY, eg. 05/25/2015. Write legibly for this is how the staff knows who has been using the microscope. Check in and Check out time is written in 24 hour format, e.g. 1pm is 13. The format for writing the bulb time is ###h ## m, eg. 100h 25m. (You will not be able to write the bulb time until you turn on the power supply.)

Our system has the startup labeled numerically with yellow stickers.

1. Turn on the mercury lamp power source. The green power light and the green lamp light will come on. (This is where you record the bulb time.) If the lamp light indicator does not light up, please contact Core facility staff. Once turned on, the lamp should remain on for a minimum of 30 minutes. Do not turn the lamp off if another user is scheduled within 2 hours after your session.



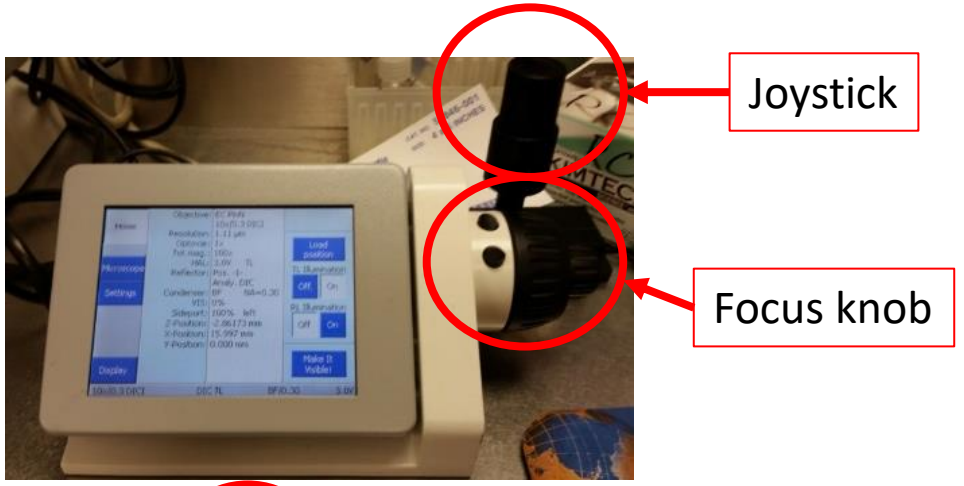
2. Turn on the Power Supply 2 by flipping the switch which will have a green light turn on.

3. Turn on the microscope (number 3). Button located at the left side of the microscope. This will turn on the Touch Screen Controller, and please wait for this display to fully load before proceeding.

4. Turn on the Apotome power supply labeled with a yellow 4 sticker if you are going to use apotome.

Touch Screen Controller

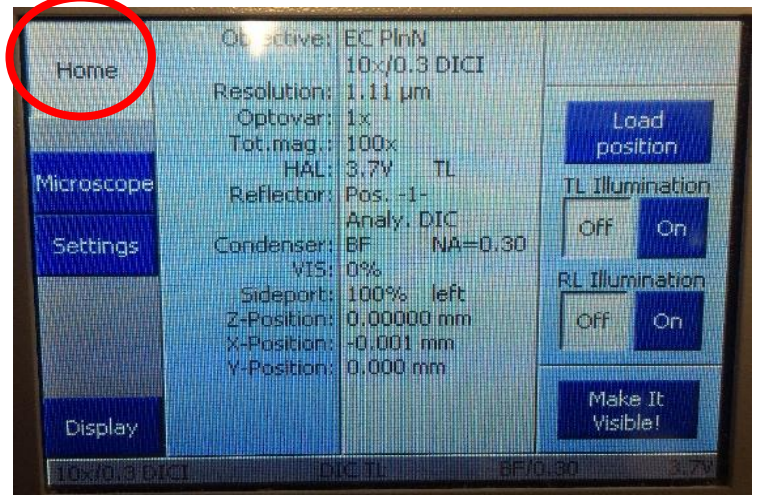
TOUCH SCREEN CONTROLLER



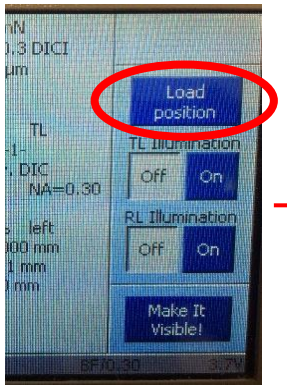
Focus knob: Find the focus (move objective up and down)
Two “wheels” for coarse and fine adjustments and controls the Z-direction of movement.
Joystick: Find the sample (move the stage). The joystick controls the X and Y direction of the stage

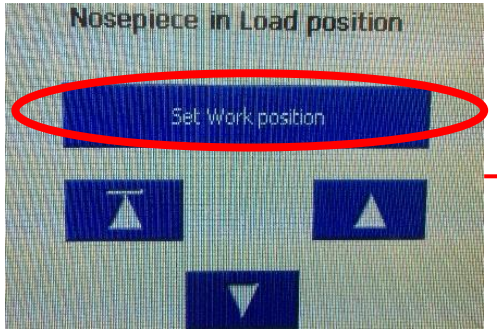
An icon is selected when it is in white and not selected when in blue.

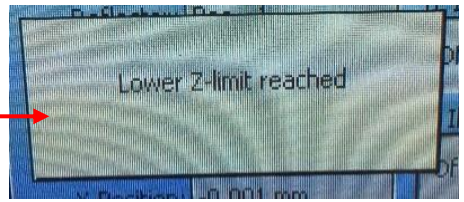
Home screen information:



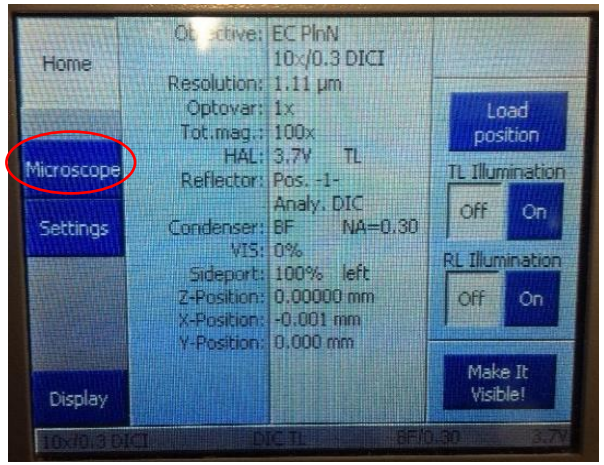
1.**Objective**: Description of the current objective in the light path along with N.A.
2.Resolution: How fine of detail the current image will appear
3.Total Magnification: magnification of objective * eyepiece magnification (10x)
4.Reflector: Displays which position of the reflector turret is in the light path and what type of cube is in that position.
5.VIS: Displays how much of the light from the sample is being sent to the eyepiece
6.Sideport: Displays how much of the light from the sample is sent to the camera/computer
7. Z-position: The bigger the number the higher the objective, the smaller the number the lower the objective.
8: X, Y-position: sample position coordinates.
9: **Load Position (on the right side of the screen): Press this will drop the objective to the bottom**.
10: **TL Illumination**: Transmitted light on or off
11: **RL Illumination**: Fluorescent light on or off

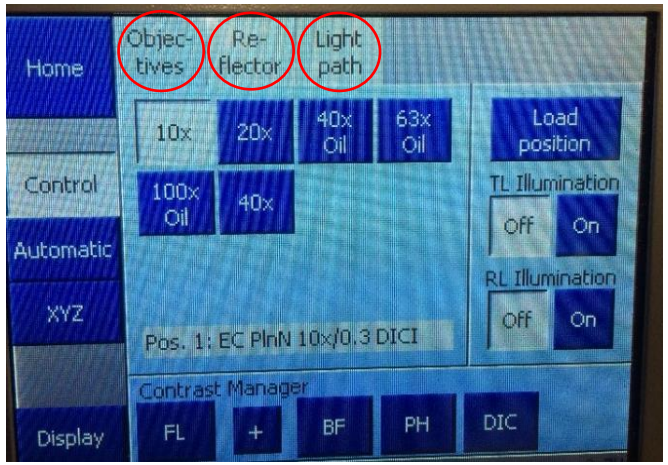






On the left of the screen, push the icon labeled **Microscope**. A new window will pull up in which you can select **Objectives**, **Reflector**, and **Light path**.



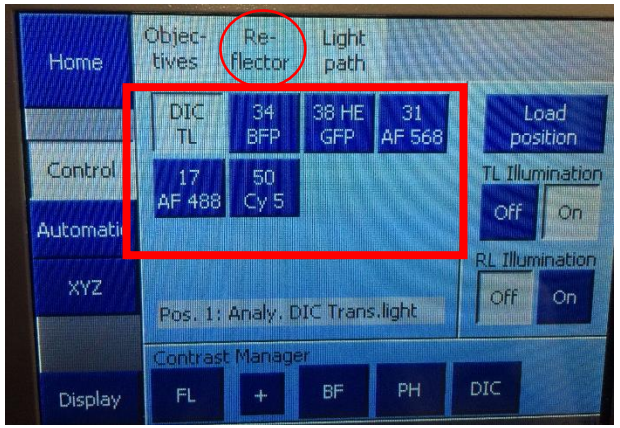


Objective Screen

In the Microscope screen, press the **Objectives** tab to enter the objectives screen. Also, ensure that you are on the **Control** display by checking the **Control** icon on the left sidebar. The **Objectives** displayed tell you which one is an air objective versus an oil objective. If you need to switch between different objectives that use different immersion, the software and TFT display will inform you that you should be prepared for a different immersion objective

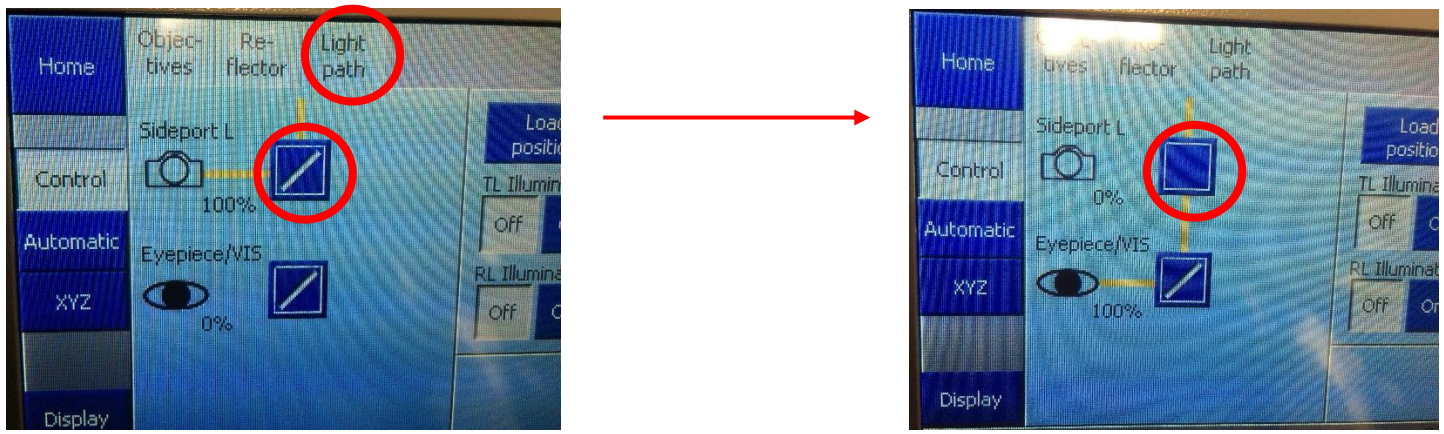
Press Reflector on the top of the microscope screen and you will enter the Reflector screen.

