Microscopy Shared Resource Facility
Icahn School of Medicine at Mount Sinai

Zeiss AxioImager.Z2 Brightfield Protocol

1) System Startup

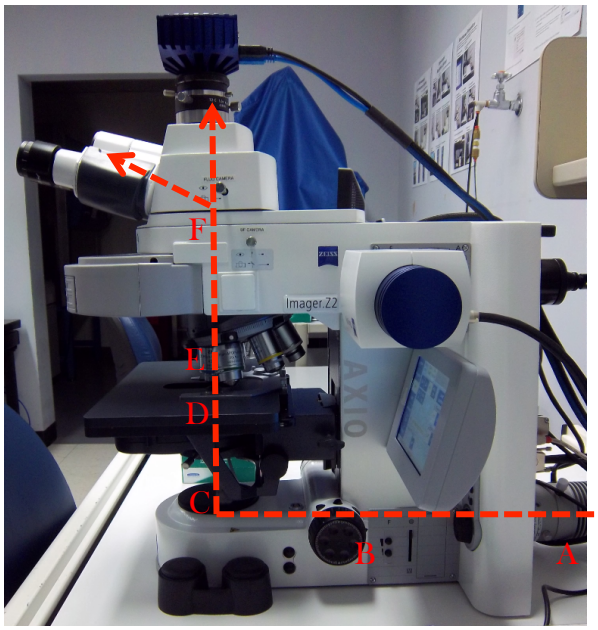
- ☞ Please note put sign-up policy. You must inform the facility at least 24 hours beforehand if you can't come; otherwise, you will receive a charge for unused time. The facility will allow for extenuating circumstances (cells dying, sick day, etc.) if you inform us in a timely fashion.
- ☞ Follow each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

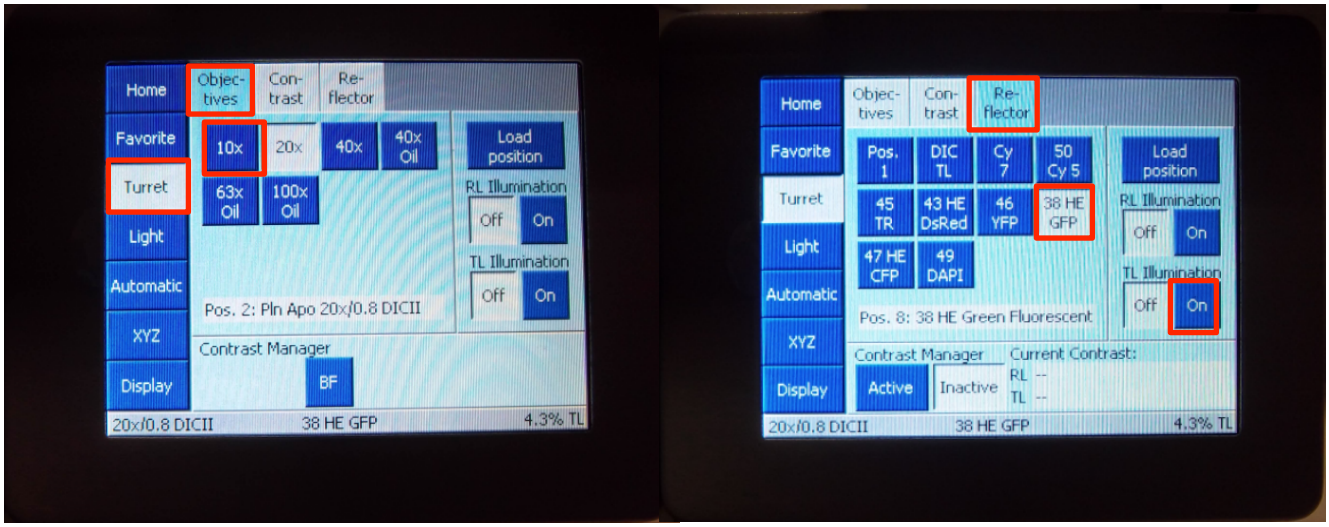
3) Microscope Control

- ☞ Take note of the microscope light path:
  - A. Light source
  - B. Direction through microscope
  - C. Condenser assembly
  - D. Slide
  - E. Lens
  - F. 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 TL 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.

