Microscopy Shared Resource Facility
Icahn School of Medicine at Mount Sinai

Zeiss AxioImager.Z2 Fluorescence Protocol

1) System Startup

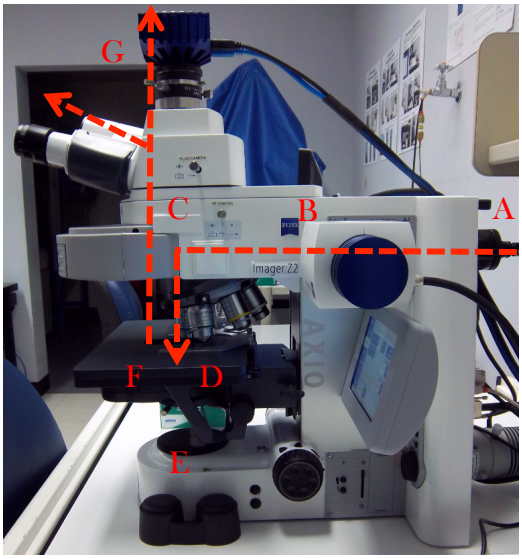
- Please note put sign-up policy. You must inform the facility at least 24 hours beforehand if you can't come; otherwise, you will receive a charge for unused time. The facility will allow for extenuating circumstances (cells dying, sick day, etc.) if you inform us in a timely fashion.
- Follow each step of the startup poster and wait for the microscope software to fully load.

2) Lens Cleaning

- Please **clean all of the lenses** (used and unused) **before and after** your session. Refer to the lens cleaning poster if you need any help recalling the rules and steps.

3) Microscope Control

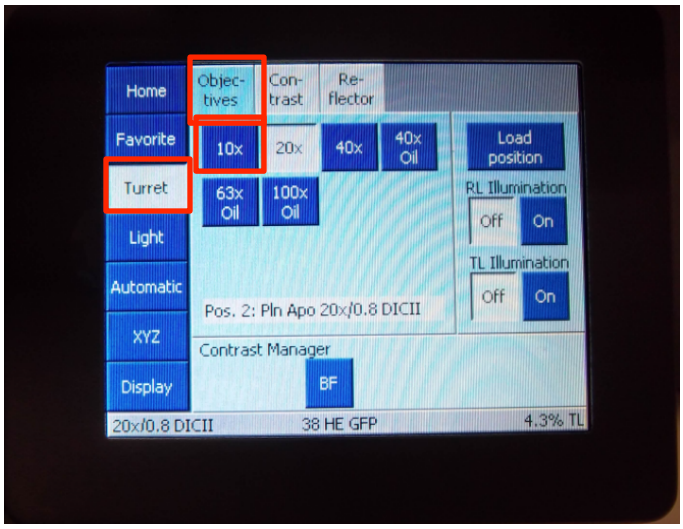
- Take note of the microscope light path:
  - A. Light source
  - B. Direction through microscope
  - C. Filter
  - D. Lens
  - E. Slide
    - a. Since we are measuring reflected light, you will not use the optics below the stage
  - F. Lens
  - G. Camera/ Eyepiece
    - a. Note the locking positions of the sliders and remember to be delicate! The slider pulls glass.

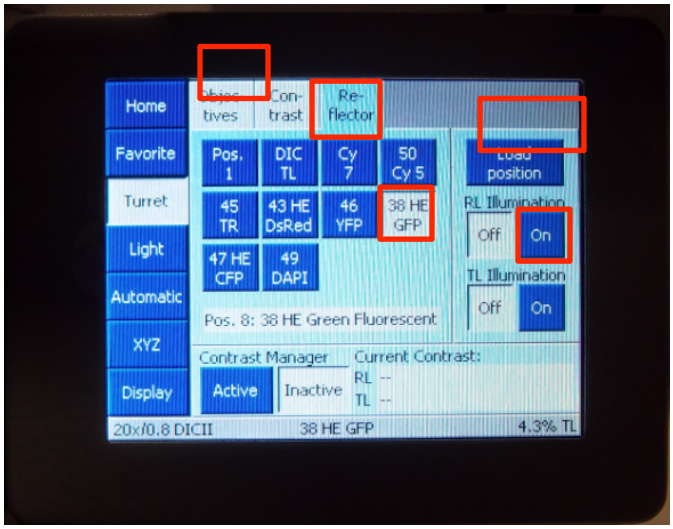


- Touch pad control
  - Within the "Home" menu, select "Microscope" and then "Turret" **(Stay within this window for the entire session and ignore other tabs)**
    - Objective Menu: always begin focusing using the **10x lens**.