DIC: Transmitted light
34 BFP: DAPI channel (EX 390/20, EM 460/60)
38 HE GFP: Green GFP channel (EX 470/40, EM 525/50)
31 AF 568: Red/ DsRed channel (EX 565/30, EM 620/60)
17 AF 488: FITC channel (EX 485/20, EM 540/25)
50 Cy5: 647 far red channel (EX 640/30, EM 690/50)



Reflector Screen

Press **Light Path** on the top of the microscope screen and you will enter the **Light Path** screen



Sideport L: Directs light to go through the camera and images will show up on the computer.

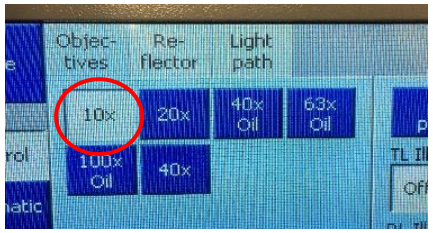
Eyepiece/VIS: Directs light to go through Eyepiece/Vision, so you can observer through eyepiece.

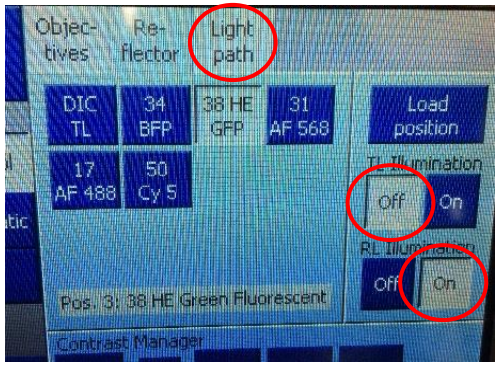
Press the square next to the Camera and you will have 3 options (100% camera, 100% eye, and 50% Camera & 50% eye). **Do not press the square next to Eye**, it won't change anything

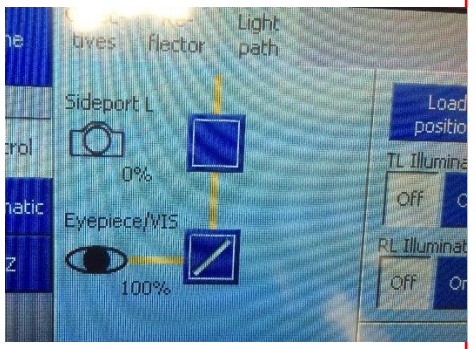
Everything on the touch screen can be controlled by the software. It is preferred to control everything through the software, but below shows you briefly how to set up for imaging if you do not use the software.

Set up for eyepiece observation

1. Select an objective through the Objective screen.
2. Go to the Reflector screen and select a channel. Then turn off TL, and turn on RL
3. Go to the Light path screen and select 100% light goes to Eyes.
4. Locate your sample and focus.
5. **When done, press RL illumination off to turn off the light (Very important!!)**

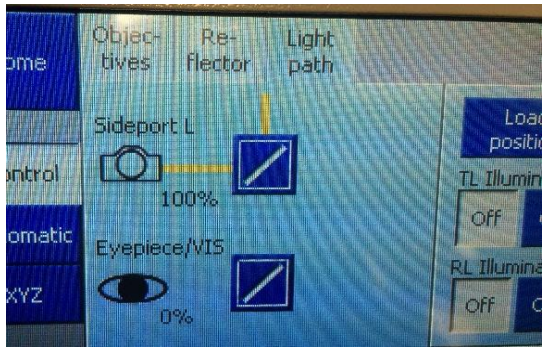






Set up for the software to acquire an image

After turning off RL illumination, swicth light path to 100% to sideport



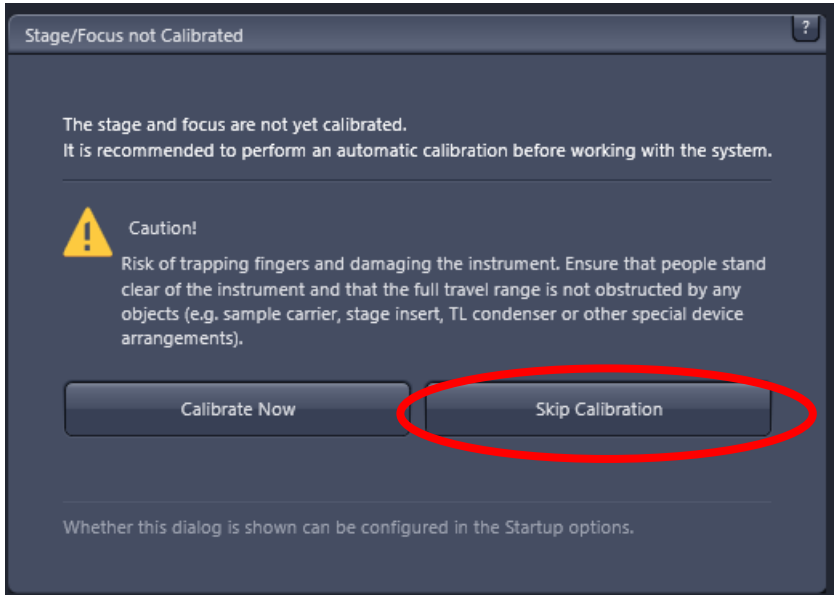
Start up the Zen software

To acquire images, all hardware MUST be turned on before you start up the software.

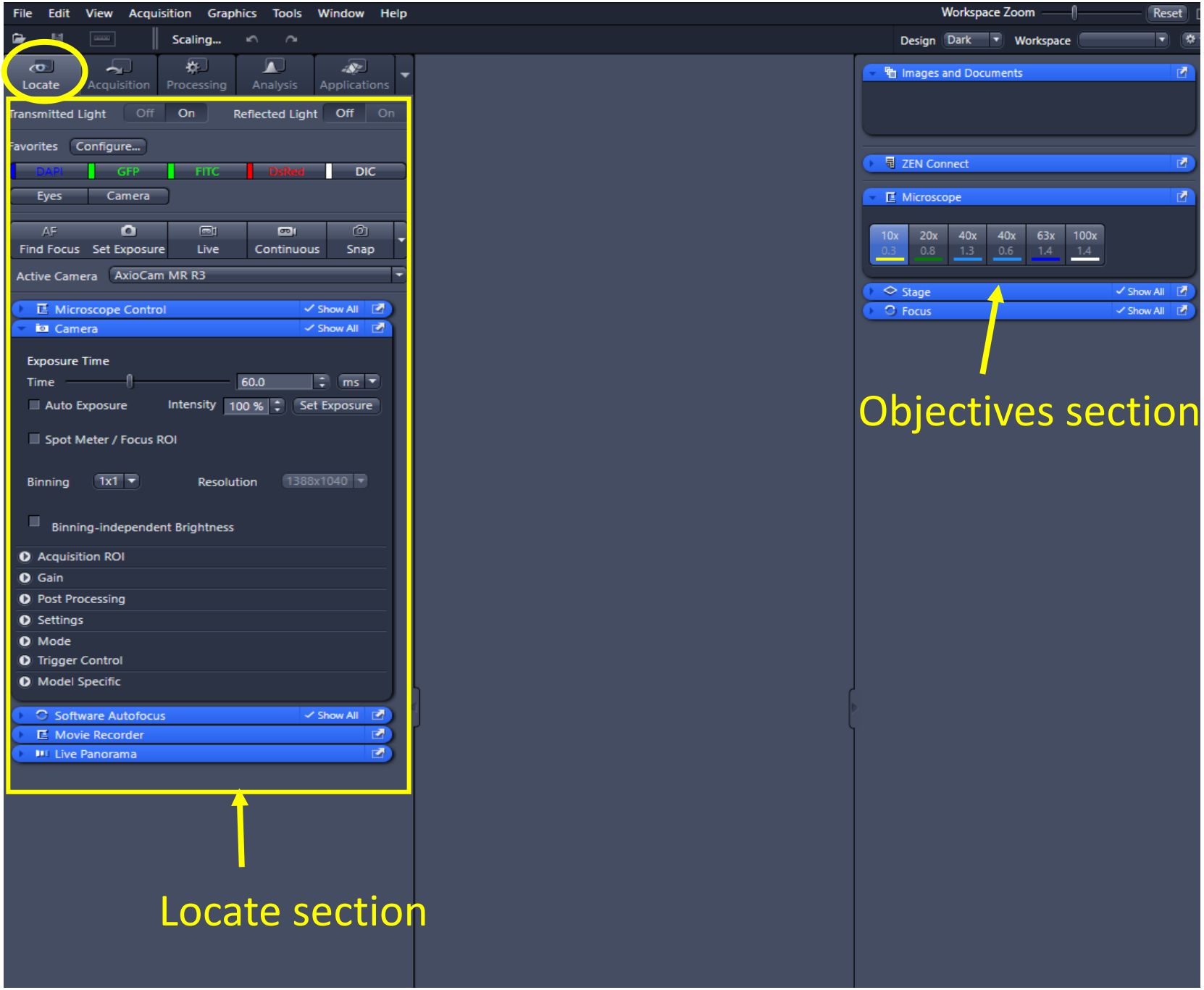
1. On the desktop, click the Zen 3.3 (blue edition) icon to launch the software.

2. Then select Zen pro for image acquisition. If you only want to look at an image, or do image analysis, click ZEN image processing.

3. Next, click Skip Calibration when you see this window pop up.



If you only want to look at an image, or do image analysis using ZEN image processing. You do not need to turn on the microscope hardware.



When software turns on, by default, it displays the Locate tab/section (on the left)

The **Locate** section allows you to turn on the fluorescent light and view the sample through eyepieces.

The Objectives section on the right allows you to change objectives.

