



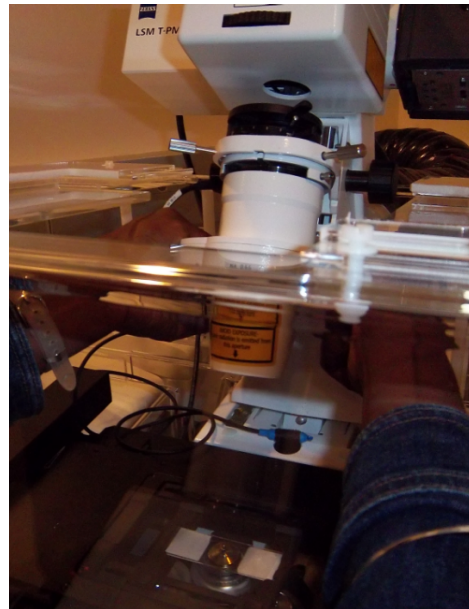
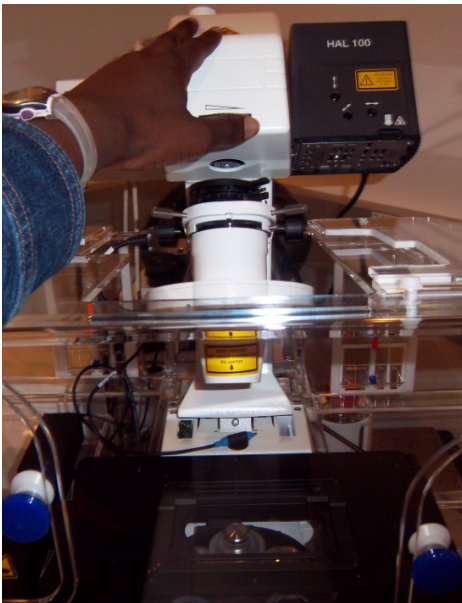
## Zeiss LSM 880 Protocol

### 1) System Startup

- ☞ Please note our 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
- ☞ **Log into** the computer with your user account.
- ☞ Open Zen black . Press  to continue and wait for the microscope software to fully load. You should see the Locate tab when the software has successfully started.

### 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. Gently push the condenser assembly back using **ONLY** the solid center points as shown below. (Lenses: 10x, 20x, 40xW, 40x Oil, 63x Oil, and 100x Oil)
- ☞ Gently pull incubation box sliders and gently push the condenser assembly back using **ONLY** the following support points:



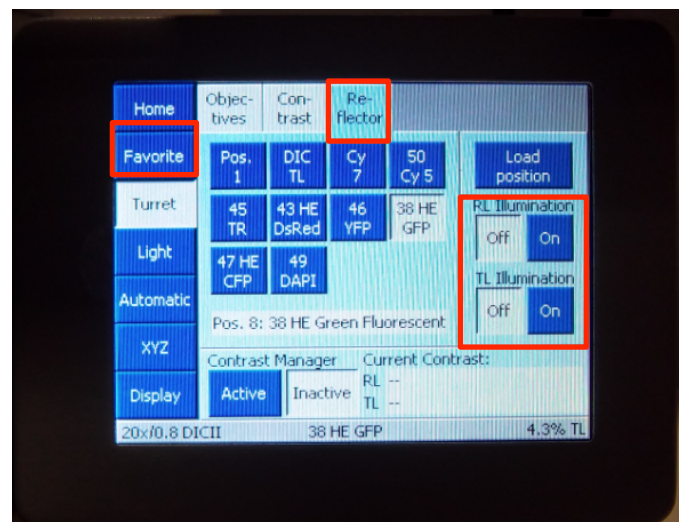
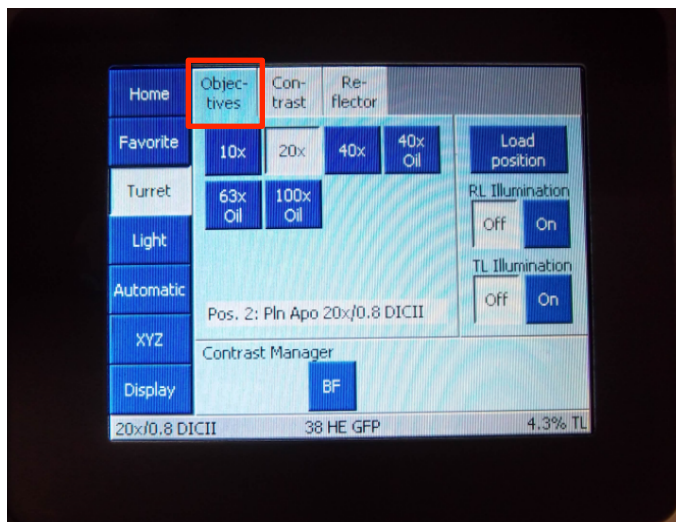
## Microscope Control