(a) **Take care to properly set your focal position with the lower power lens before going to a higher power lens.** Proper focusing from low power to high power will help prevent the microscope from breaking your slide.

- Reflector Menu: select your first filter channel
- To open the shutter, turn the RL illumination on the far right of the screen "On"
  (a) Remember to **close the shutter** as often as you open it to prevent rapid bleaching of your sample.
  (b) Note: The active option is always white.



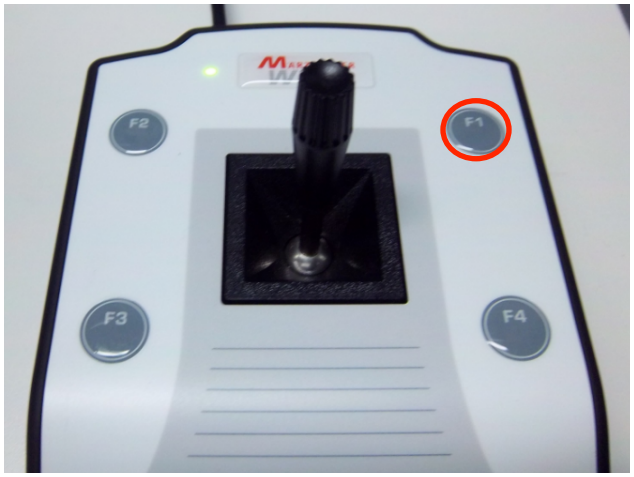


☞ Always begin imaging with the 10x objective and take care for the following issues:

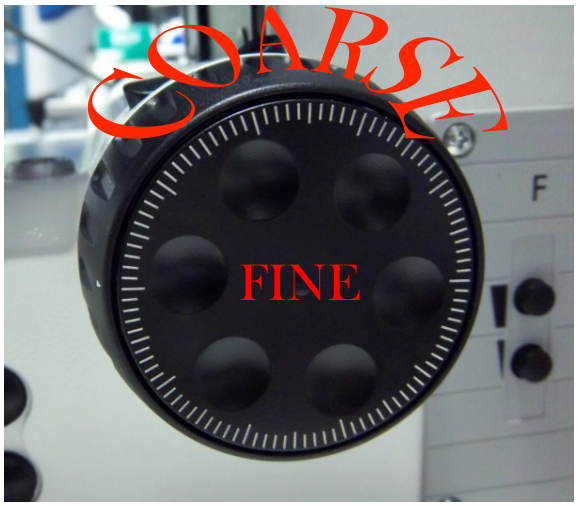
- **Select and inspect each slide**
  - If it is dirty, gently clean with a Kim Wipes and/or cotton swab. You should do this with all your slides before you come.
- **Load the slide securely into the stage clip.**
  - Focusing will become difficult when the slide is uneven.

☞ Joystick Control

- Use the joystick to find your region of interest
- Use the F1 button in the upper right to toggle between course and fine x and y control



- ☞ Focus Control
  - Course and fine focus control are located on both sides of the microscope.
  - If you are focusing on the right side of the microscope, the fine focus is located INSIDE the coarse focus. You can adjust by using the finger positions within the coarse.