Set up eyepiece observation through software

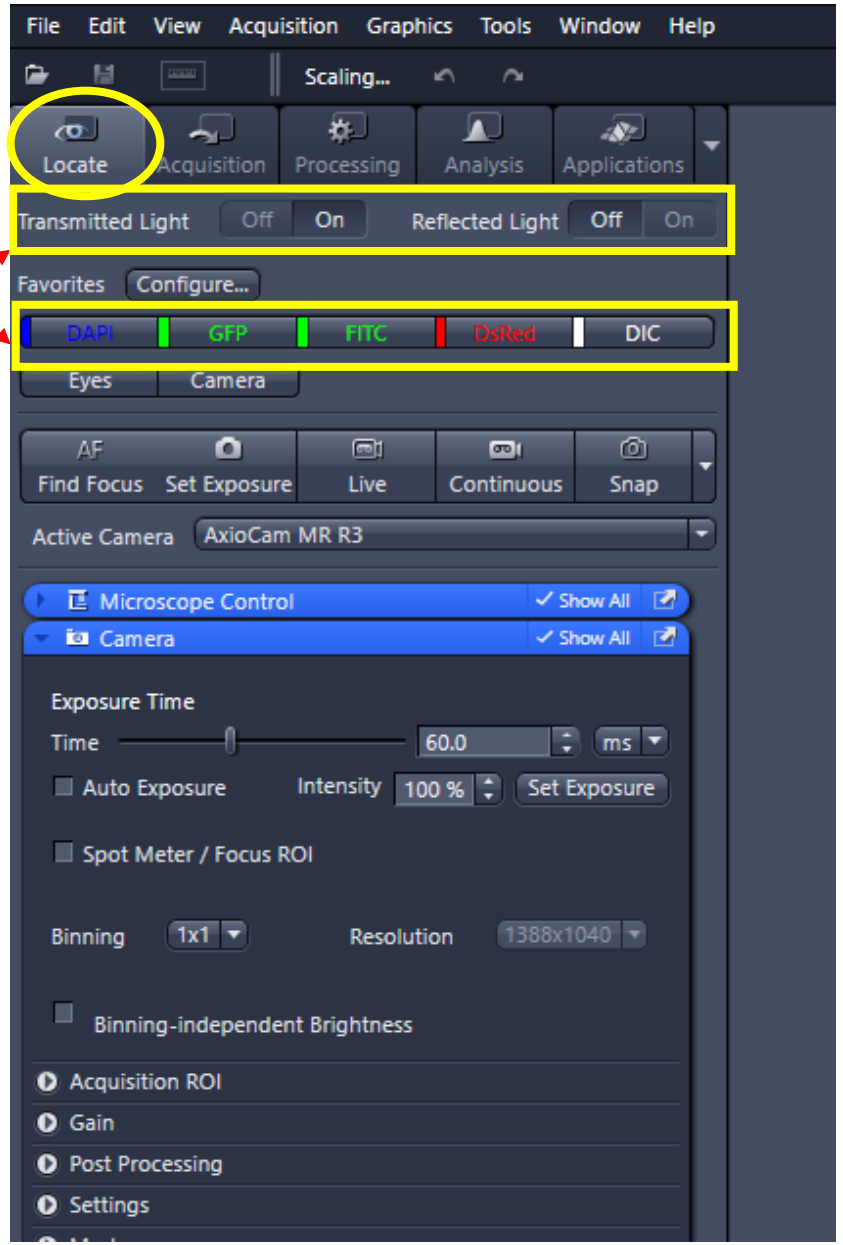
1. In the Locate section, click to select a fluorescent/Bright field channel (DAPI, GFP, FITC, DsRed, DIC). This will turn on the light on the microscope.

2. When light is turned on, the lightpath automatically switch to eyes, you do not need to press anything to make this happen.

3. Then use the joystick to locate your sample. Use the focus knob to focus on your sample.

4. When done, turn off the fluorescent light either through pressing the RL/TL Illumination Off on the touch screen controller, or turn off the light through pressing the Off button on the software.

<u>It is VERY IMPORTANT to turn off the light after observation, so light does not photobleach your sample.</u>

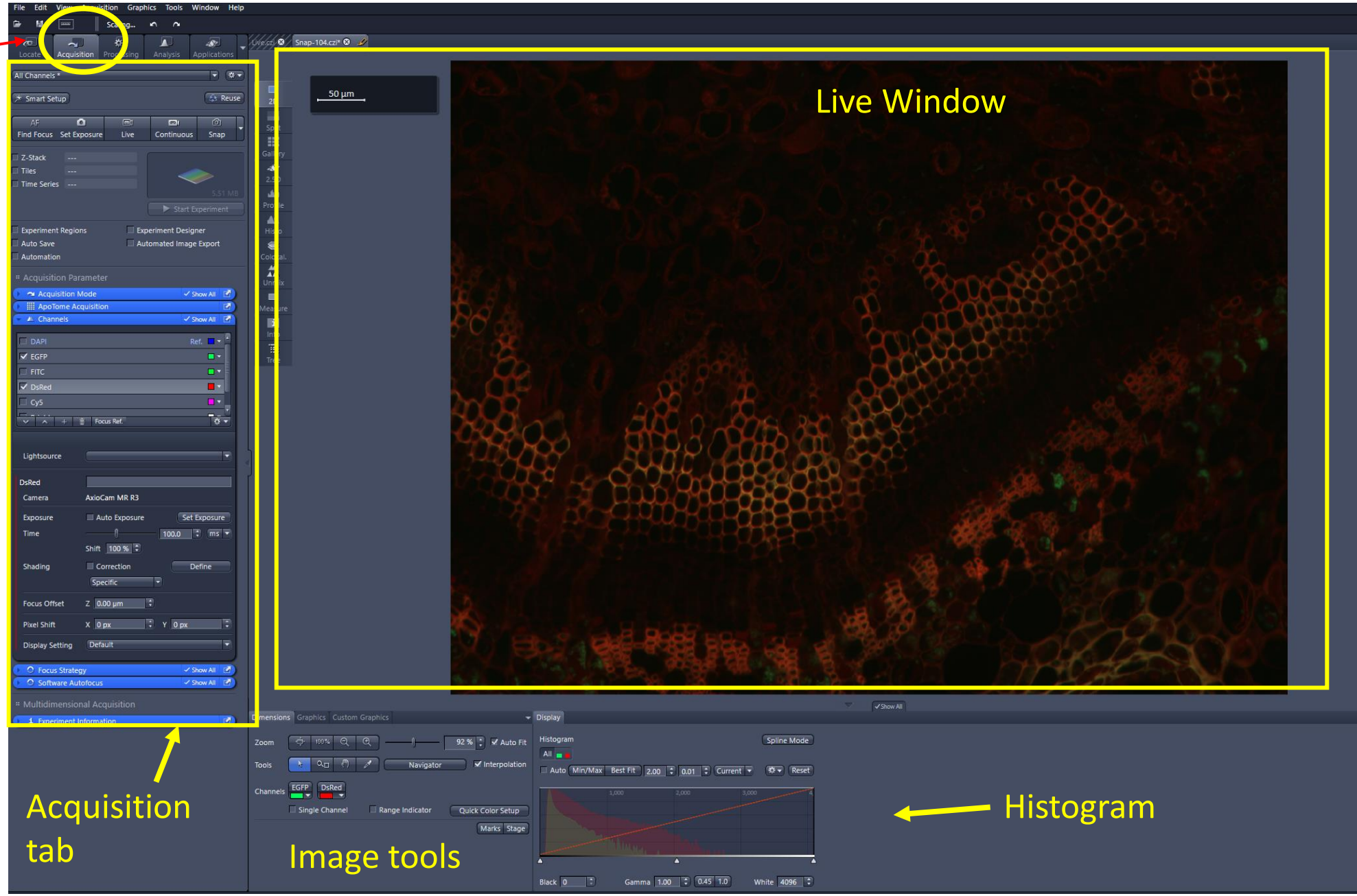


View and acquire image through the software:

1. Click on Acquisition to switch to the Acquisition section.

2. The middle window is where the live image will display.

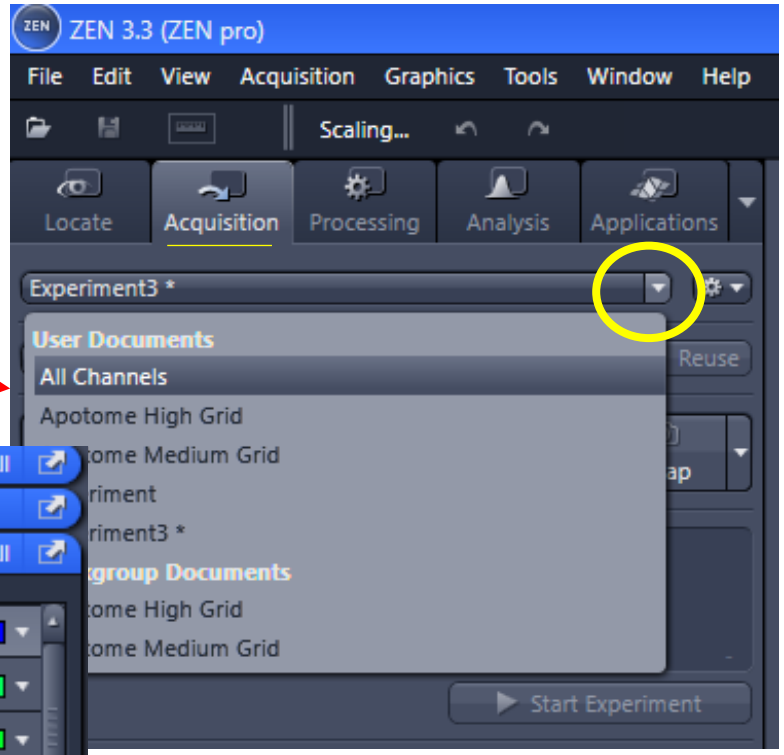
3. The bottom is the histogram and image tools.

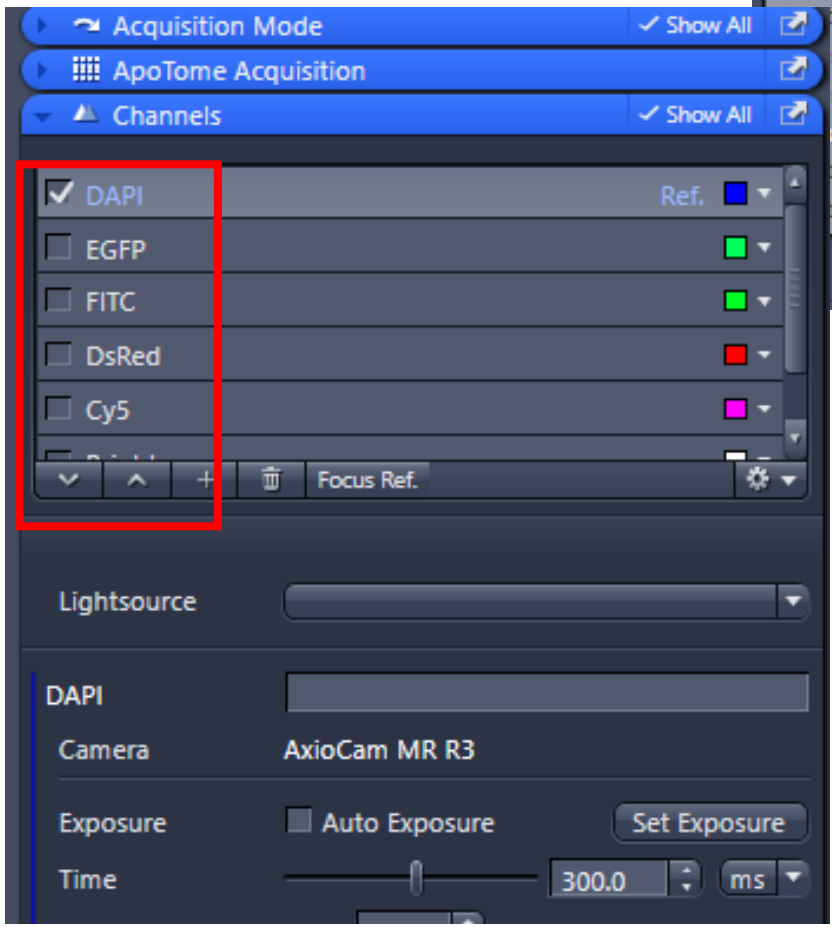


The **Acquisition** section allows you to view and capture images through the software.

