Zeiss AxioObserver with ApoTome
Zen software

User Guide - short

LSU Health-Shreveport

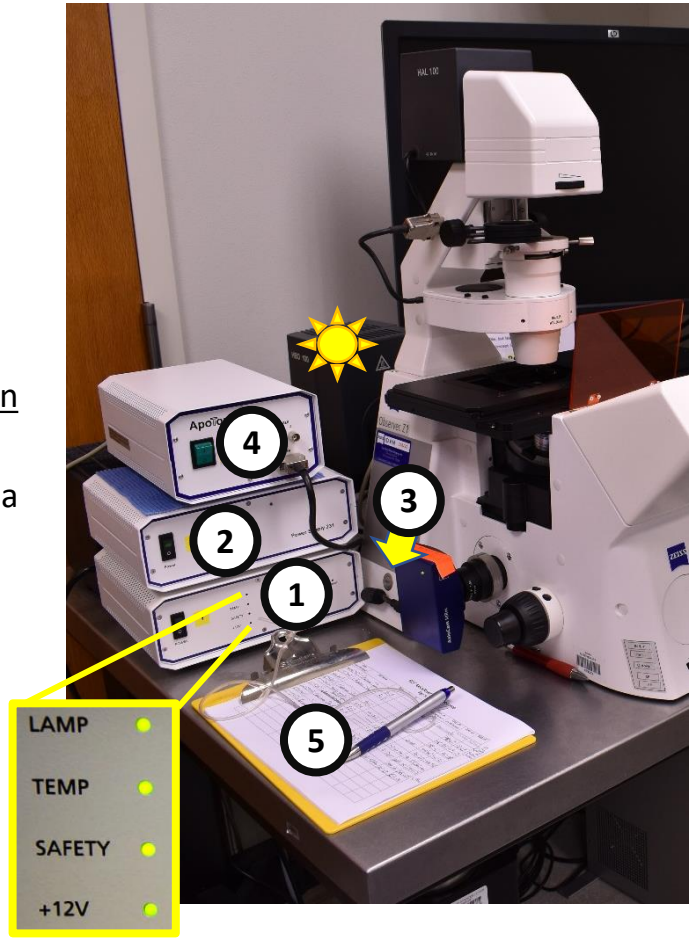
Research Core Facility (RCF)

MBHaba March 2022
based on Chaowei Shang, July 2021

START UP THE SYSTEM / ZEN SOFTWARE

- Turn on the system in the order (yellow stickers):
    1. **the mercury lamp** power source [1]. The green power light and 4 green indicator lights will come on. If “lamp” light indicator does not light up, please contact Core facility staff. **Wait at least 5-10min** for the lamp to warm up before using any light. Once turned on, the lamp should remain on for a minimum of 30 minutes.
    2. **the microscope power supply** [2] by flipping the switch which will have a green light turn on.
    3. **the microscope** [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. **the Apotome** power supply labeled with a yellow 4 sticker if you are going to use apotome.

- Sign in on the login sheet.
- Log in with your **LSUHSC ID and password**. Make sure the domain is set to **LSUMC-MASTER\username**, 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.
- Start Zen software



ORANGE - warming up (≈5min)

FLASHIG GREEN - automatic adjustment (≈1min)

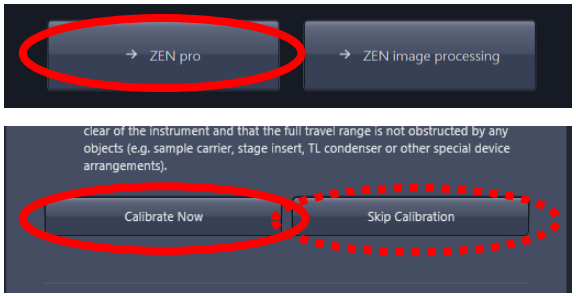
SOLID GREEN - ready to use

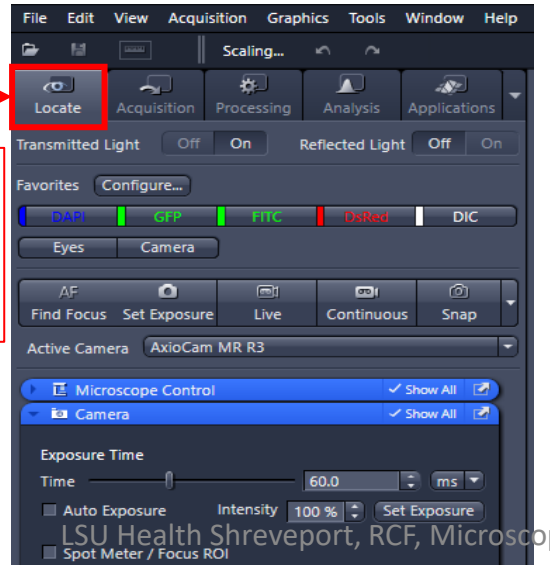
START UP THE SYSTEM / ZEN SOFTWARE

- On the desktop, click the **Zen 3.3 (blue edition)** 

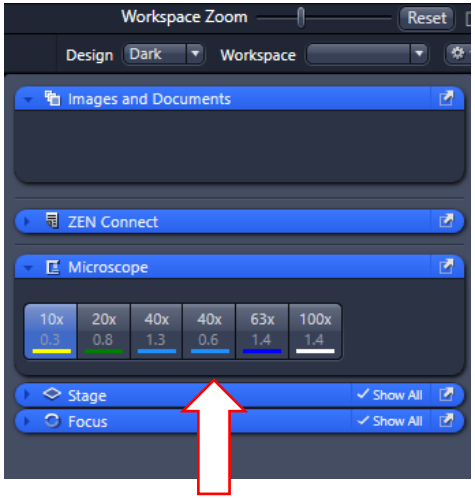
icon to launch the software.
- Then select **Zen pro** for image acquisition. If you only want to look at an image, or do image analysis, click ZEN image processing.
- Next, click **Skip Calibration** if you are going to acquire only **single images** or **Calibrate Now** if you are planning to run **Z-stack or Tile.**

  !!!BEFORE CALIBRATION YOU MUST ENSURE THAT THE OBJECTIVES ARE ON THE LOWEST POSITION!!!
- **Calibration** may be done later.
- By default, layout displays the **Locate** tab/section (on the left) and the **Objective** section (on the right)