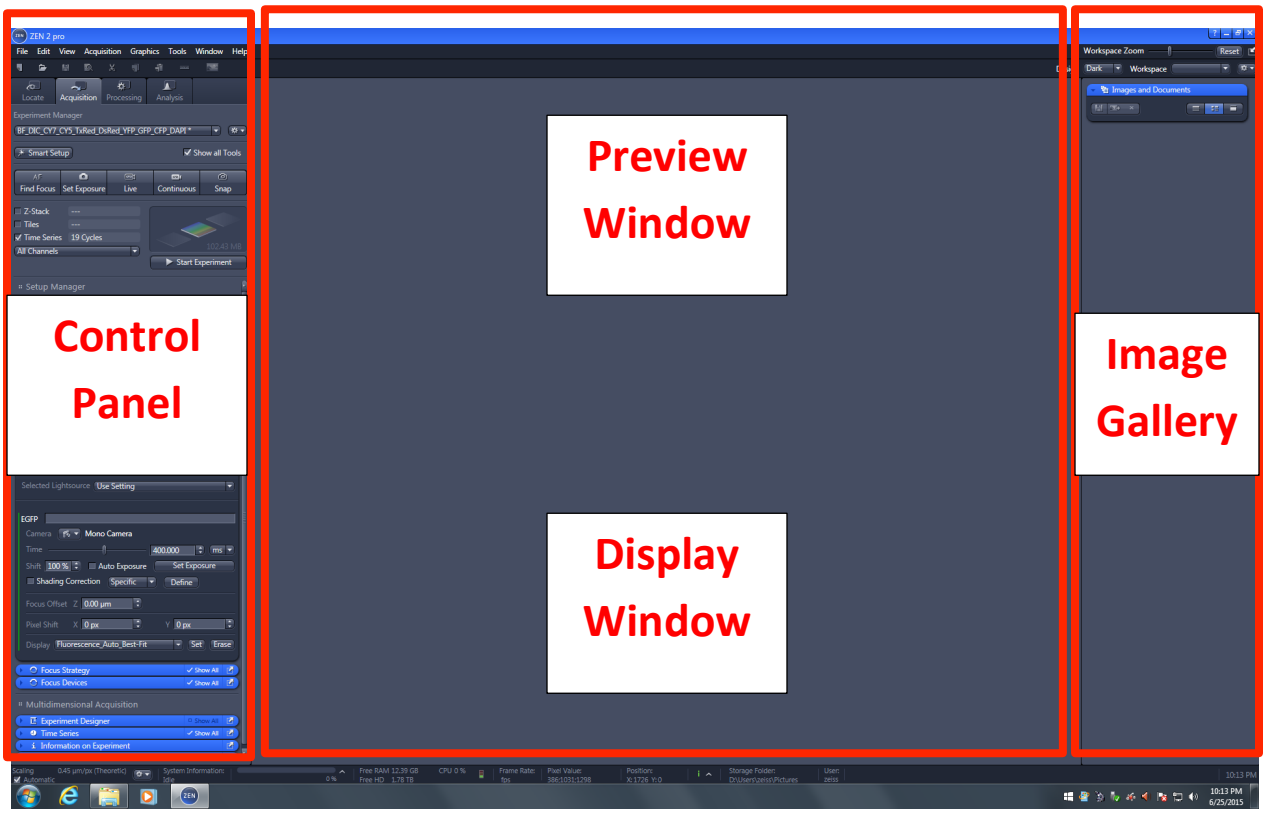


  - Focus your sample and check all the channels before imaging.