☞ Take note of the microscope light path: [insert image and letters]

- A. Excitation laser lines
- B. Main beam splitter
- C. Galvo scanning mirrors
- D. Objective lens
- E. Pinhole
- F. Secondary beam splitter
- G. Detection unit
- H. Emission filters

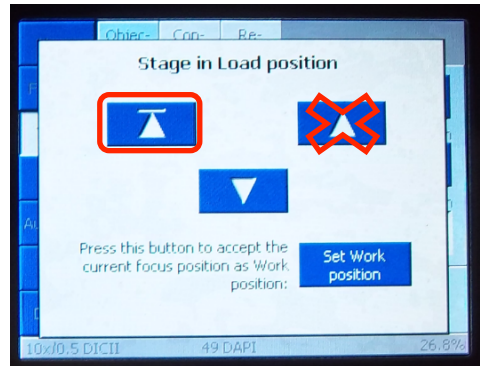
☞ Touch pad control

- Within the “Home” menu, select “Microscope.” The “Microscope” window turns into “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. 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 using “Load position.”**
  - The stage will drop allowing you to insert your slide.
  - Once your slide is secure, press the top left arrow to reestablish your z-position.



- 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 left 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.

3) Software Control



☞ Start Zen 2 Black

- Click Start System



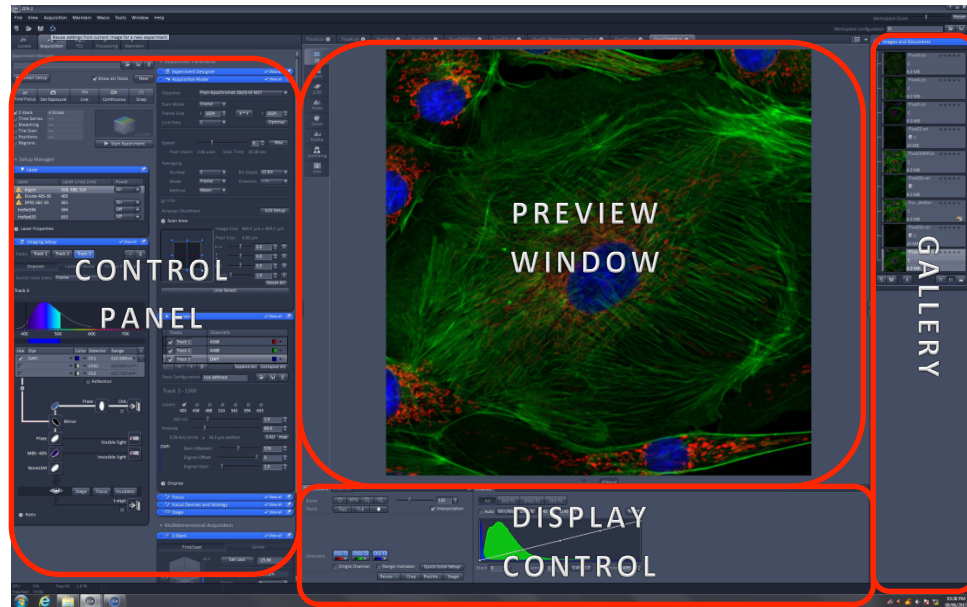
- During the System Startup, the incubation system may produce an error message in the lower left hand corner.