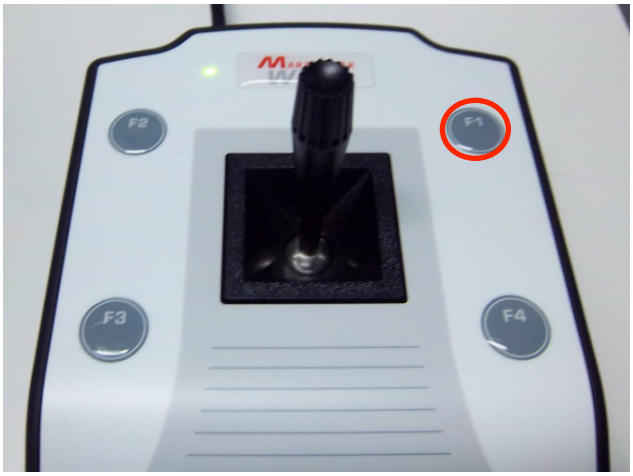


☞ Always begin imaging with the 10x objective and take care for the following issues:

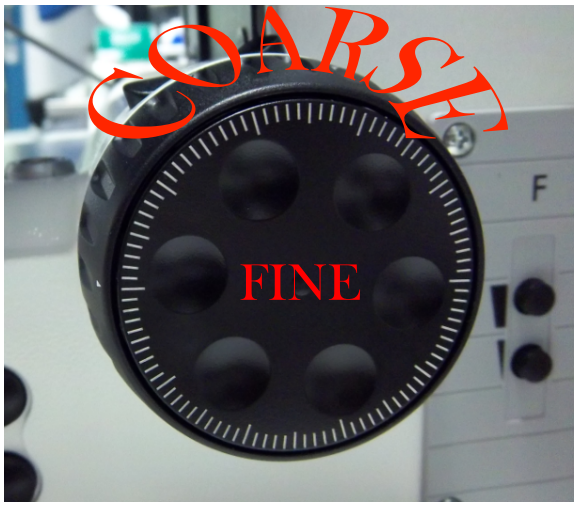
- **Select and inspect each slide**
  - If it is dirty, gently clean with a Kim Wipes and/or cotton swab. You should do this with all your slides before you come.
- **Load the slide securely into the stage clip.**
  - Focusing will become difficult when the slide is uneven.

☞ 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



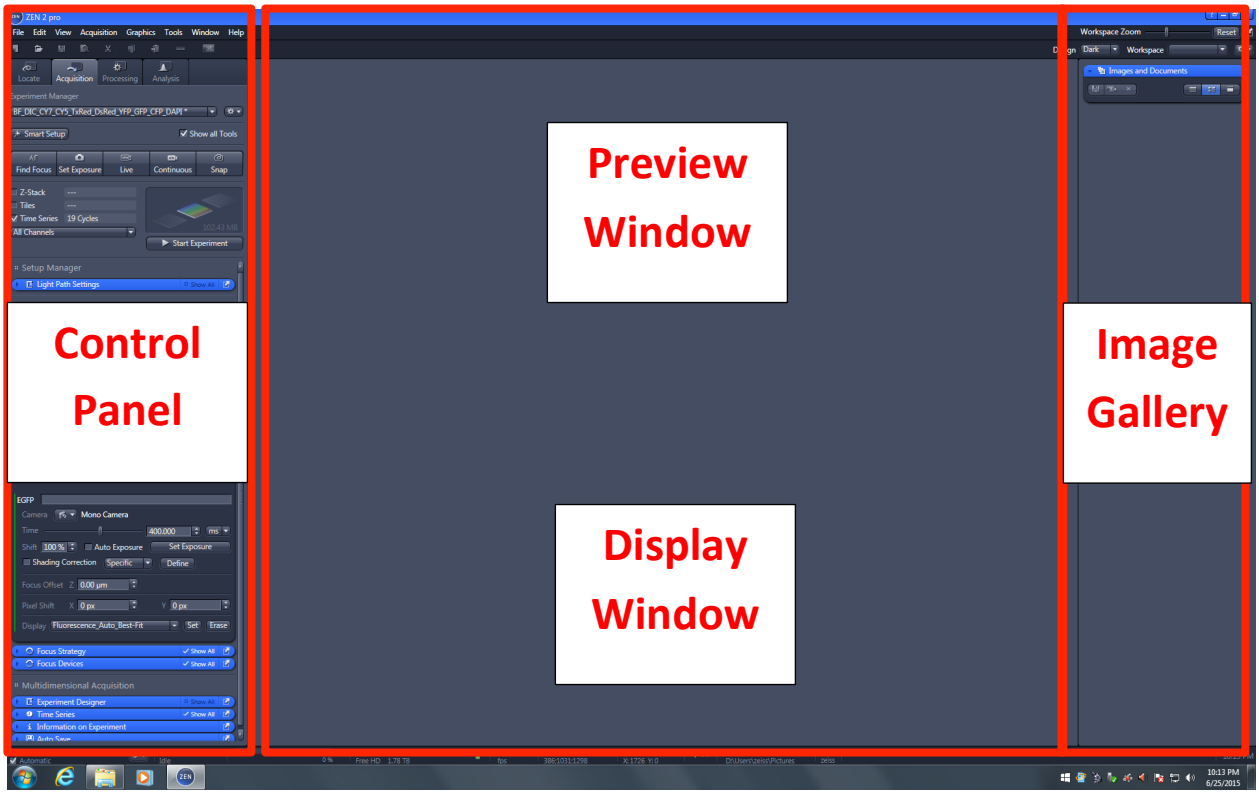
- ☞ Focus Control
  - Course and fine focus control are located on both sides of the microscope.
  - If you are focusing on the right side of the microscope, the fine focus is located INSIDE the coarse focus. You can adjust by using the finger positions within the coarse.
  - Focus your sample and check 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: acquisition control
  - Middle:
    - Top: image preview and acquired image