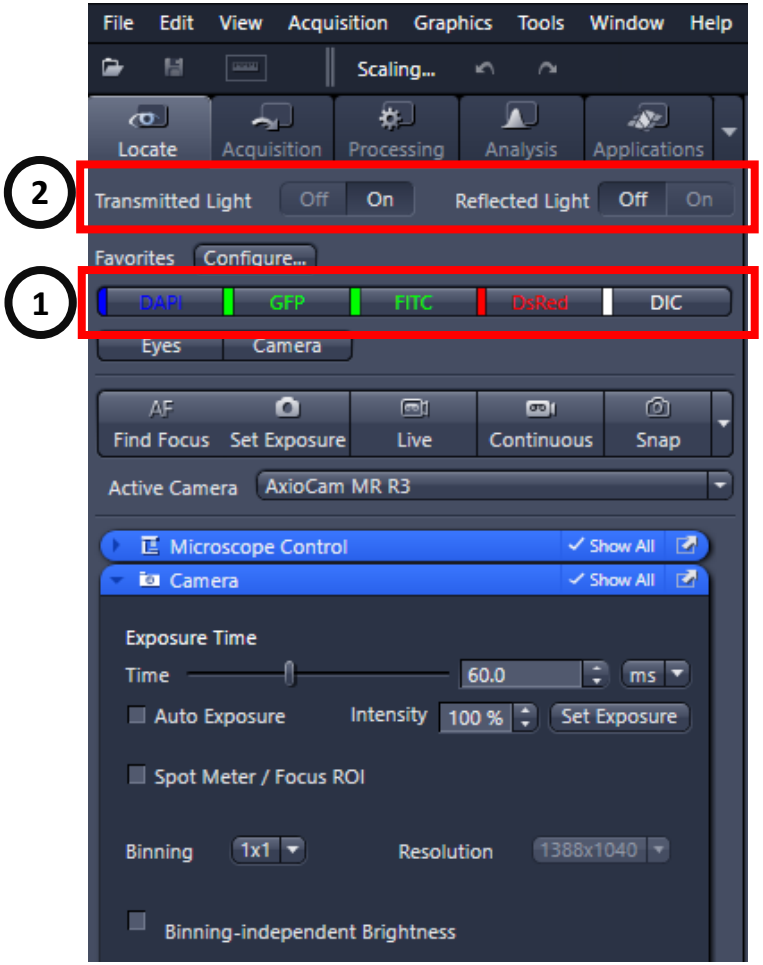
The **Locate** section allows you to turn on the fluorescent light and view the sample through eyepieces.



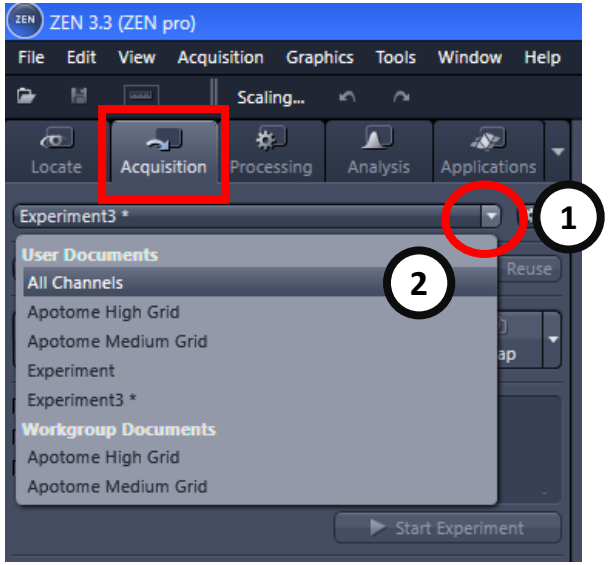
The Objectives section on the right allows you to change objectives

FIND YOUR SAMPLE USING EYEPIECE - LOCATE

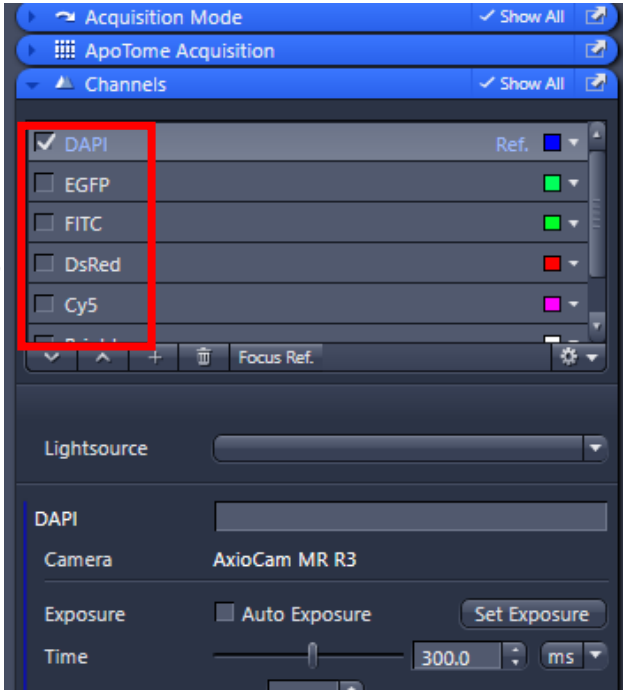
1. Pull the **Apotome slider** out at the first stop or pull it out fully if you do not need to use the Apotome.
2. Start from 10x objective to find focus/sample
3. Select a fluorescent/Bright field channel (DAPI, GFP, FITC, DsRed, DIC). This will turn on the light on the microscope.
4. When light is turned on, the light path automatically switch to eyes.
5. Use the joystick and the focus knob to locate your sample.
6. Turn off the fluorescent light by pressing the Off button on the software.
7. Change for higher magnification objective and re-focus.
8. If needed change objective, add immersion oil, turn on light and again re-focus.
9. **<u>!To avoid photobleaching always turn off the fluorescent light when done with focusing!</u>**
10. Switch to Acquisition Tab