☞ Upon Zen's completed startup, please note the 4 quadrants of the interface:

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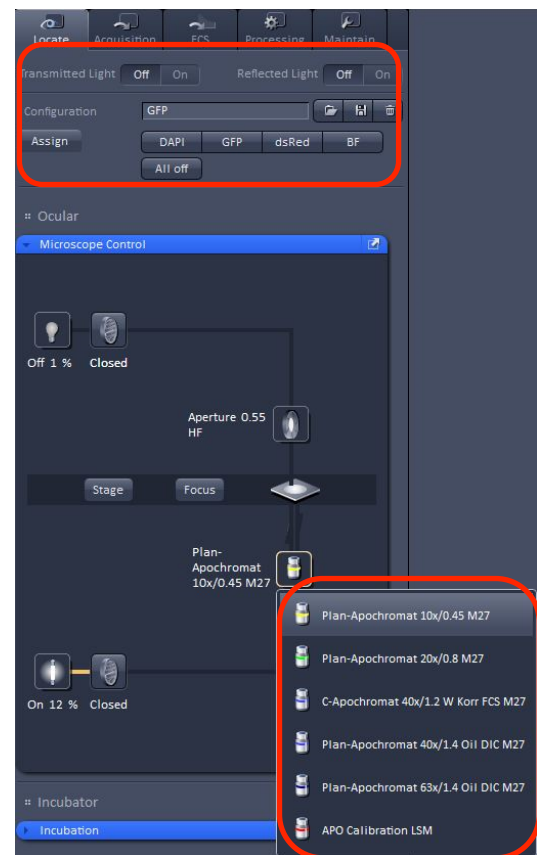
- Left: control panel
- Middle Top: image preview and acquired image
- Middle Bottom: display options
- Right: image gallery



#### 4) Your first image

While on the Microscope:

- ☞ Use the touchpad or the locate tab to:
- ☞ **Select the 10x lens** in the objective menu Locate tab control
  - Within the Locate tab, use the preconfigured filter cubes to activate light. Open and close filter using the following [insert configuration]
  - [icon] Change objectives using the light path
  - **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 or the locate tab “Off” icon to **close the shutter**.

Within the Zen software:

- ☞ Setup your experiment in the **Acquisition Tab**

- Click Smart Setup
  - Click “+” to add the number of channels you are imaging
  - Click drop down on each channel and select the appropriate fluorophore
  - Select Smart Setup imaging option

- (a) Fastest: simultaneous
- (b) **Best signal: sequential (choose this option)**