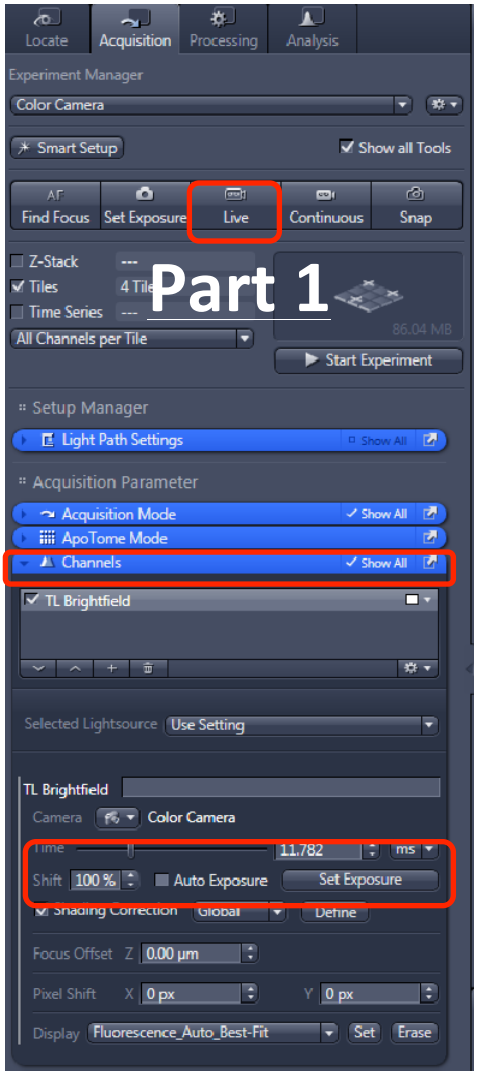
- Bottom: basic measurements and manipulations
- Right: image gallery