4) Software Control

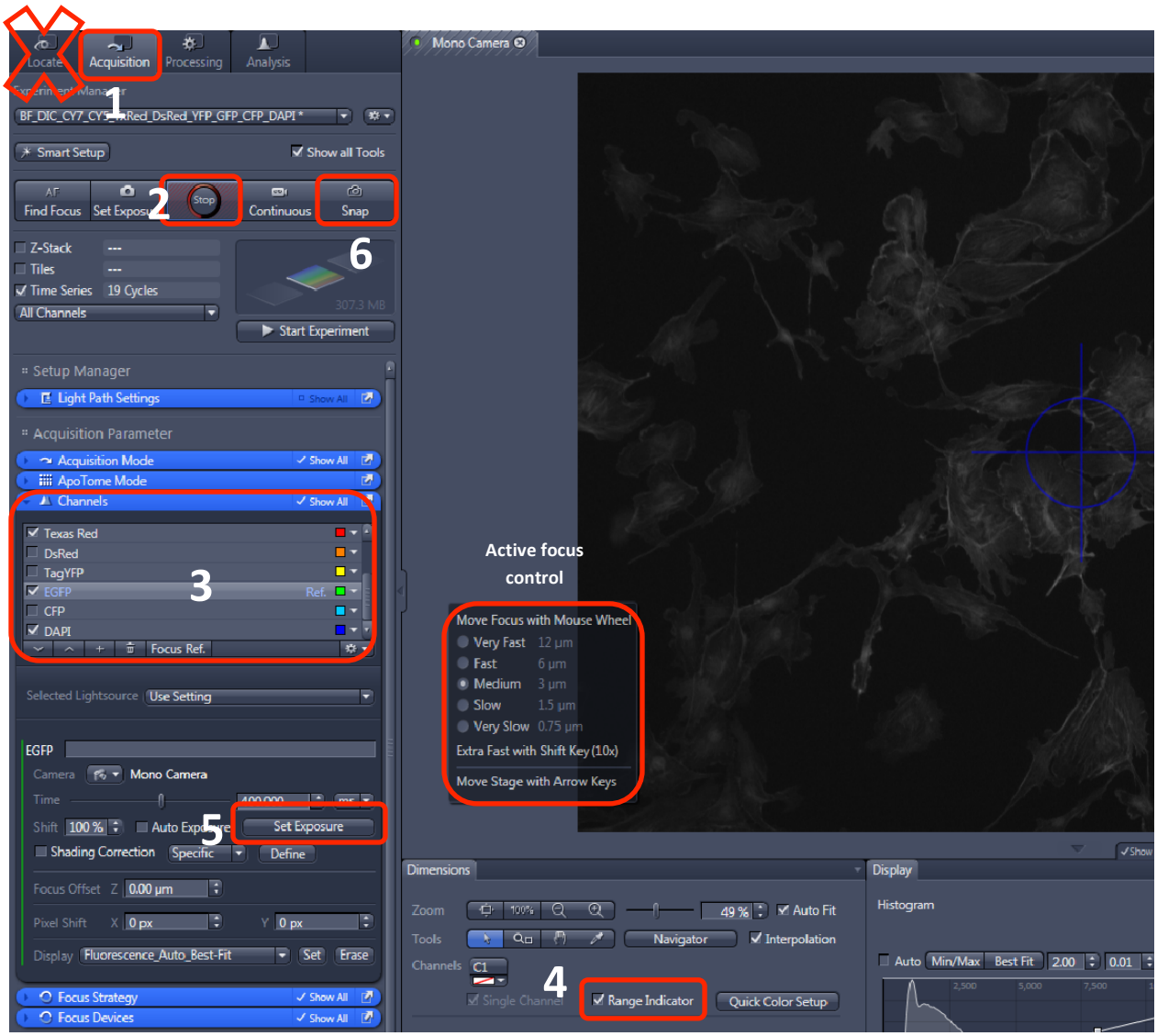
- ☞ Open the Zen Software
  - Select the "Zen Pro" imaging option
  - Click "Skip calibration"
- ☞ Upon Zen's completed startup, please note the 3 divisions of the interface:
  - Left: control panel
  - Middle:
    - Top: image preview and acquired image

- Bottom: display options
- Right: image gallery



5) Your first image

- ☞ Gently push both sliders all the way in to send the light to the eyepiece.
- ☞ Use the touchpad to:
  - **Select the 10x lens** in the objective menu
  - **Select your first filter channel and open the shutter** in the reflector menu
- ☞ Use the stage movement wand and focus knob to **focus and center your specimen** on a region of interest.
- ☞ Be sure to **check all fluorophores** in your specimen. You need to have an idea of what to expect before imaging with the camera.
- ☞ Use the touchpad RL illumination option to **close the shutter.**
- ☞ **Pull the TOP camera/eyepiece slider out** to send light to the camera.



Within the Zen software:

- Setup your experiment in the **Control Panel:**
- AVOID THE LOCATE TAB!
- Click the **"Acquisition"** tab
- Select the **"Channels"** window.
- Choose the wavelengths by checking channel names. (Channels are listed by descending wavelengths.)
- Make your most important channel your focus reference by clicking on the channel until it turns gray, and select **"Focus Ref."**
- In the "Live" mode, **focus** on the reference channel.
  - Focus using the computer keyboard

- Put your cursor over the image and hit "Ctrl" on the keyboard; use the mouse roller to fine tune the focus (to signify focus activation, a dialog box will appear).

- Focus using the microscope fine focus

☞ Click **"Range Indicator"** at the bottom of the screen.

☞ Click **"Set Exposure"** within the channels window.

☞ Click **"Stop"** (the same icon as "Live")

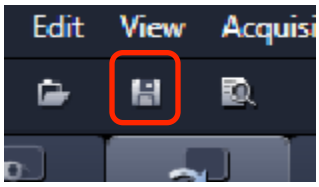
☞ Click the remaining channels and set exposure times.

☞ When all exposure times are set, Click **"Snap"** to acquire the image.