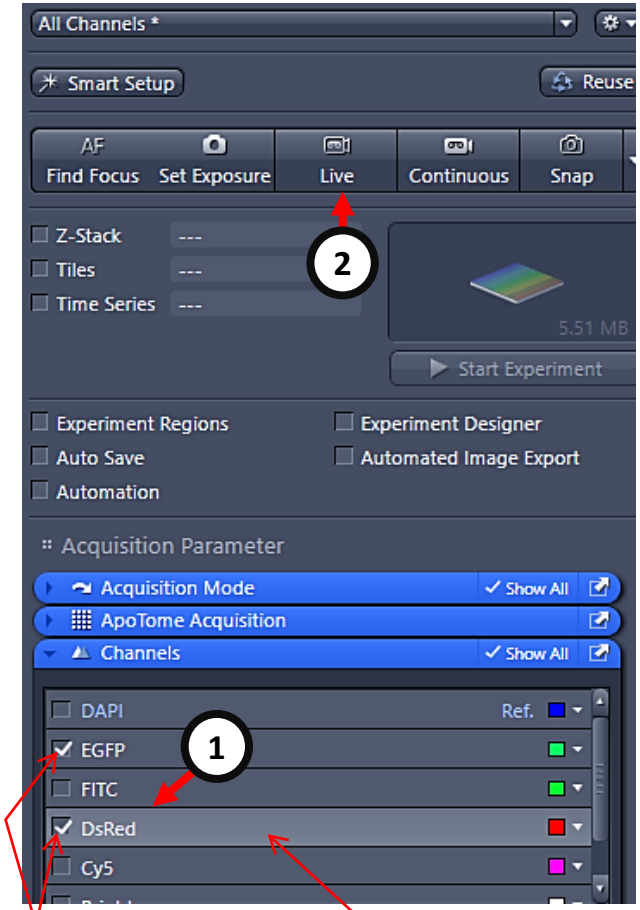
CAPTURE YOUR IMAGES – ACQUISITION



- 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.
- Check the box in front of the channel to select the channels you need.
- Channel highlighted on gray is the active channel – it will show in live image

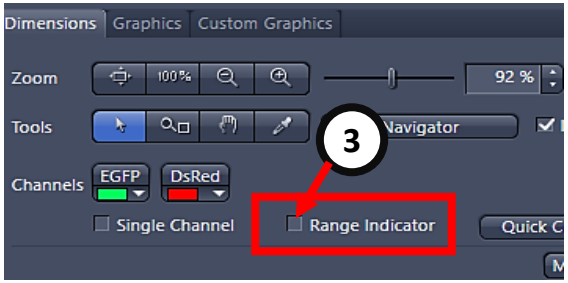


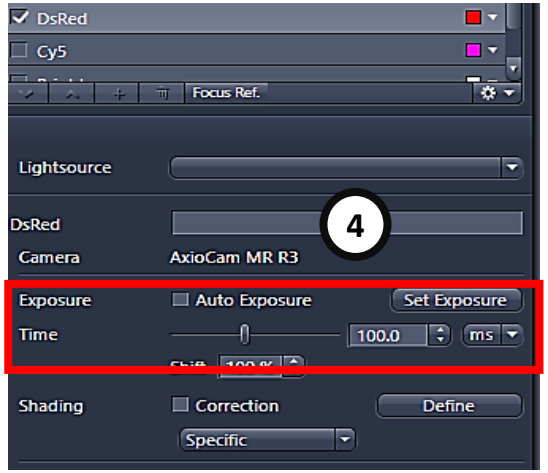
CAPTURE YOUR IMAGES – ACQUISITION (channel adjustment)



- Click on one of the selected channels for adjustment (active channel is highlighted in light grey color).
- Click on **Live**, and you will see the image in the live window. Readjust the focus of the image on the screen. (**CTRL+ mouse scroll**)
- Turn on **Range Indicator** (panel bellow the live view) to check for overexposure.
- Change the exposure time to adjust the brightness of the image. Click Stop to stop the live view

- Click on a different channel to view and adjust exposure time. Repeat for all selected channels.
- Stop when all adjustments are done.
- All channels with checked boxes will be included in the image

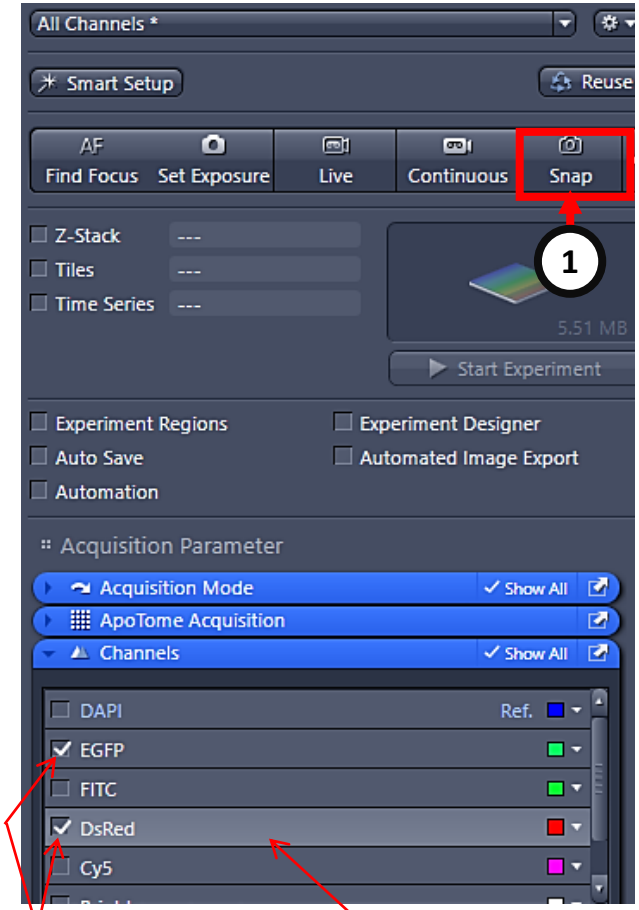




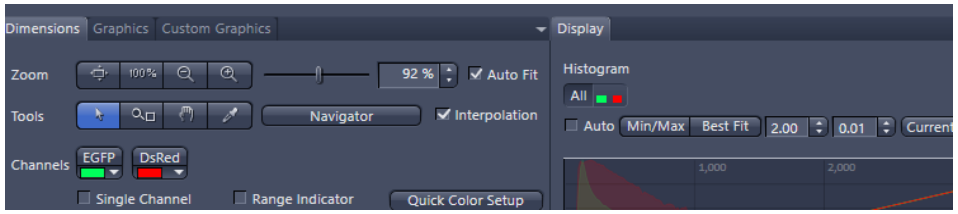
Active for adjustments

Included in the image

CAPTURE YOUR IMAGES – ACQUISITION (multichannel imaging)