- (c) Smartest: fastest speed with some sequential

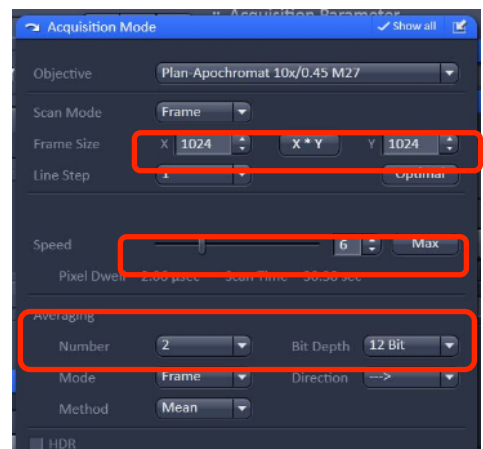
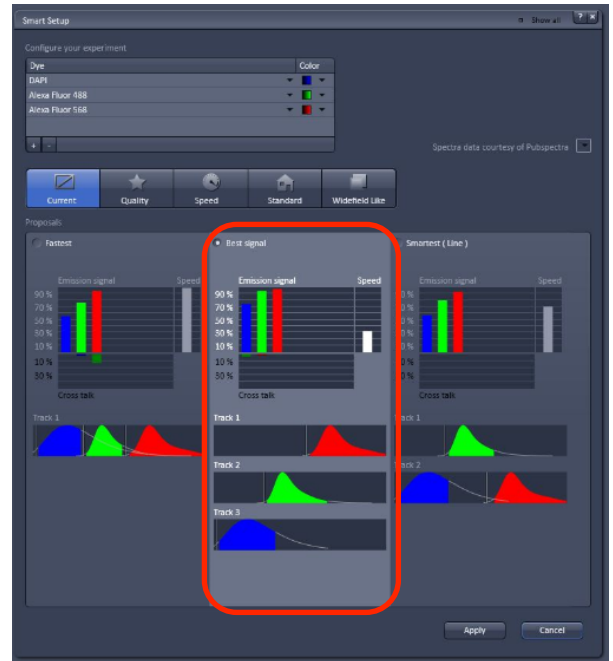
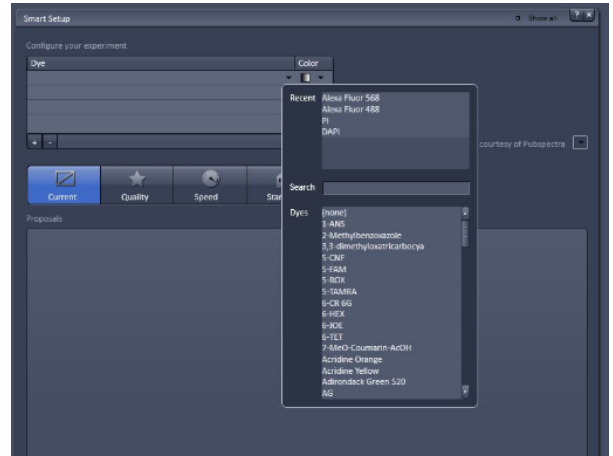
- Click Apply. Program will ask you if you want to turn on the Argon laser if green is active. Click Yes.

- Open the following control windows:

- Laser Window
  - (a) Make sure the appropriate lasers are on.
  - (b) You do not need to activate the Argon laser. It will automatically change from Standby to ON

- Acquisition Mode Window

- (a) Set the parameters for imaging here:
  - (i) Frame Size: 1024 x 1024
  - (ii) Speed: 6
  - (iii) Averaging: 2
  - (iv) Bit Depth: 12



- (v) Zoom: 1
- (vi) \*Optimal option adjusts laser wavelength to the numerical aperture, and zoom factors
- (vii) Don't change objective here

- Image Setup Window

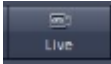
- (a) If necessary, adjust bandwidths or change from frame to line scanning in this window.

- Channels Window

- (a) Adjust gain settings for each channel
  - (i) First, decide which channel you'll set first and disable the other channels in the List of Tracks. Deselect the channels you do not want to visualize in the live view
  - (ii) Make sure the channel you want to visualize is highlighted in gray