Image Saving Options:

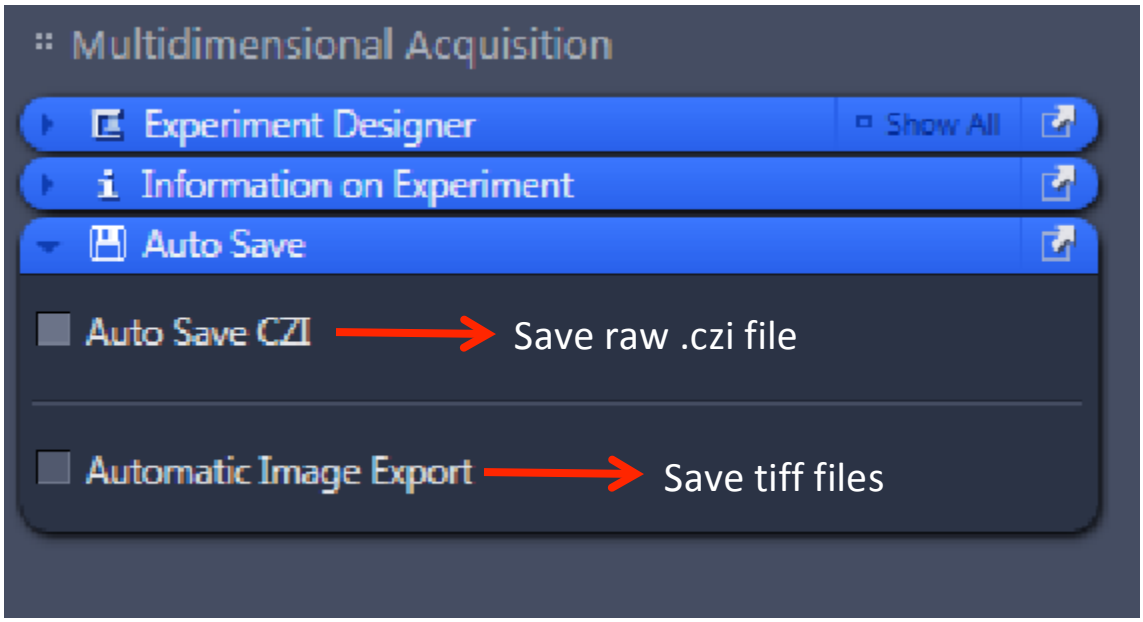
☞ Click the **"Save As"** button to save the image in your directory within the specified User Data folder.

- Note: All data is stored in the D: drive under the "User Data" folder. Be sure to save to the "User Data" folder and NOT the "Users" folder.



☞ Optional: Auto-save function

- Note: There is also an auto save function that must be enabled AND disabled before and after your session. You CANNOT export overlay images using this tool.

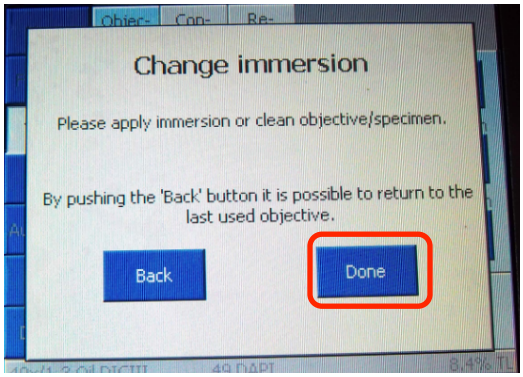


- Use the same parameters from the batch protocol to export your single channel images.

6) Higher magnification and switching slides

☞ If you switch to the higher magnification oil immersion lenses from a dry immersion lens, go back to the microscope and enter the objective menu on the touchpad.

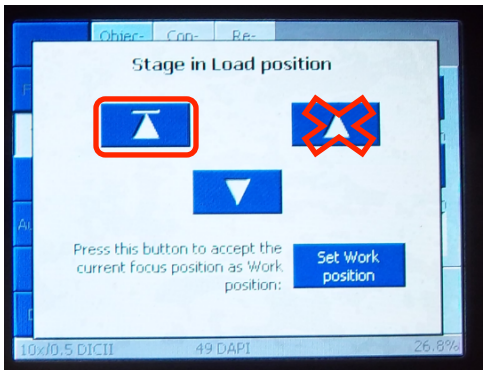
- Press the high power lens you want to use.
- The lens will then move to the correct objective and the stage will drop.
- The touchpad will prompt you to put immersion on the specimen.
- At this point, apply **one drop of oil** and click "**Done**" to raise the stage back up.