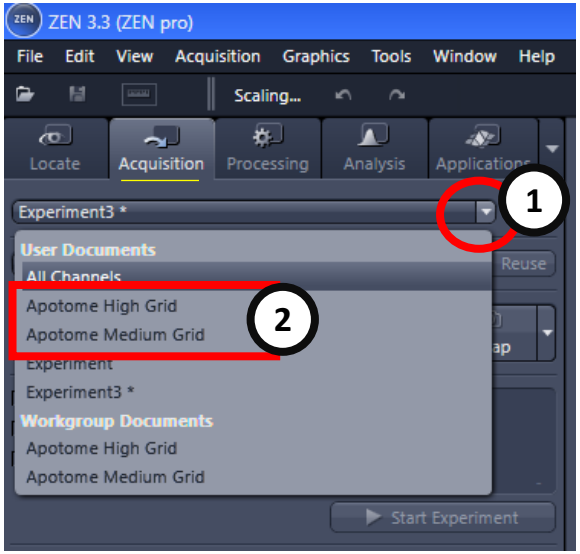
- To capture the image with all selected channels, click **Snap.**
- After acquisition, you will see a merged channel image. To view single channel instead of multiple channels simultaneously, check the single channel option, and click on the corresponding channel you wish to view.



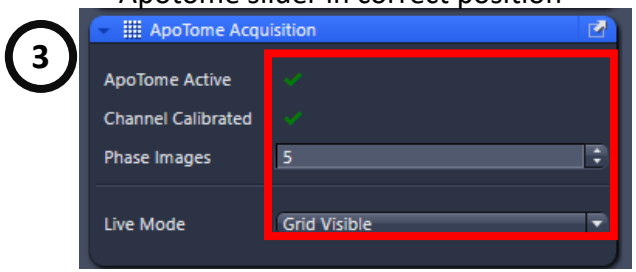
- If needed adjust the image brightness through the histogram option.

CAPTURE YOUR IMAGES WITH APOTOME – ACQUISITION - multichannel imaging

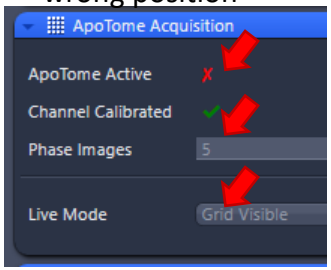
- To activate the **Apotome** push the **Apotome slider** completely into the side of the microscope.
- Click on the drop-down acquisition menu and select **Apotome Medium Grid** or **Apotome High Grid (for 40-100x objectives)**.
- Make sure you pushed in the apotome slider. Check under **ApoTome Acquisition**



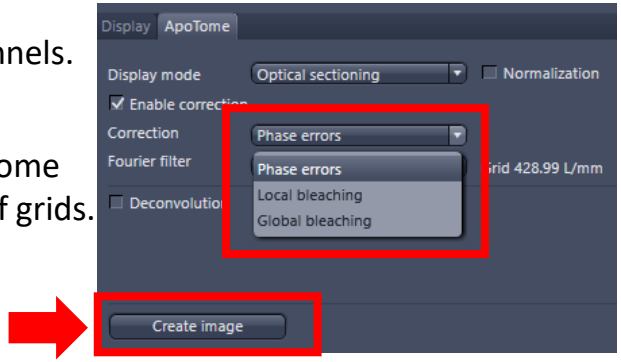
Apotome slider in correct position



wrong position

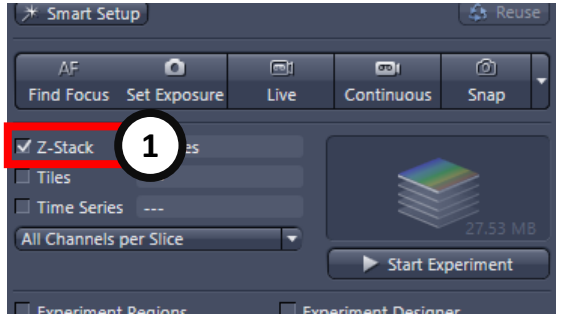


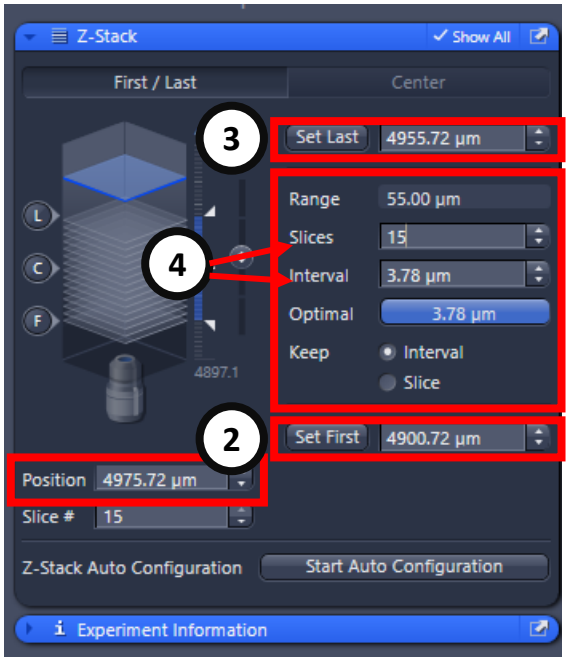
- Make the time exposure adjustment (as previously described) for each used channels.
- Capture image (as previously described) by clicking on Snap.
- After an apotome image is acquired, you can adjust the results through the Apotome options listed below the captured image. Phase errors are often used to get rid of grids.
- Always **Create Image** to generate reconstructed picture.