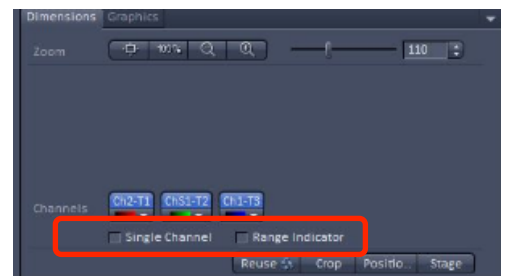
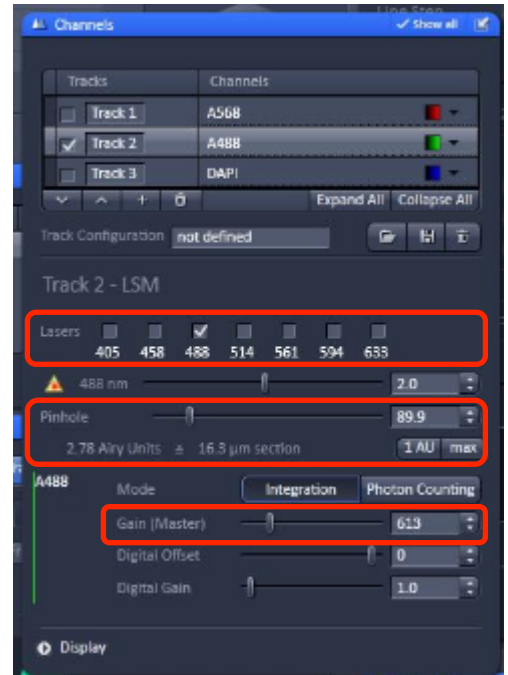
- (b) Gain adjustments


- (i) Manual gain adjustment

1. Click  to start your live preview scan
2. Click "Range indicator" at the bottom of the display menu
  - a. Red pixels: saturation
  - b. Green pixels: black
3. Adjust the Detector Gain slider until you see your image appear. Use the fine focus to locate the brightest focal plane. Readjust the gain until you see only a few red pixels.
4. Click the remaining channels and set exposure times.



- (ii) Automatic gain adjustment

1. Click  instead of  (this option will set the software recommended exposure time)



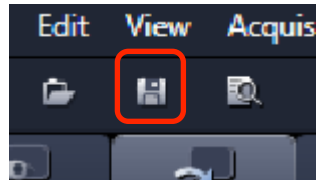
2. Click the remaining channels and set exposure times.
- (c) Pinhole adjustments (important for colocalization)
- (i) Click the 1AU button while the longest wavelength channel is selected.
  - (ii) Record the AU size and adjust the lower wavelengths to match the same value.
- Click **“Stop”** (the same icon as “Live”)
  - When all exposure times are set, Click  to acquire the image.

☞ Take note of the following viewing options:

- Click  to see all of your channels and an overlay channel
- Click  to find all image information

### Image Saving Options:

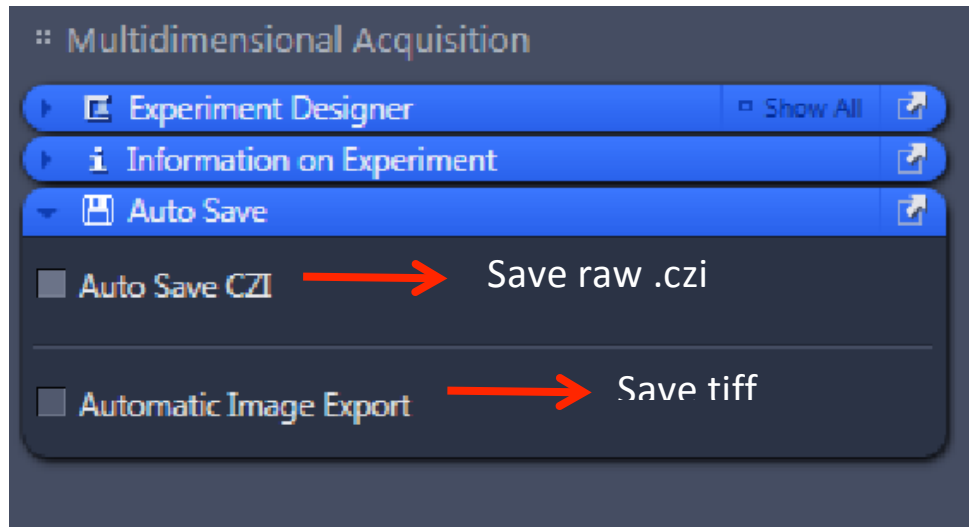
- ☞ Click the **“Save As”** button to save the image in your directory within the specified User Data folder.
- Note: All data is stored in the D: drive under the “User Data” folder. Be sure to save to the “User Data” folder and NOT the “Users” folder.