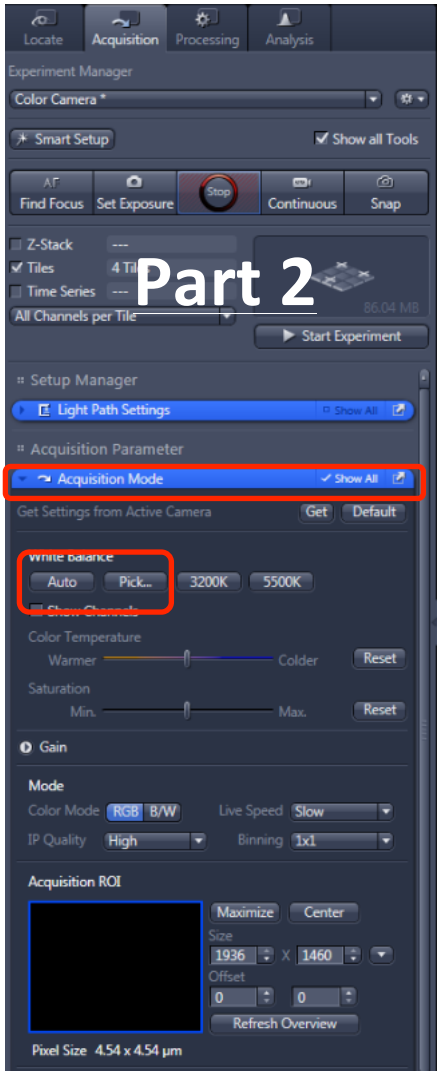


5) Your first image

- ☞ Select the H condenser filter and readjust light intensity using the LED dial
- ☞ 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 the DIC/TL filter channel and open the TL illumination**
- ☞ Use the stage movement wand and focus knobs to **focus and center your specimen** on a region of interest.
- ☞ Setup Kohler illumination; you can refer to the poster for help. This step is critical and necessary for uniform field illumination in imaging and must be done every time you switch lenses.
- ☞ With one hand on focus and the other on the condenser iris for your desired level of brightness, contrast, and morphological detail.
  - Higher Lens NA → larger condenser aperture
  - Lower Lens NA → smaller condenser aperture

- Use the touchpad RL illumination option to **close the shutter.**
- **Pull the BOTTOM 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.

☞ Enter the **"Live"** mode,

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

☞ **Focus** on your sample using one of the following options:

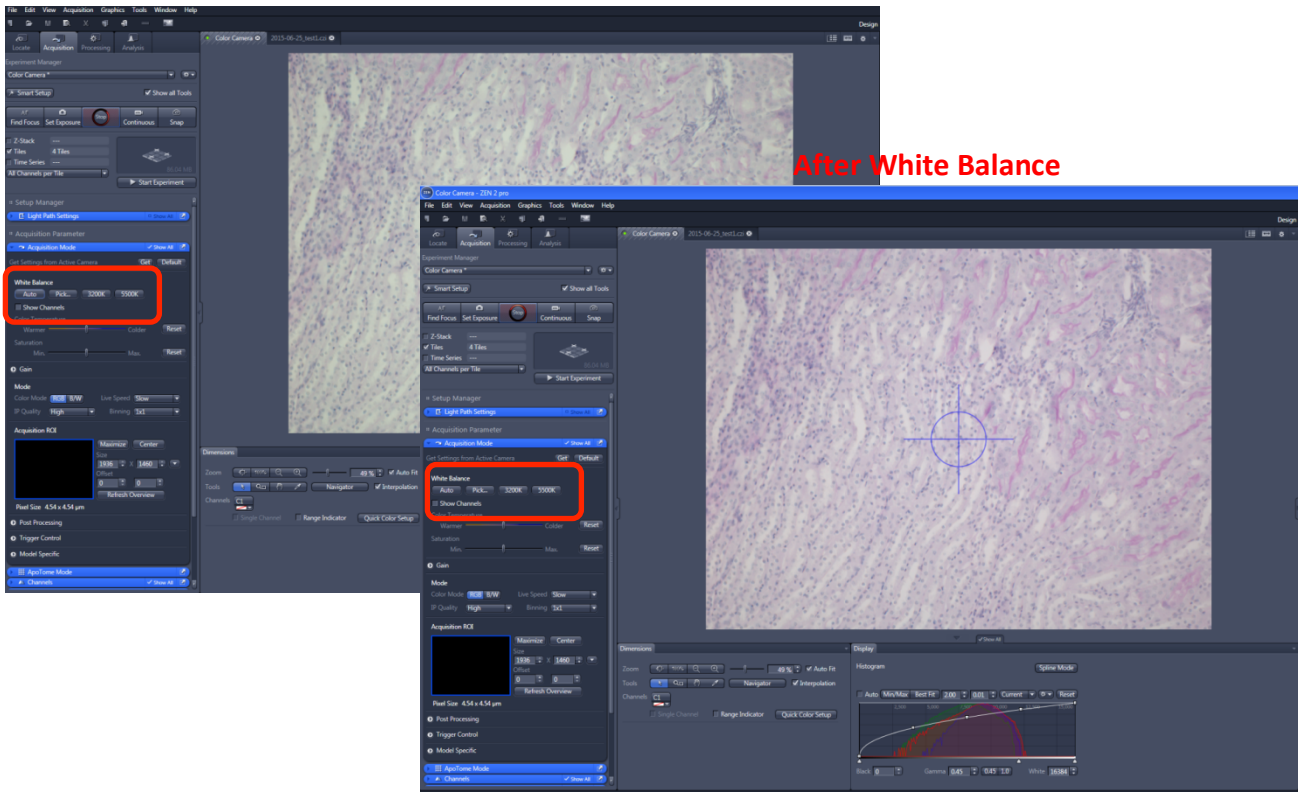
- Focus using the computer keyboard
  - Put your cursor over the image and hit "Ctrl" on the keyboard; use the mouse roller to fine tune the focus (to signify focus activation, a dialog box will appear).
- Focus using the microscope fine focus