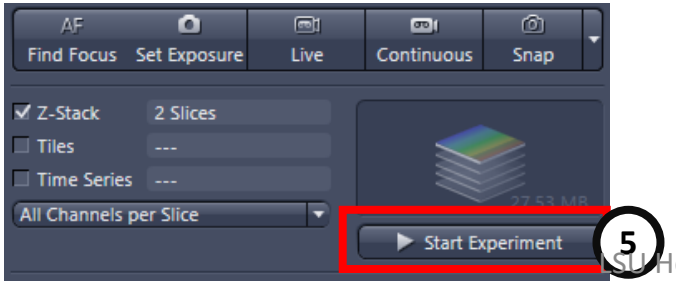


CAPTURE YOUR IMAGES – Z-STACK ACQUISITION (multichannel imaging)
RUN CALIBRATION IF USING Z-STACK

- To enable Z-stack imaging, check the **Z-stack box** to activate the Z-stack window
- Lower the objective and run Calibration.
- Adjust the exposure time for the brightest focal plane to make sure the channels do not overexpose at this focal plane.
- To define the z range, stay on live view, and using Ctrl+mouse scroll change the focal plane. The real time z-position value is located below range diagram, and it changes while scrolling.
- First find **the bottom z position** of the cell/tissue and click **Set First**. Then scroll the mouse wheel the opposite direction and find **the top z position** of the cell/tissue, then click **Set Last**.
- After the z range is defined, to change number of slices, first click **Keep Slice**, and type in the number of slices you prefer. To change interval, click **Keep Interval** first, then type in the value for interval.
- Click **Start Experiment** to acquire the Z-stack image**.** Do not use Snap option**.**

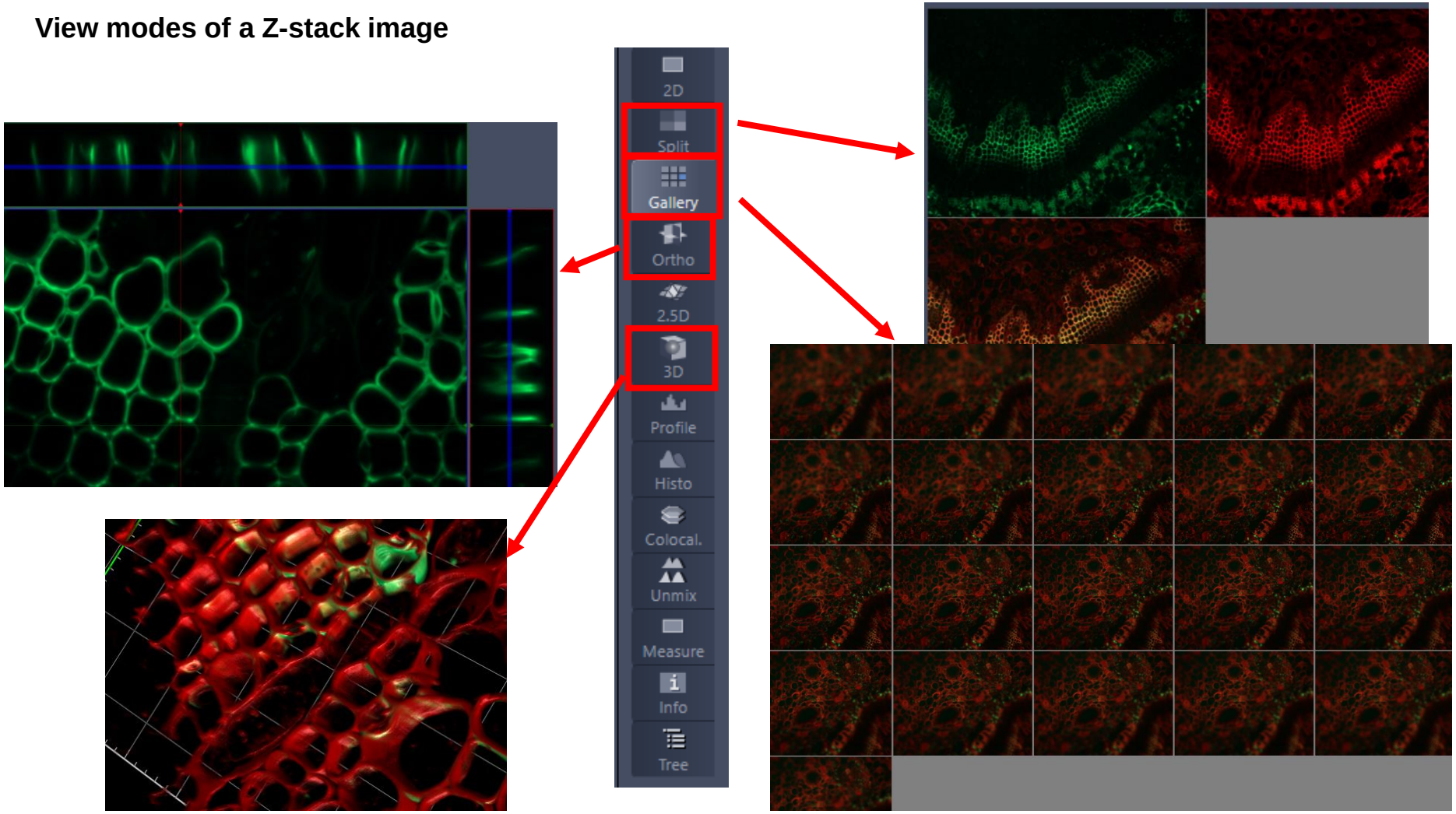




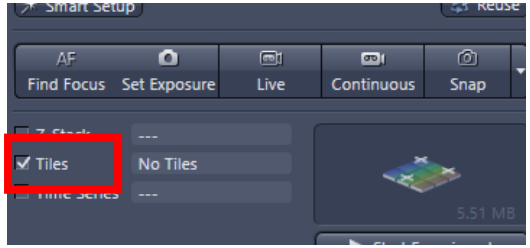


Once you click Start Experiment, all the checked boxes will apply.