☞ If you switch from an oil lenses to a dry lens, after the touchpad prompts you to remove the immersion, take the slide out, wipe off the oil with a cotton swab. Replace the slide into the stage clip and click "**Done**" to raise the stage back up.

☞ When you need to switch slides,

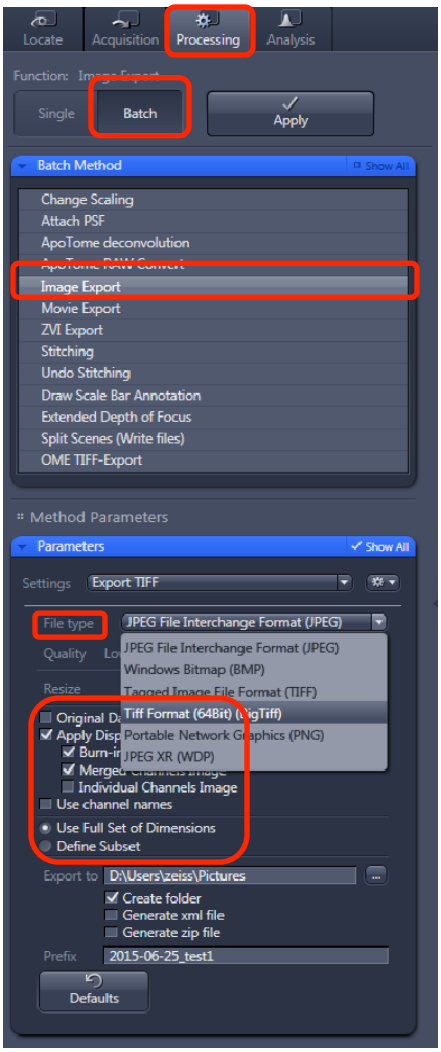
- Go back to the **10x** and refocus the new sample
- If you are imaging a similar sample using the same objective lens, click the **"Load Position"** button located in the upper right hand corner of the touchpad. This will allow you to lower the stage, replace your slide with a new one, and restore the same focal plane.

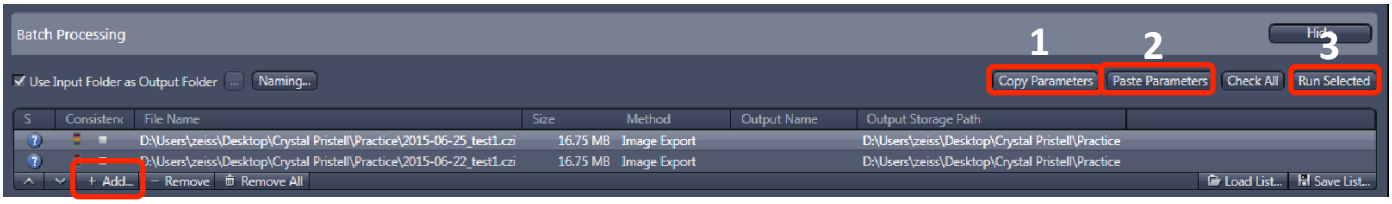


7) **Data Export**

☞ Go to the **"Processing"** tab (located to the right of the "Acquisition" tab)

- Click **"Batch"**
- Under **"Batch Method"** select **"Image Export"**
- In the middle of the screen under "Batch Processing," click **"Add"** and then select all the images you want to export.
- Highlight one image
- Under parameters, make the following selections
  - File type: Tagged Image File (.tiff)
  - Compression: None
  - Unclick convert to 8 bit
  - Check "Use Full Set of Dimensions"
  - Unclick "Create project folder" unless you would like all images in separate folders
- Exporting options: also within the parameters tab, you can choose the display of your images
  - Select "Orignal Data" to export the raw data in gray
  - Select "Individual Channel Images" to export the individual channels with pseudocolor
  - Select " Merge Channels Images" to export the overlay
    (a) Note: All image display types **CAN** be exported at the same time.
    (b) Note: You can batch several groups of images differently and run them simultaneously.





☞ Underneath Batch Processing,

- Click "**Copy Parameters**"
- Select all images. (Ctrl +Shift A)
- Click **"Paste Parameters"**
- Click **"Run Selected"**

8) System Shutdown

- ☞ Back up all your data.
- ☞ Clean all the lenses.
- ☞ Check the microscope calendar to see when the next user has an appointment
- ☞ If the user comes within 2 hours, log off your account
  - Otherwise, follow shutdown poster steps.