1. Click on the drop down menu as shown in the picture. Select All Channels. This will bring up a list of all the fluorescent channels.

2. Check the box in front of the channel to select the channels you need.





To start a live view of the image

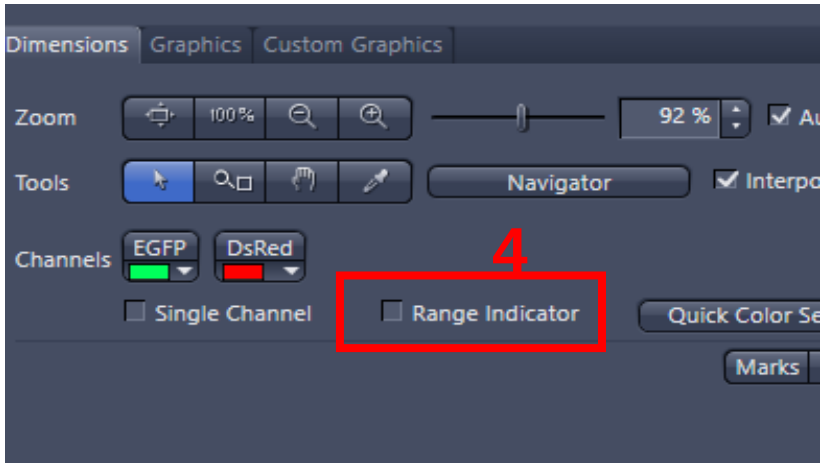
1. Click on the selected channel so that it turns light grey color.

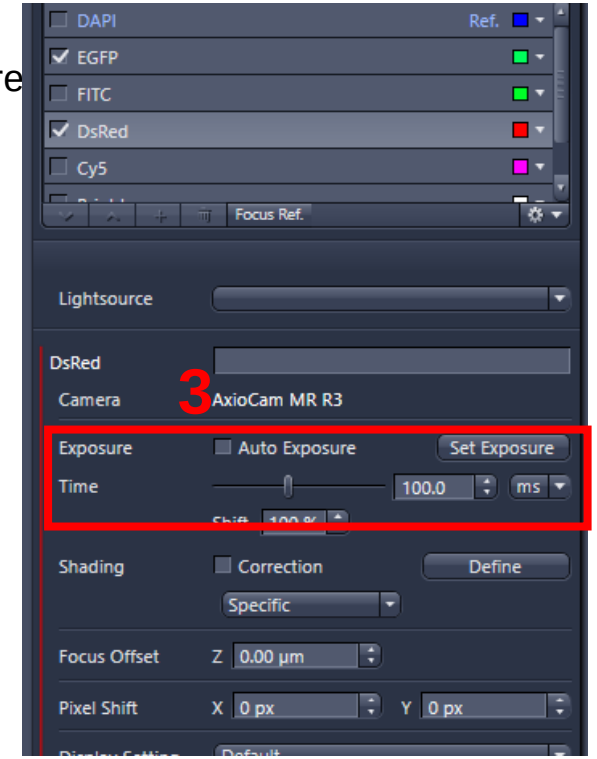
2. Then click on Live, and you will see the image in the live window. Do not forget to readjust the focus of the image on the screen. (Use Control + mouse scroll to adjust the focus)

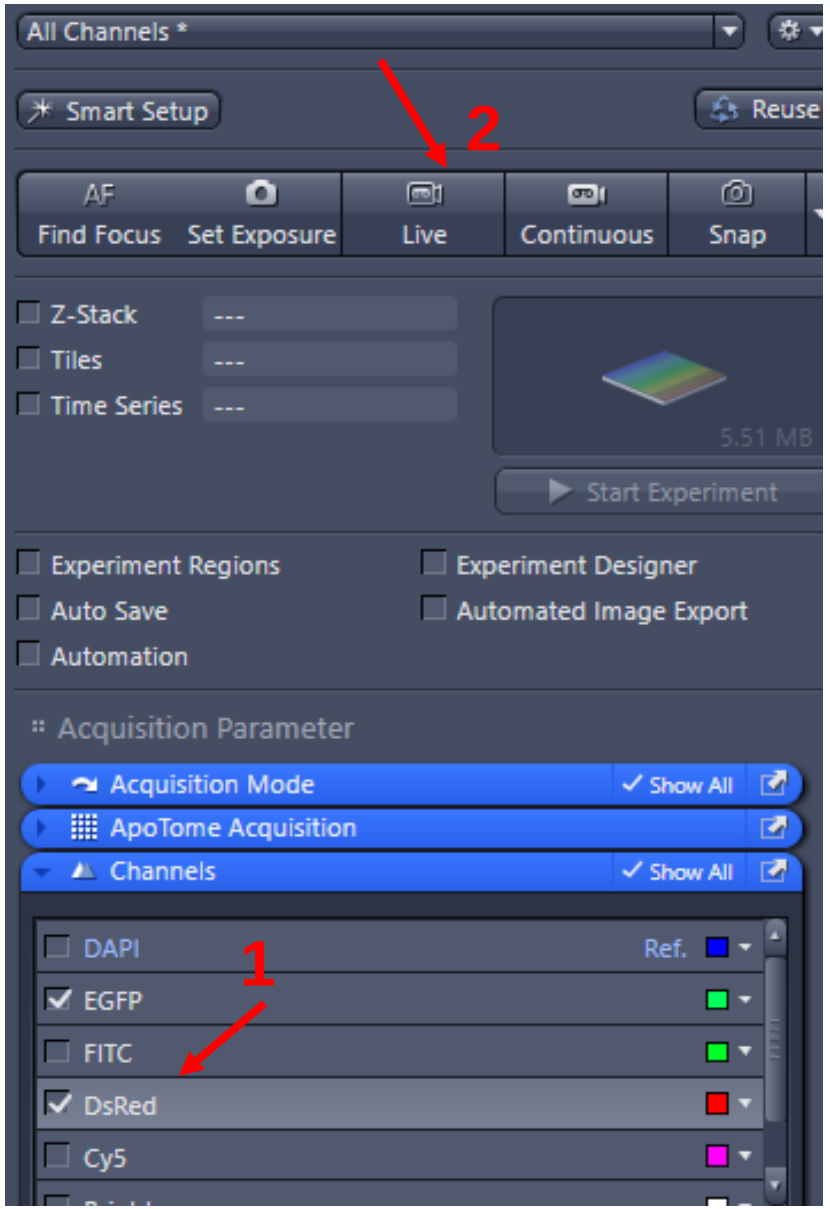
3. Turn on Range Indicator at the bottom allows you to see whether there is overexposure. **<u>Avoid overexposure!!!</u>**

4. Change the exposure time to adjust the brightness of the image. Then click Stop to stop the live view 

5. Then click on a different channel to view and adjust exposure time for that channel. Do this for all the channels you selected.