View modes of a Z-stack image

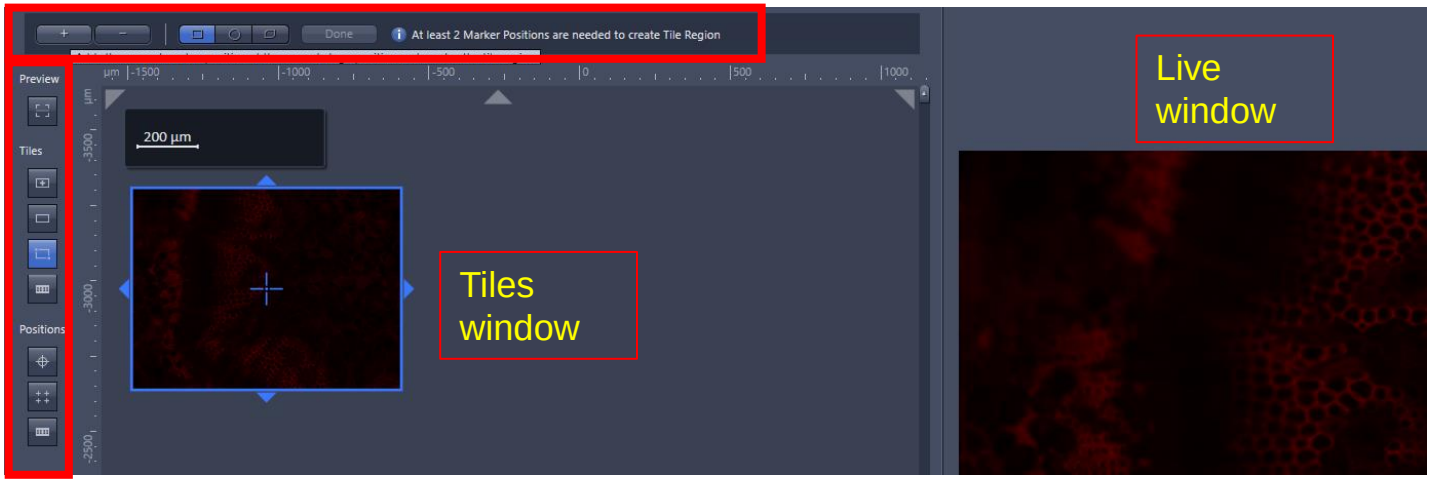


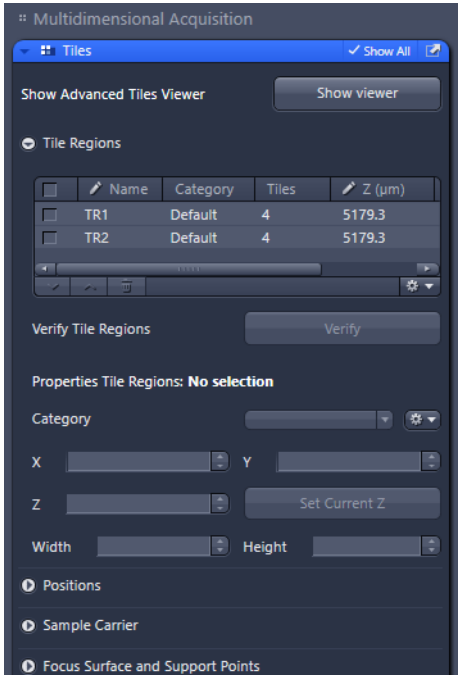
CAPTURE YOUR IMAGES – ACQUISITION (Large scan - Tiles)
RUN CALIBRATION IF USING Z-STACK



- For large scan area use tile images - check the Tiles box.
- Then you will find a new Tiles window and a Tiles section.
- Then click Live to view the tile window simultaneously with the live window.
- Use the tool bar on top and the tool bar on the left Tiles functions to set up Large image scan area.
- Use positions area to set up multi-points.
- After an area is set, click **Start Experiment** to start acquisition. <u>Do not use Snap option</u>

Once you click Start Experiment, all the checked boxes will apply.

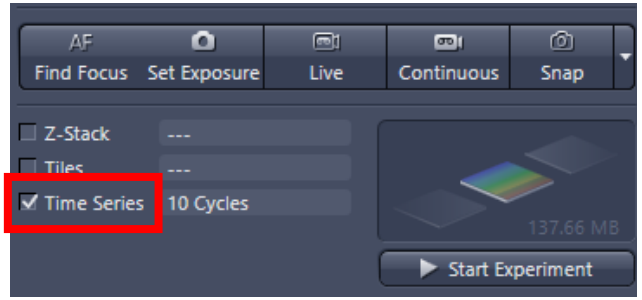


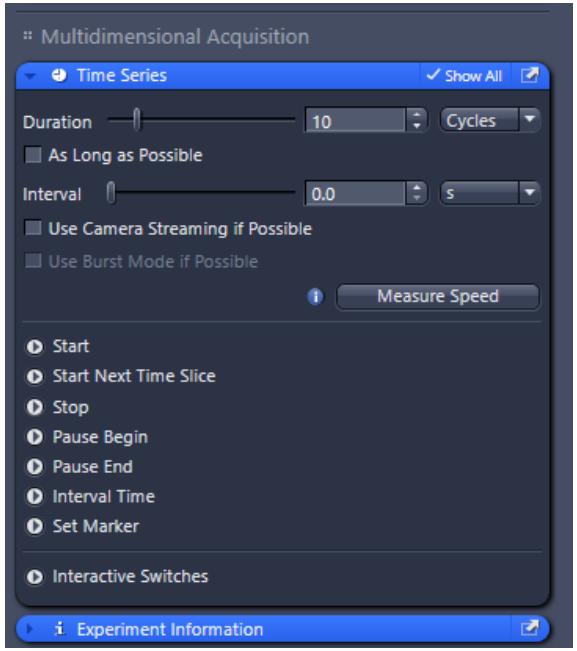


CAPTURE YOUR IMAGES – ACQUISITION (Time Series)

- 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)
- In the **Time Series** window, set up the **Duration** (over all imaging time,) and the **Interval** (time gap between each image.)
- Click **Start Experiment** to start acquisition. Do not use Snap option.

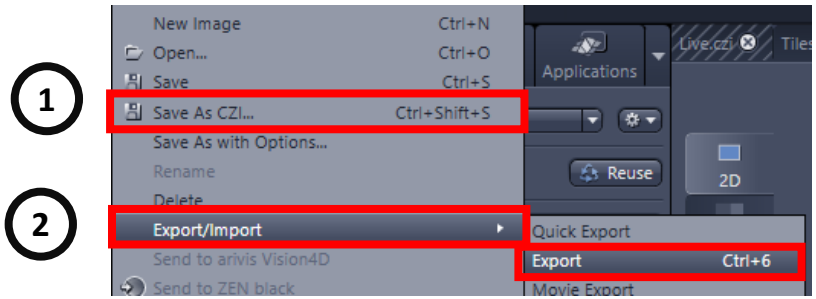
Once you click Start Experiment, all the checked boxes will apply.