☞ Click the **"Acquisition Mode"** window to set the white balance.

- Select either the auto option or picker option; find a white region of your sample and click using the picker option.

Before White Balance

After White Balance



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

☞ When all exposure times are set, Click **"Snap"** to acquire the image. Before saving your image **set your gamma = 1** using the display tab at the bottom of the display window.

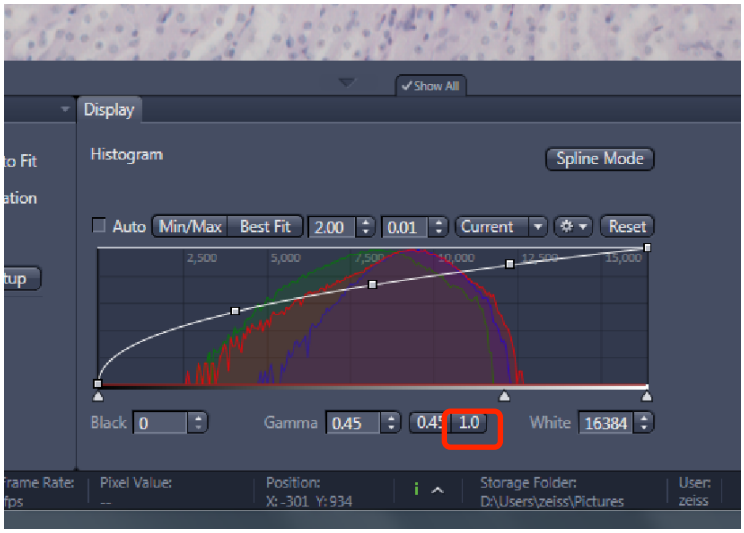
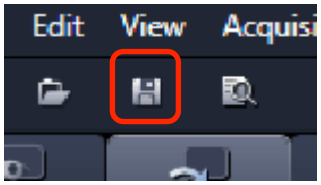
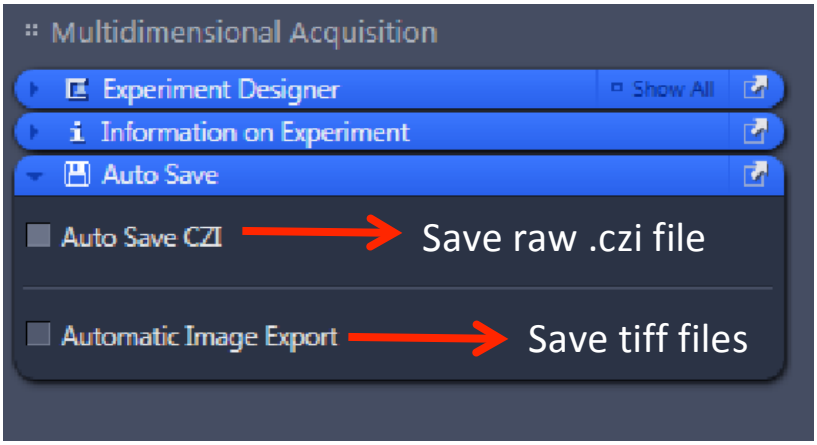


Image Saving Options:

- ☞ 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.



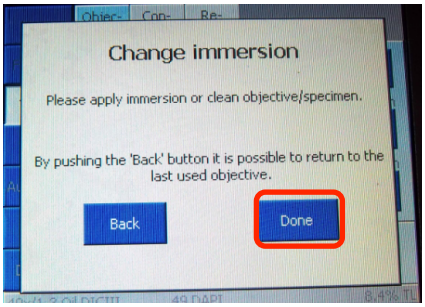
  - Use the same parameters from the batch protocol to export your images correctly.