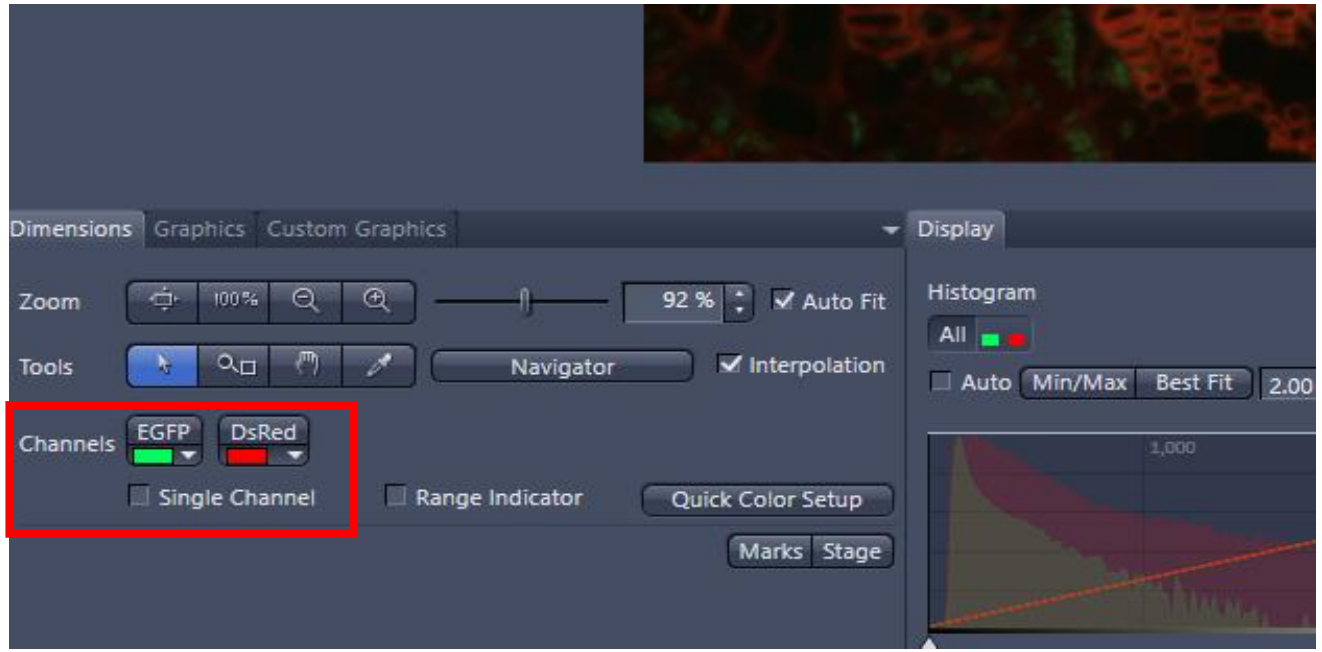


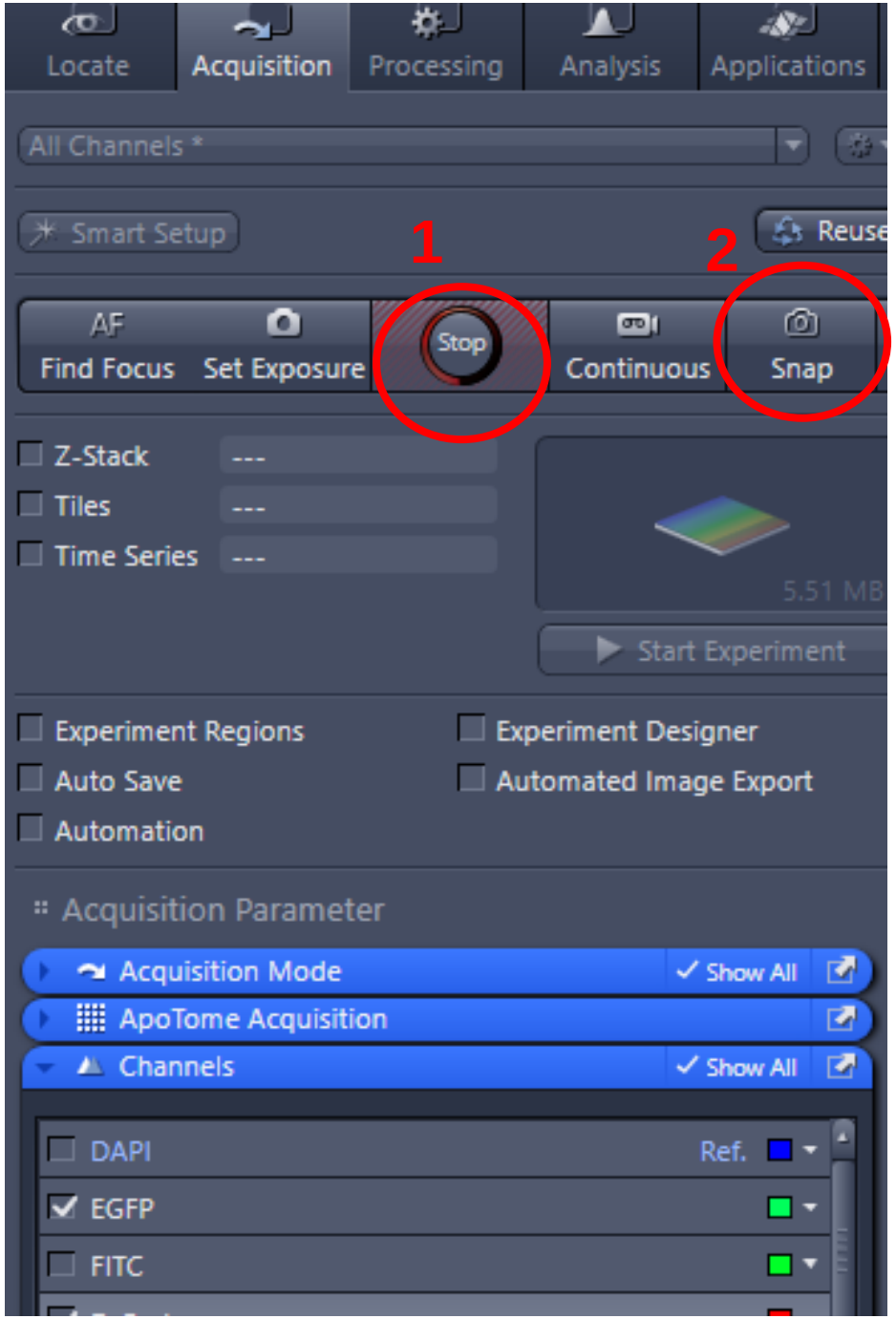
Acquire a multichannel image

1. After adjusting the exposure times for all the channels, click on Stop to stop the live view.

2. Then click Snap to capture the image with the channels you've selected.

3. After acquisiton, you will see a merged channel image. To view single channel instead of multiple channels simultaneously, check the single channel option, and click on the corresponding channel you wish to view.

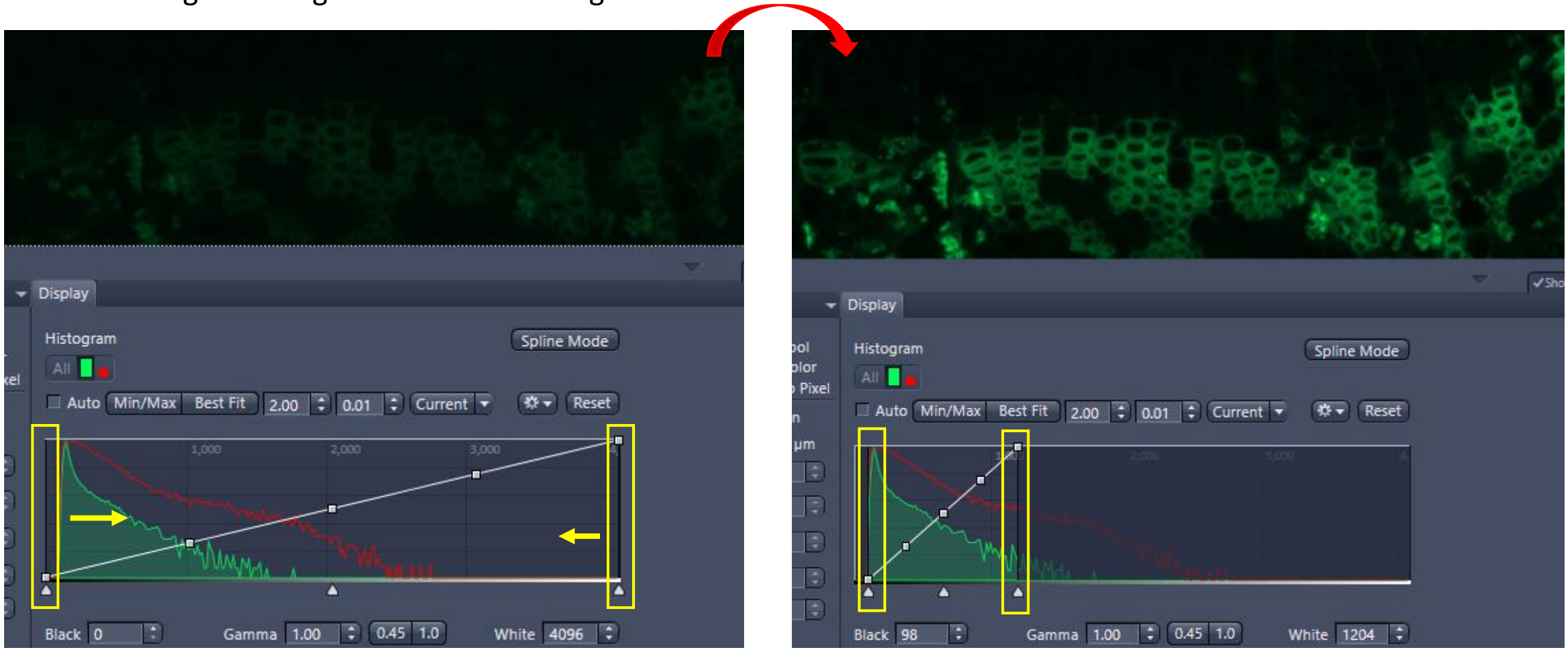




Adjust image brightness and background post-acquisition

1. After image acquisition, you can adjust the image brightness through the histogram at the bottom of the software.

2. On the histogram, the right vertical bar moving to the left increases the intensity. The left vertical bar moving to the right darkens the background.



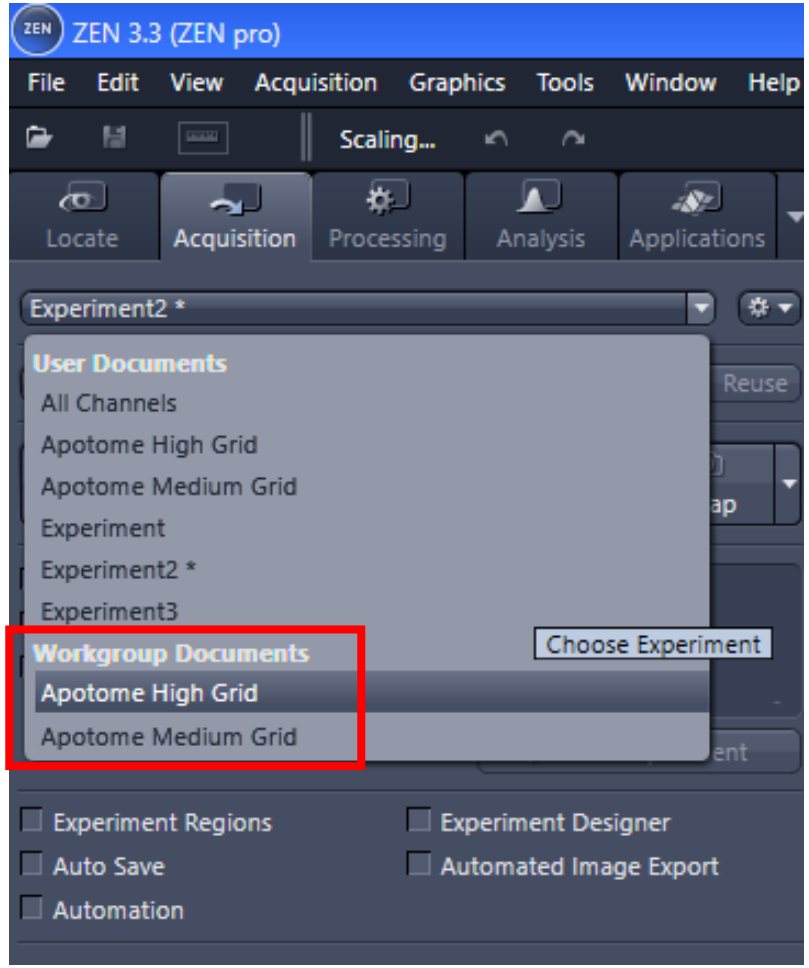
Acquire apotome images

1. Push the Apotome slider completely in to activate the apotome. Pull the slider out at the first stop or pull it out fully if you do not need to use the apotome.

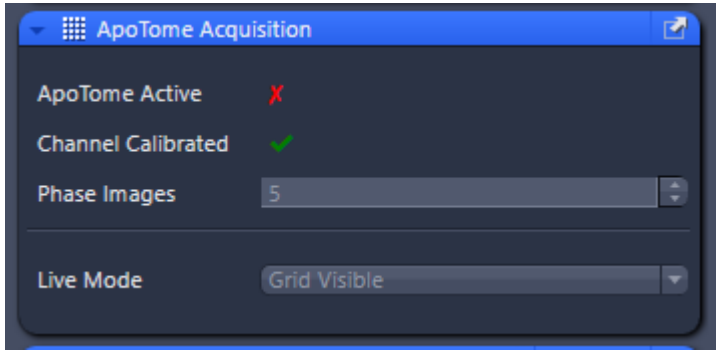
2. You can acquire apotome images (Large scan) by checking the ApoTome High Grid or ApoTome Medium Grid acquisition settings.

3. **For our 40x-100x objectives, use AptoTome High Grid settings, otherwise the outcome image is not good. (**Very important!!)

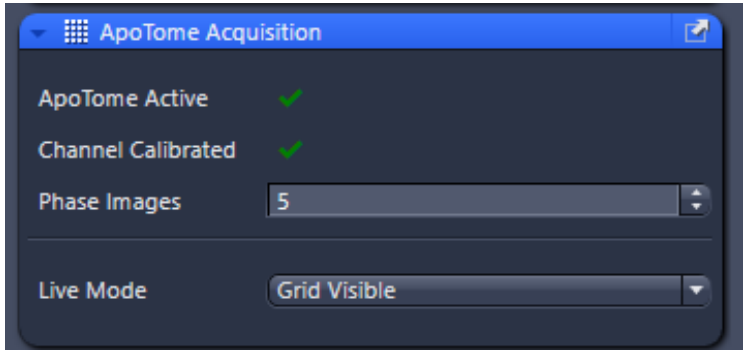
4. Also make sure you pushed in the apotome slider.