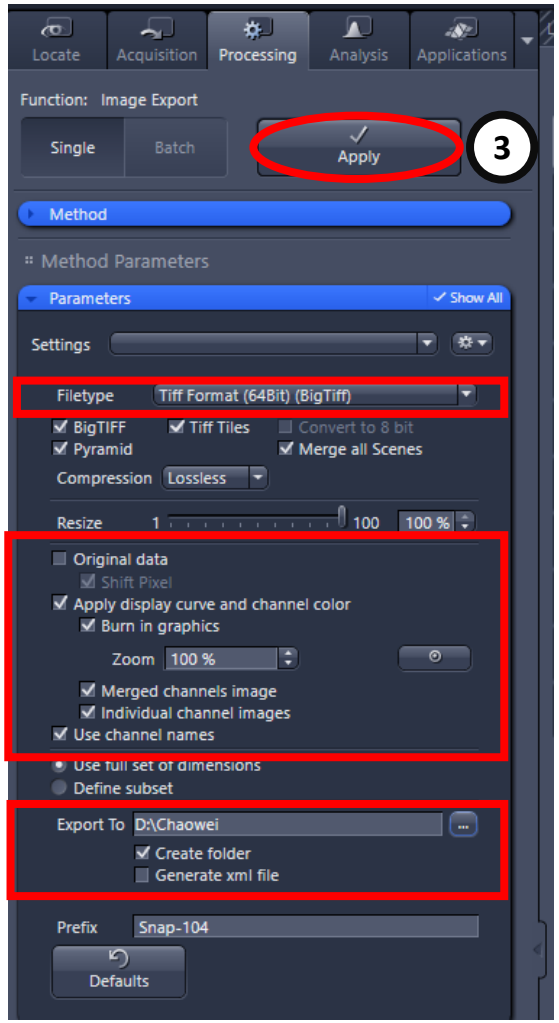




Save and Export

1. **ALWAYS FIRST SAVE** the image through File →Save. Save as .czi format.
2. Save everything ONLY 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. Once saved **Export as TIF** file through File →Export/Import → Export.
5. This will bring you to the export window.
6. Confirm / Select: - Tiff,
   - Merged channels image,
   - Individual channel images
   - Use channel names.
7. Select the correct folder for exported images.
8. Then click Apply to export.

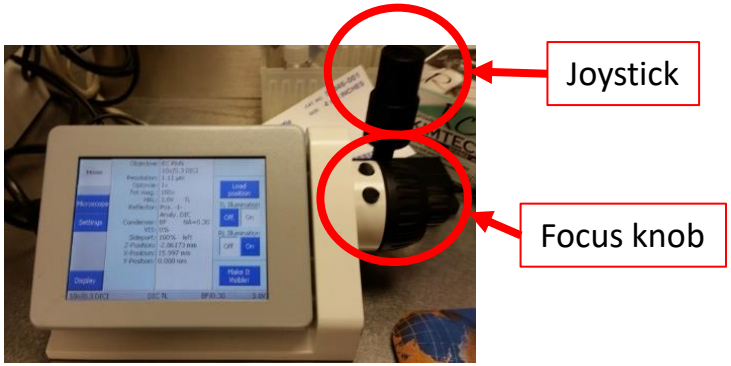




FINISH / SHUTTING DOWN THE SYSTEM

- Check if all images are saved and exported.
- Lower the objective, take out your slide and clean the objective.
  Always use provided Lens Paper and Lens Cleaner. Wet the paper with cleaner, wipe very gently immersion oil and again, gently dry it with dry lens paper. Please, be careful and do not touch the lens with your fingers.
- Switch to 10x objective.
- Close Zen software.
- Check calendar for next user.
  - If someone is signed up within **2 hours**, please just sign out and **leave the system on**. Remember to fill the time on log sheet.
  - If **not**, please sign out, fill the time on log sheet and **turn off the system** in the order: 4 ->3 ->2 ->1. Cover the microscope and check for any left behind items.

TOUCH SCREEN CONTROLLER

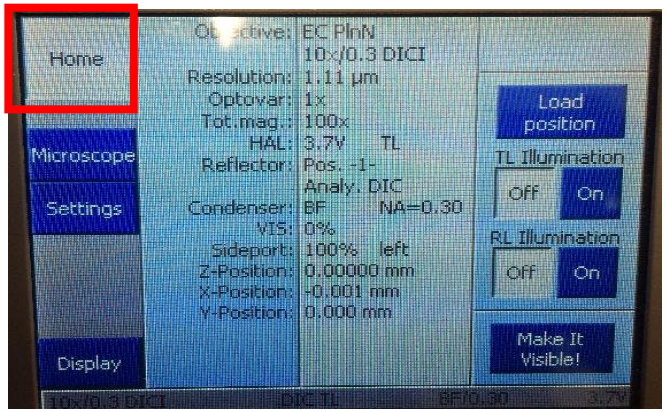


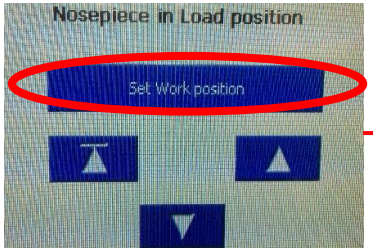
Focus knob – Z direction: for coarse and fine adjustments and controls the Z-direction of movement.
Joystick - X, Y direction: controls the X and Y direction of the stage

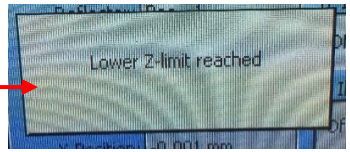
SELECTED ICON IS IN WHITE, NOT SELECTED IN BLUE.