- Exporting options: be sure that you select the following image options to generate the same image you acquired.
  (a) DO NOT select "Original Data" export
  (b) Select "Apply Display Curve and Channel Color"

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.

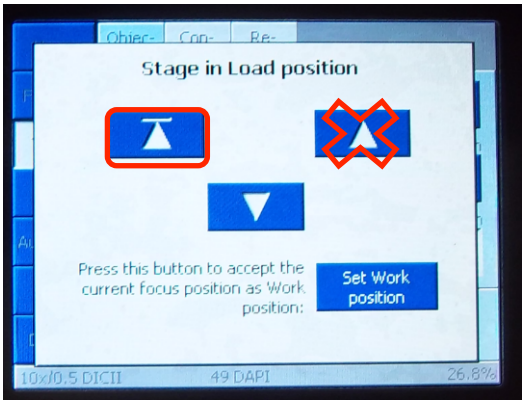
- Press the high power lens you want to use.
- The lens will then move to the correct objective and the stage will drop.
- The touchpad will prompt you to put immersion on the specimen.
- At this point, apply **one drop of oil** and click "**Done**" to raise the stage back up.



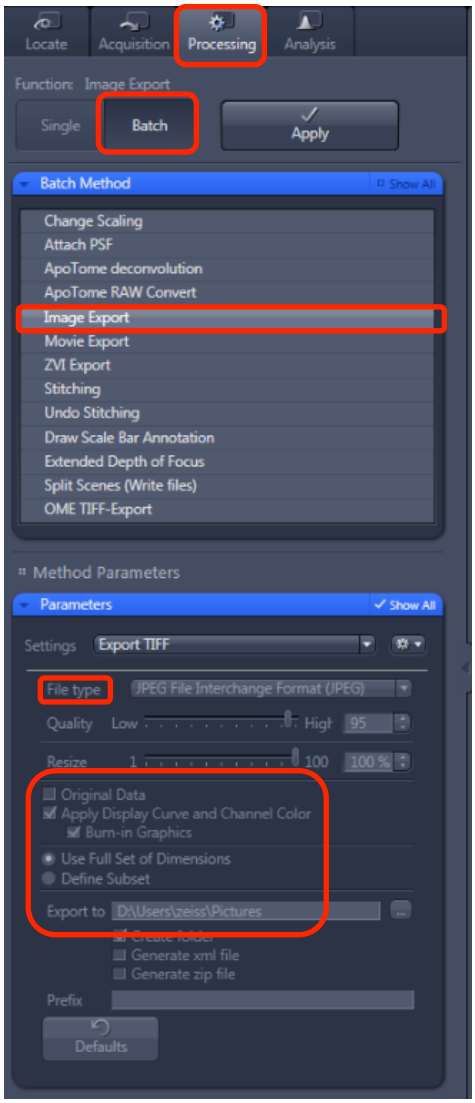
☞ If you switch from an oil lenses to a dry lens, after the touchpad prompts you to remove the immersion, take the slide out, wipe off the oil with a cotton swab. Replace the slide into the stage clip and click **up arrow on the left** to raise the stage back up.

☞ 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.

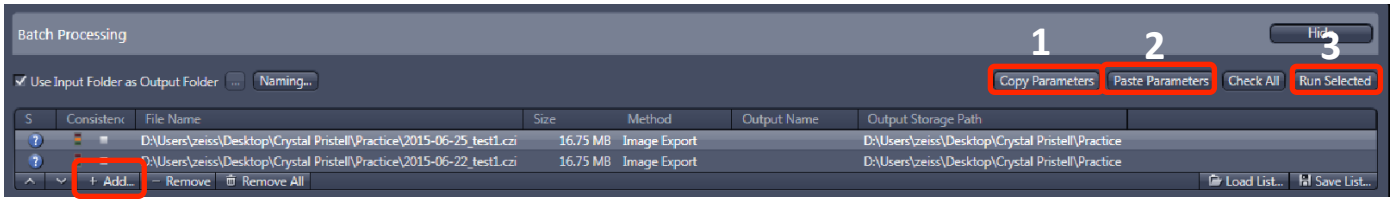


7) Data Export



☞ 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: be sure that you select the following image options to generate the same image you acquired.
  - DO NOT select "Original Data" export
  - Select "Apply Display Curve and Channel Color"



☞ Underneath Batch Processing

- Click "**Copy Parameters**"
- Select all images. (Ctrl +Shift)
- 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.