Apotome slider not pushed in



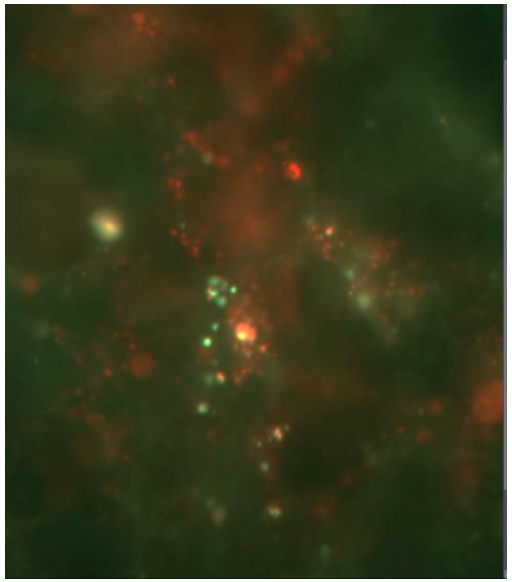
Apotome slider pushed in



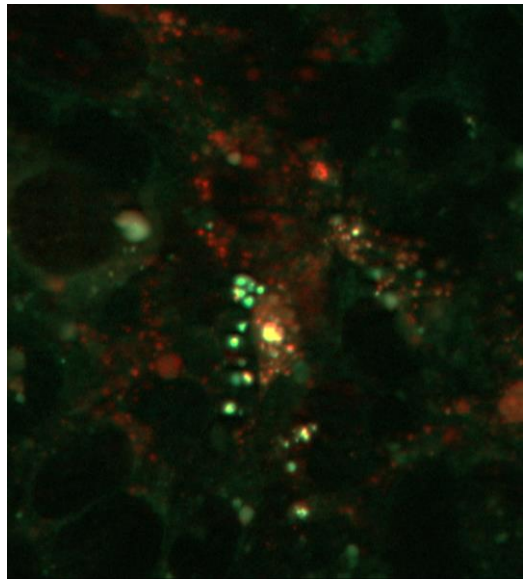
Acquire apotome images

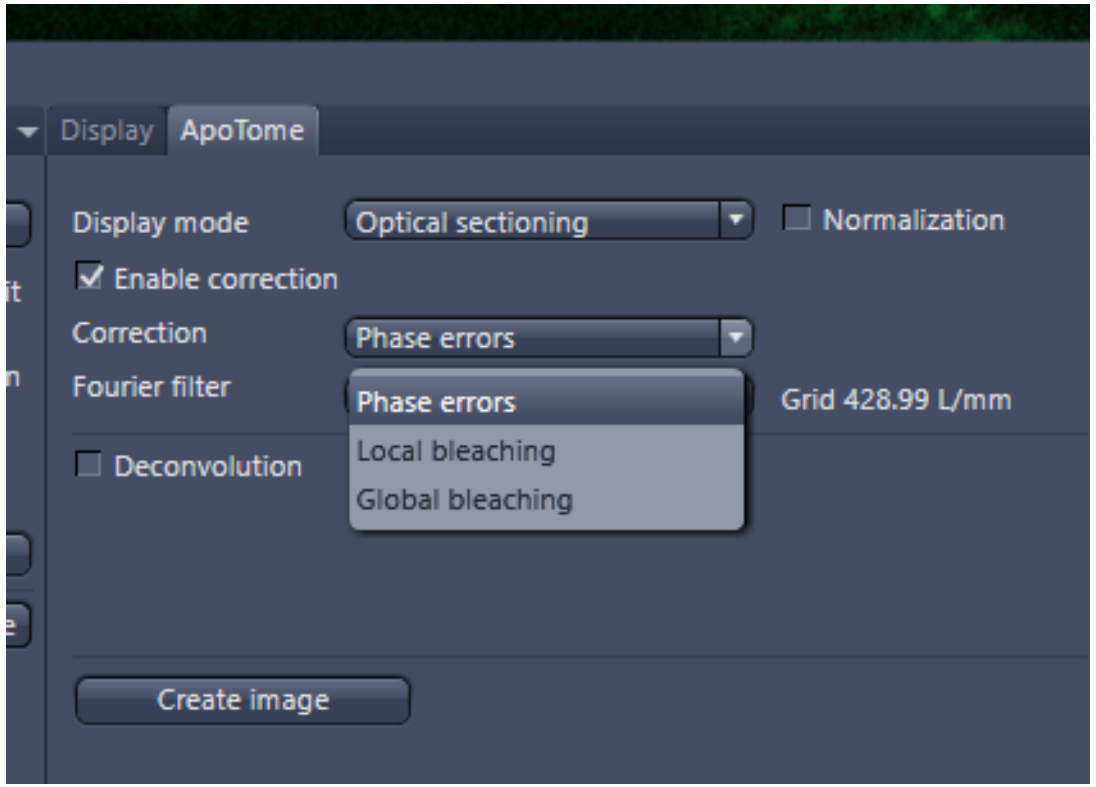
5. After an apotome image is acquired, you can adjust the results through the Apotome options listed below the captured image. Phase errors are often used to get rid of grids.

No Apotome



With Apotome





Acquire a z-stack image

1. To enable Z-stack imaging, check the Z-stack box to activate the Z-stack window

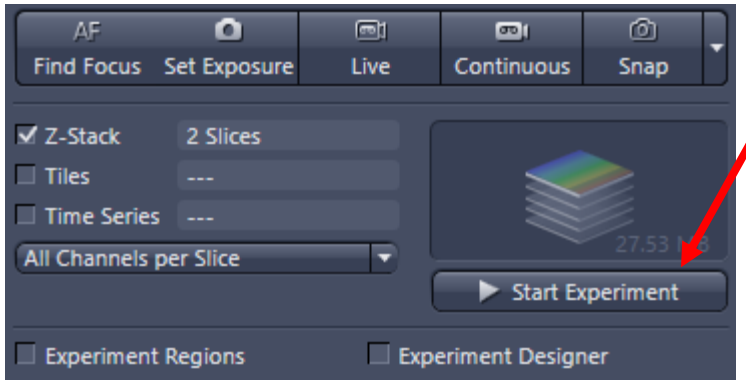
2. Before defining the Z range, first adjust the exposure time for the brightest focal plane to make sure the channels do not overexpose at this focal plane.

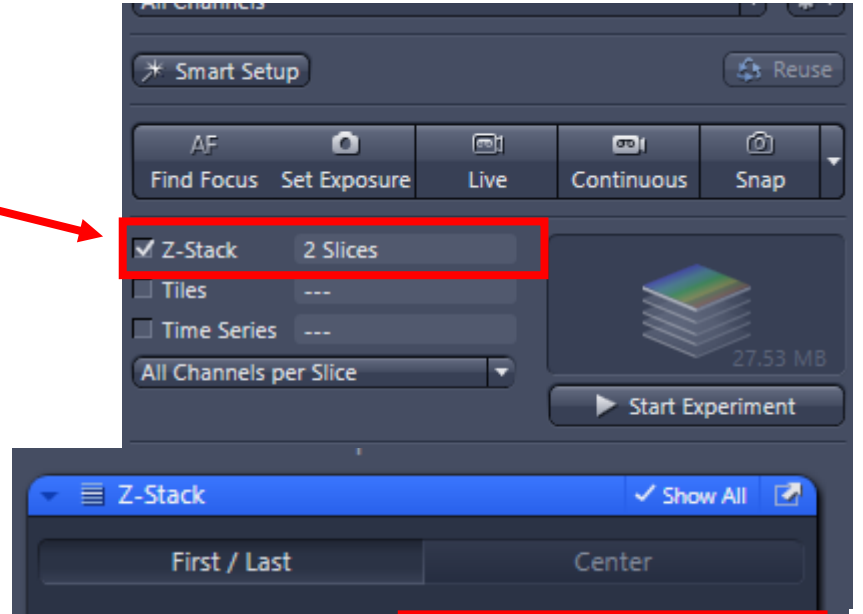
3. To define the top and bottom of the z range, stay on live view, press Ctrl, and scroll the mouse wheel to change the focal plane. When you change the focal plane, you will find the real time z-position in the Z-stack window changes as you go.

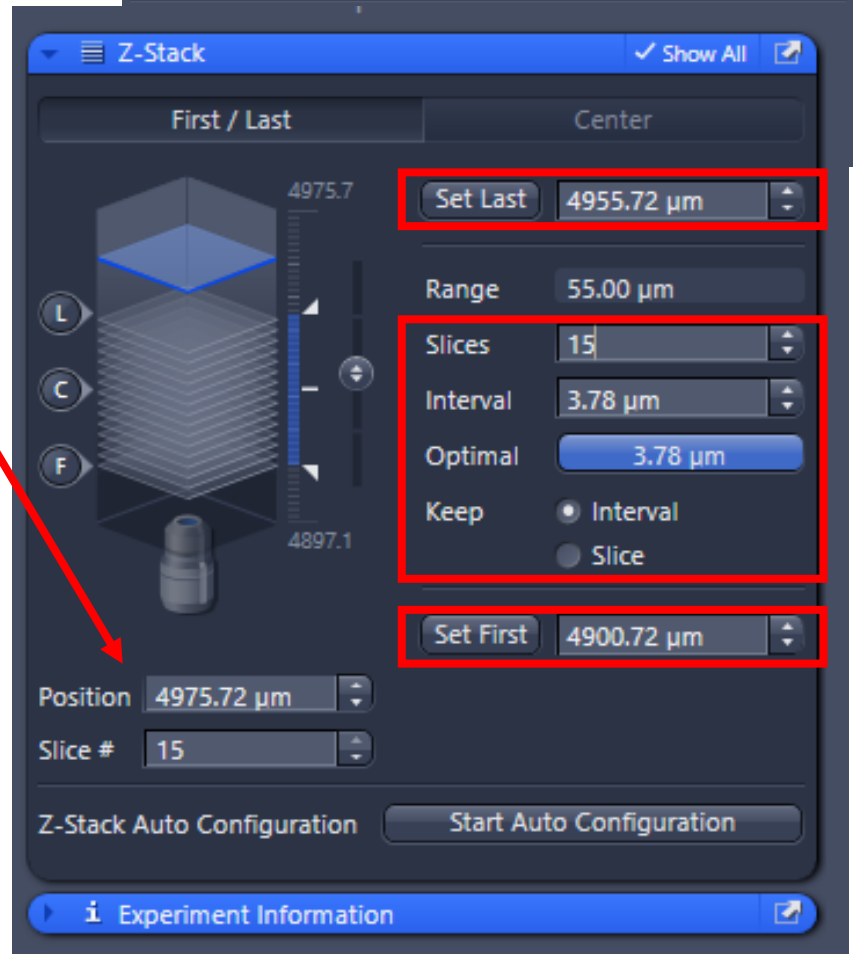
4. First find the bottom z position of the cell/tissue and click Set First to record this position. Then scroll the mouse wheel the opposite direction and find the top z position of the cell/tissue, then click Set Last to record this position.