HOME screen information:

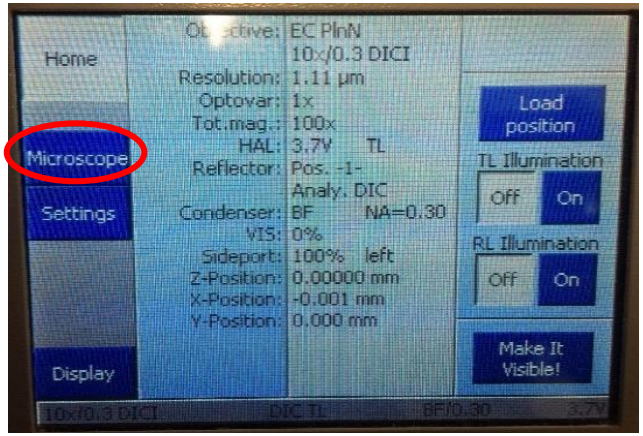
- **Objective:** Description of the current objective in the light path along with N.A.
- **Resolution**: How fine of detail the current image will appear
- **Total Magnification**: magnification of objective x eyepiece magnification (10x)
- **Reflector:** Displays which position of the reflector turret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





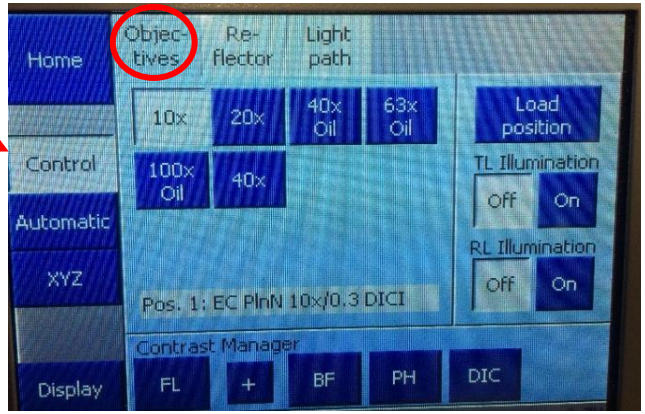


On the left of the screen, push the icon labeled **MICROSCOPE**. A new window will pull up in which you can select: **Objectives**, **Reflector**, and **Light path**.



Ensure that you are on the **Control** display.

Objective Screen



Objectives screen: display tells you which objective is used; you may change objective by pressing appropriate icon.

Reflector screen:

DIC: Transmitted light

34 BFP = DAPI channel (EX 390/20, EM 460/60)

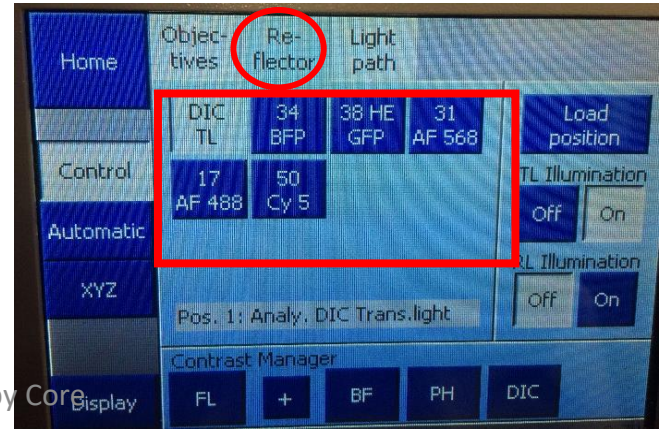
38 HE GFP = Green GFP channel (EX 470/40, EM 525/50)

31 AF 568 = Red/ DsRed channel (EX 565/30, EM 620/60)

17 AF 488 = FITC channel (EX 485/20, EM 540/25)

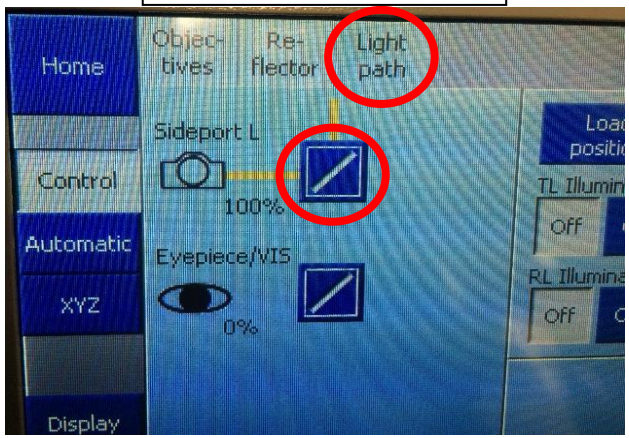
50 Cy5 = 647 far red channel (EX 640/30, EM 690/50)

Reflector Screen

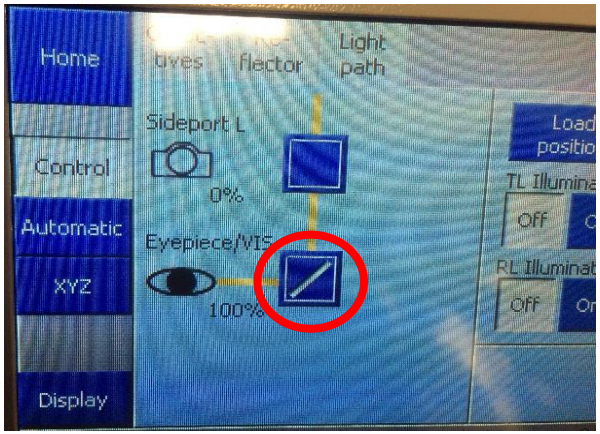


Press **Light Path** on the top of the microscope screen and you will enter the **Light Path** screen

Light Path Screen



Light Path Screen



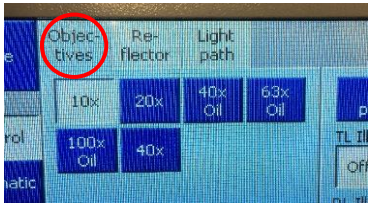
Sideport L: Directs light to go through the camera and images will show up on the computer.

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.

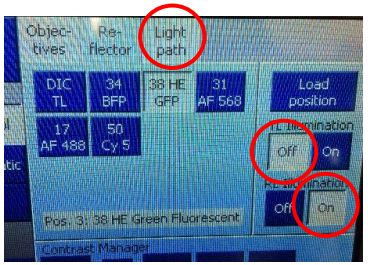
Everything on the touch screen can be controlled by the software.
It is preferred to control everything through the software.

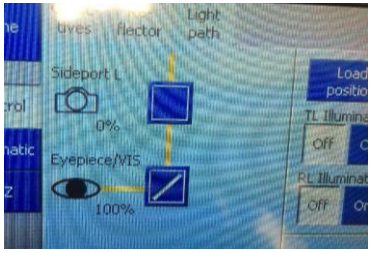
If needed, you can do all set up for imaging if you do not use the software:



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:

6. After turning off RL illumination, switch light path to 100% to Sideport L