5. After the z range is defined, to change number of slices, first click Keep Slice, then type in the number of slices you prefer. To change Interval, click Keep Interval first, then type in the number for interval.

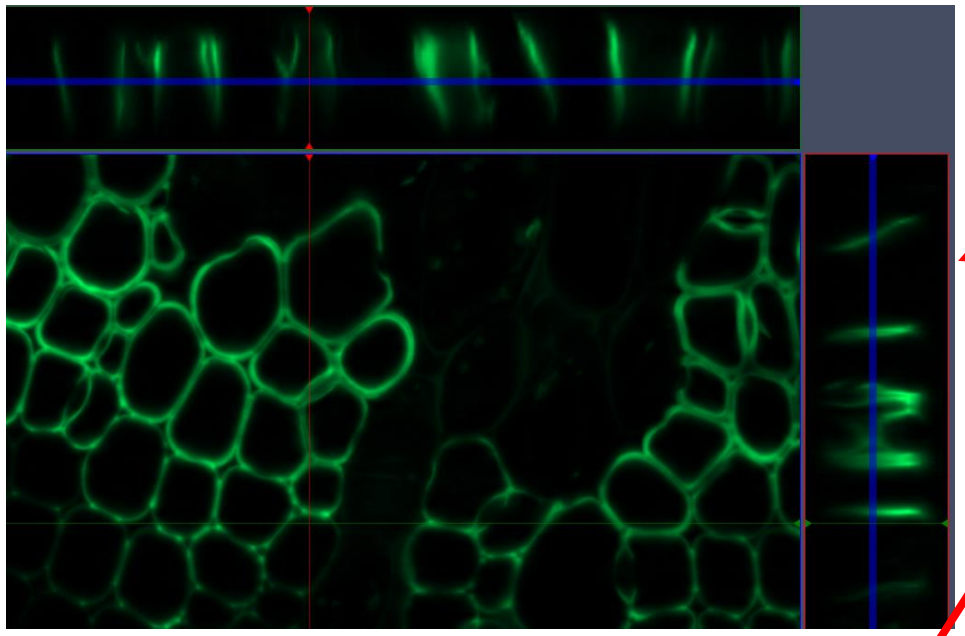
6. **Then click Start Experiment to acquire the Z-stack image. Do not click Snap.**

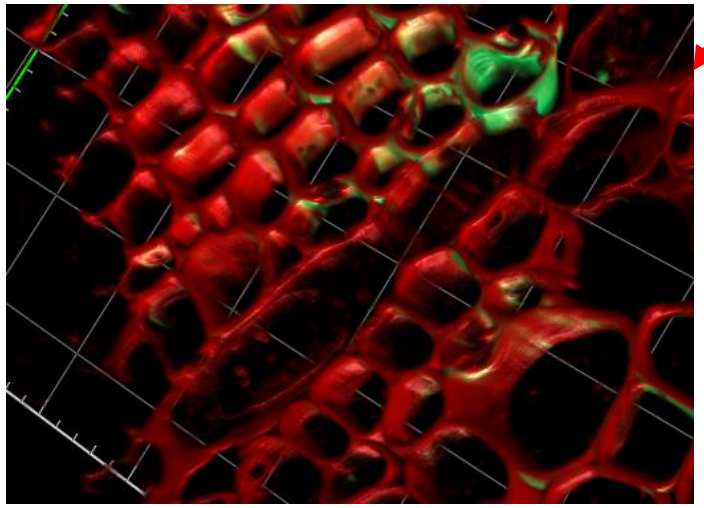


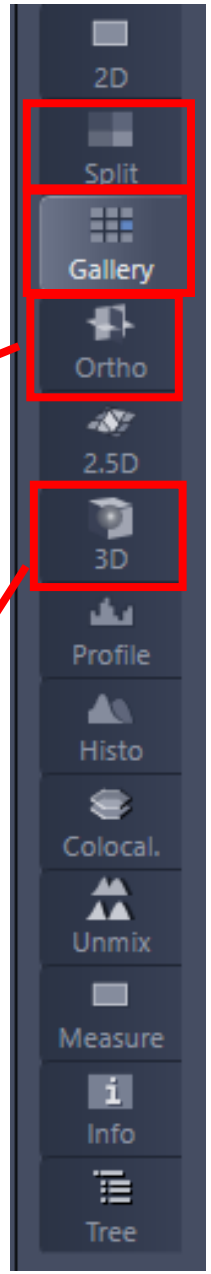


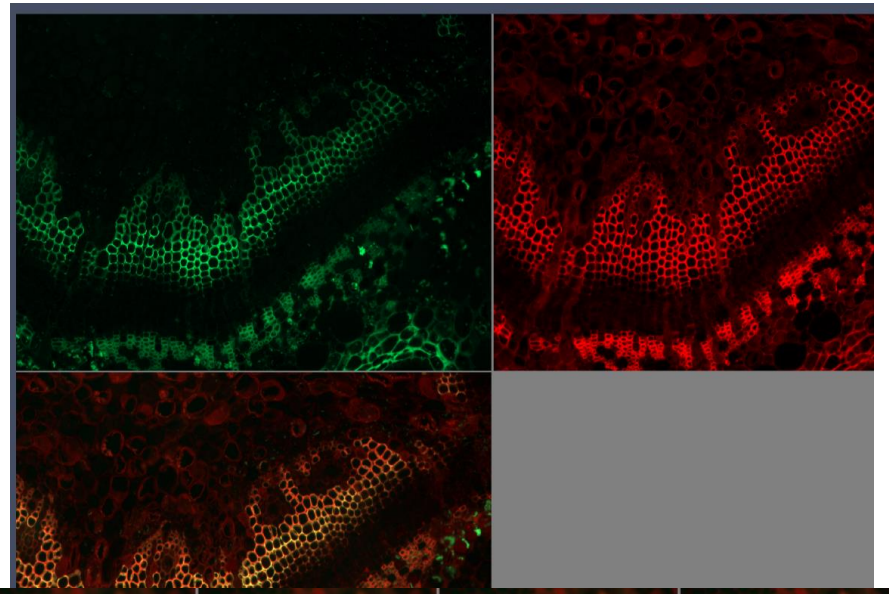


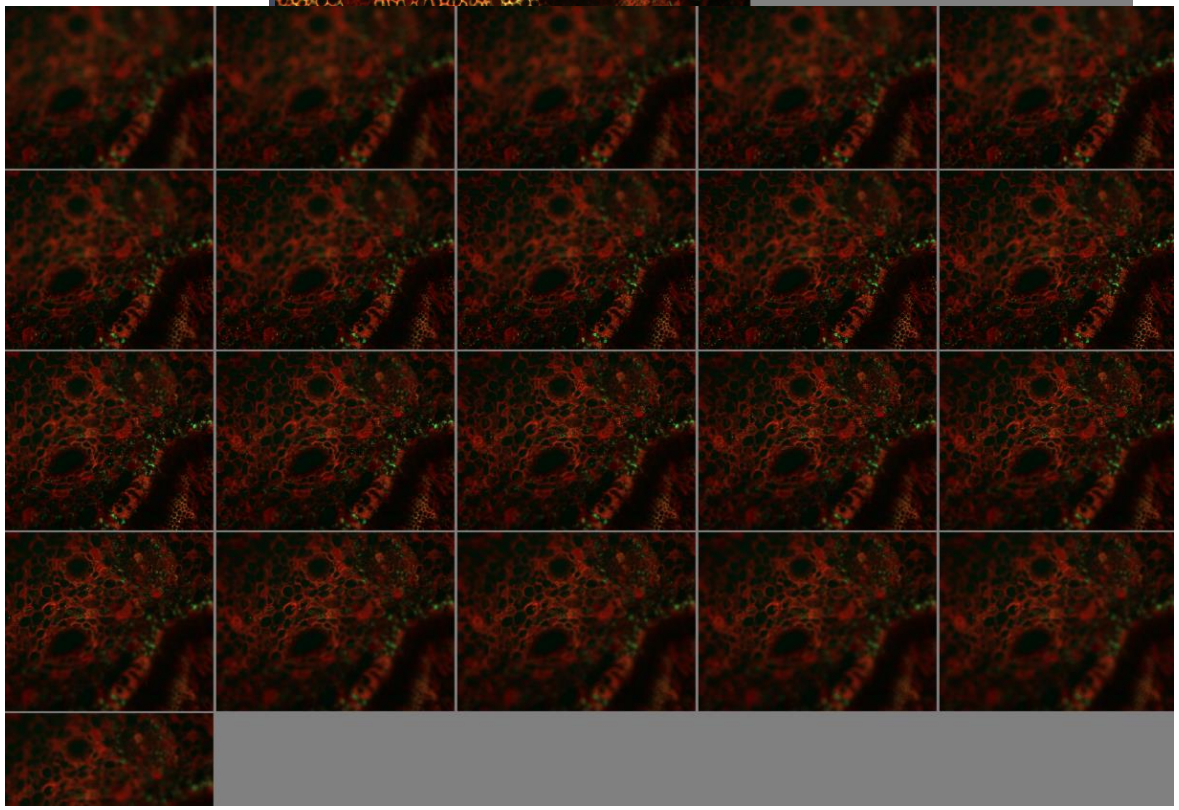
View modes of a Z-stack image









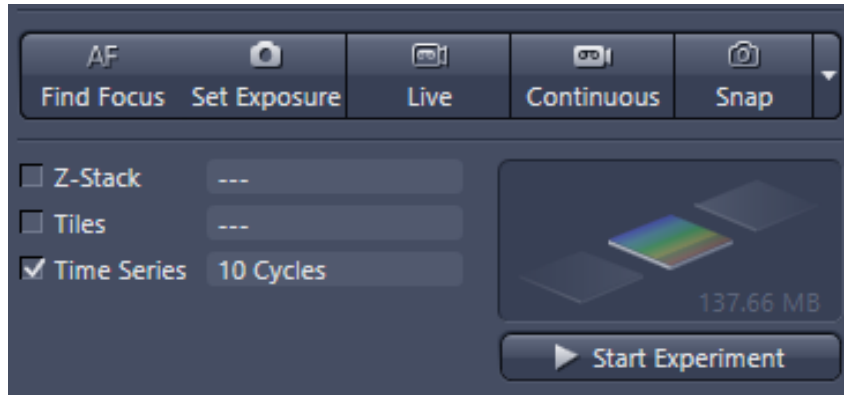


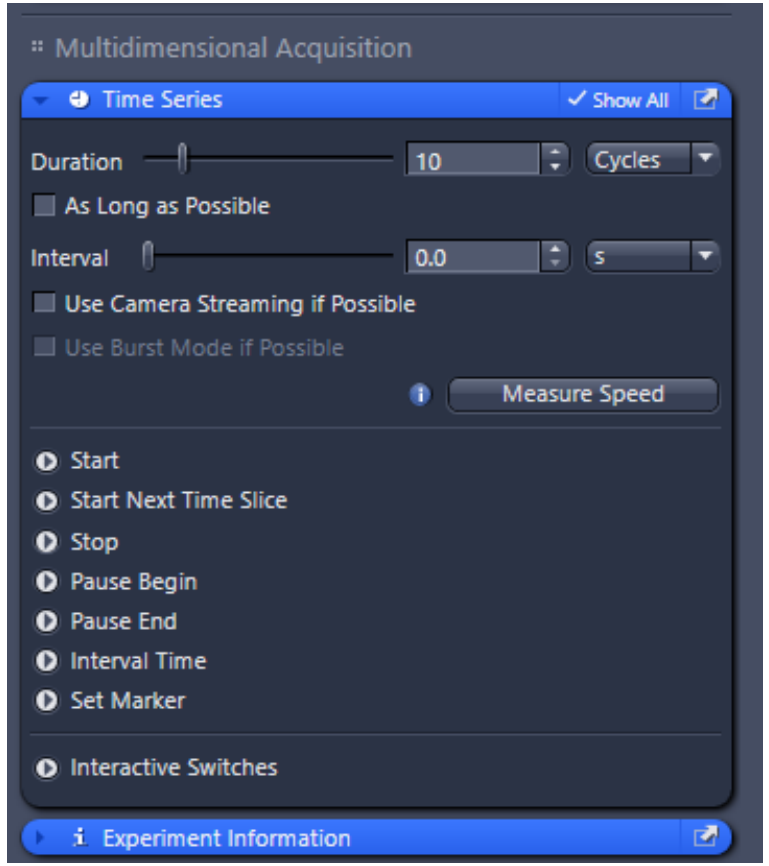
Acquire a Time-Series image

1. To enable time lapse imaging, check the Time Series box to activate the Time Series window. Uncheck others functions that you do not want to use. (Z-Stack, Tiles, Time Series)

2. In the Time Series window, set up the Duration (over all imaging time,) and the interval (time gap between each image.)

3. Then click Start Experiment.





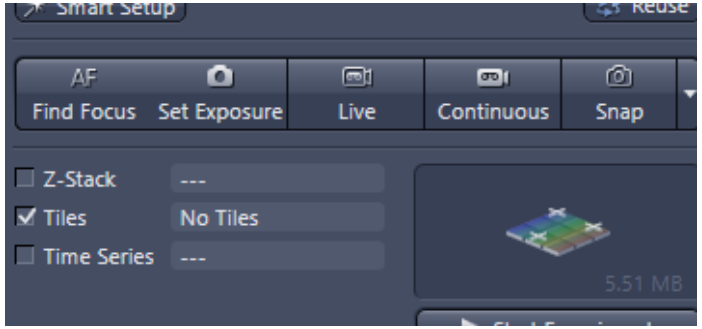
Once you click Start Experiment, all the checked boxes will apply. So you can combine Z-stack with Time series and with Tiles.

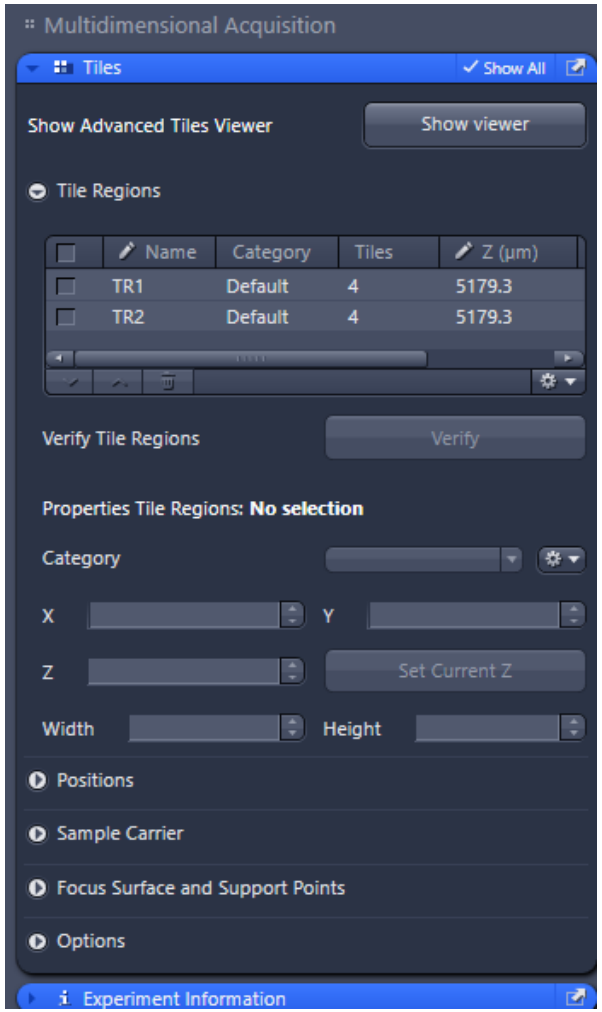
Acquire a Tile image

1. You can acquire tile images (Large scan) by checking the Tiles box.

2. Then you will find a new Tiles window and a Tiles section.

3. Then click Live to view the tile window simultaneously with the live window. (See next page)

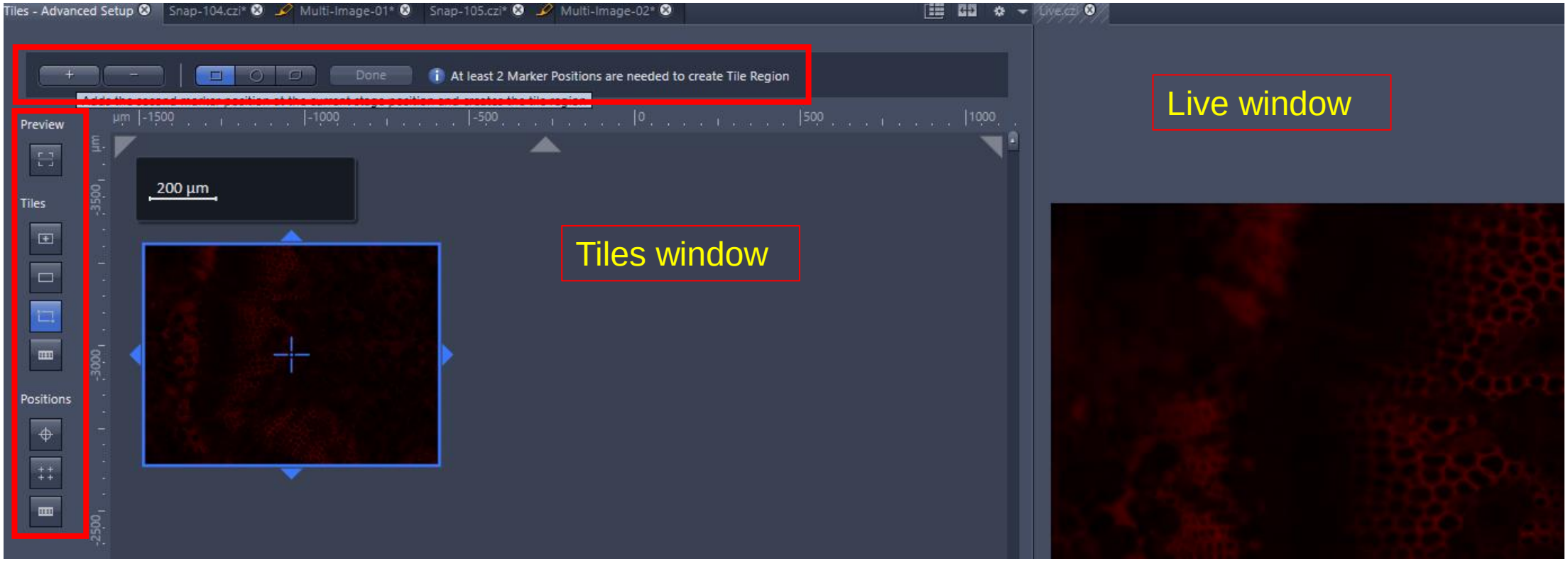




4. Use the tool bar on top and the tool bar on the left Tiles functions to set up Large image scan area.

5. Use positions area to set up multi-points.

6. After an area is set, click Start Experiment to start acquisition.



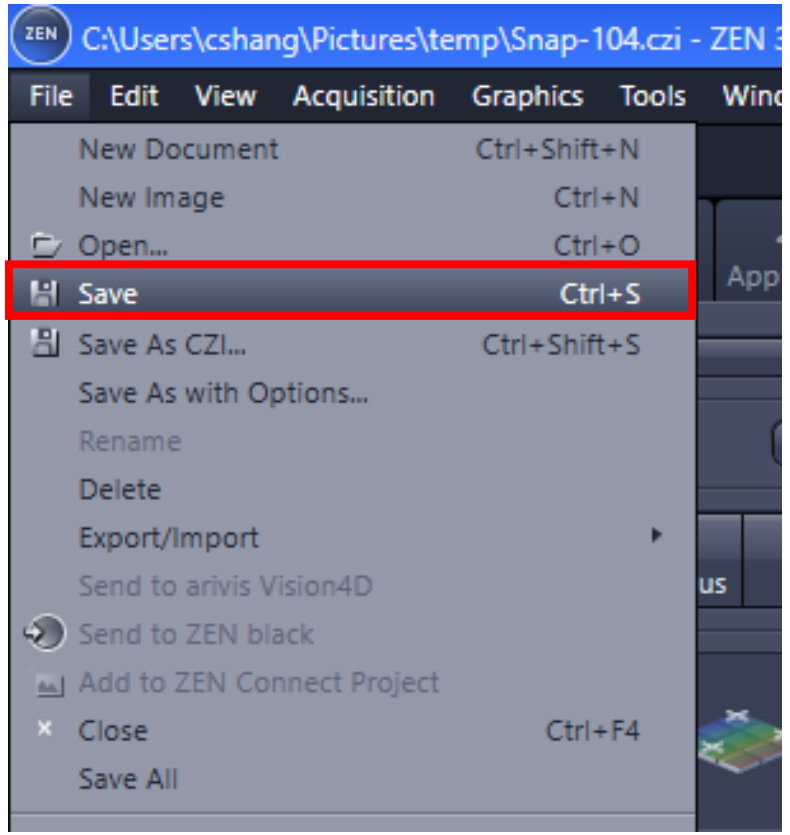
Save and Export

1. After image acquisition, save the image through File→Save. Save as .czi format.

2. **DO NOT** save anything on the desktop or C drive. Save everything in the Data drive (D drive) under your own folder.

3. Make sure you save your data as .cvi before exporting it to Tiff files.

4. Please copy your data when imaging done. Due to limited computer space, the core does not store image data for users.



Save and Export

1. After saving the original image data. Export as TIF file through File→Export/Import→ Export.

2. This will bring you to the export window.

3. Select Tiff, Merged channels image, Individual channel images and use channel names.

4. Select the correct folder for exported images.

5. Then click Apply to export.