☞ 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.

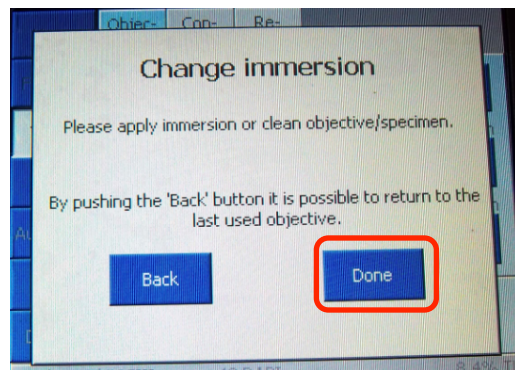




- Use the same parameters from the batch protocol to export your single channel images.

### 5) Higher magnification and switching slides

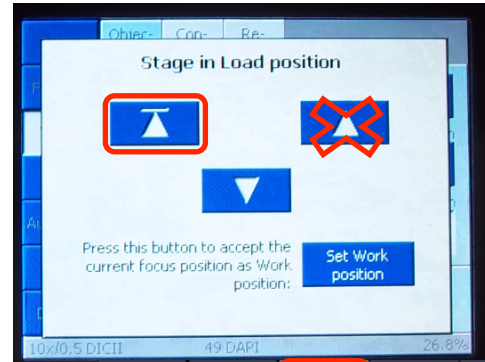
- ☞ If you switch to the higher magnification oil immersion lenses from a dry immersion lens, go back to the microscope and enter the objective menu on the touchpad.
  - Press the high power lens you want to use.
  - The lens will then move to the correct objective and the stage will drop.
  - The touchpad 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.
- ☞ It is also wise at the beginning of your session to put a drop of oil on the objective you think you will use.
- ☞ When you need to switch slides,

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- 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.



## 6) Data

### Export

#### ☞ Single Image

- File
- Image Export
- Choose a Directory
- As Tiff

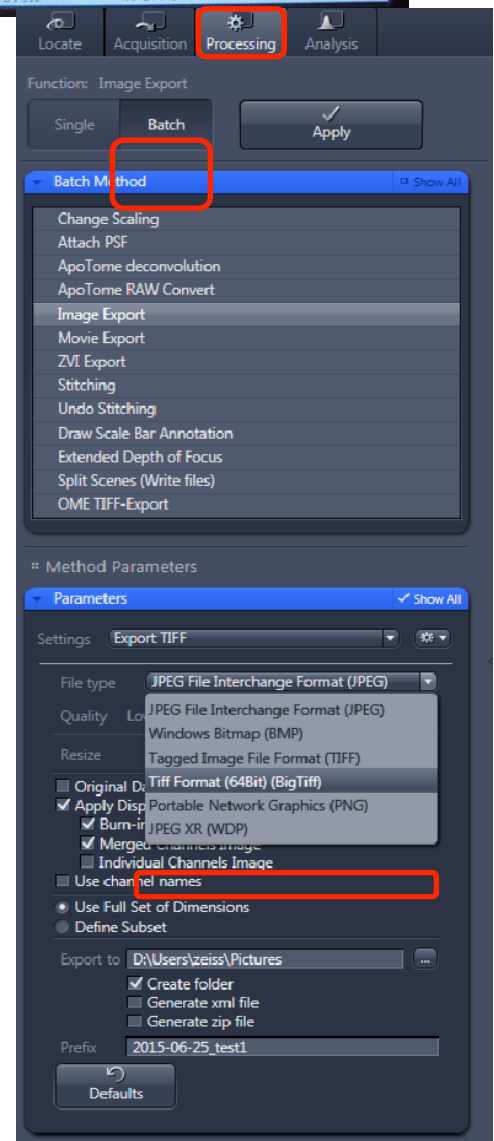
#### ☞ Multiple Image



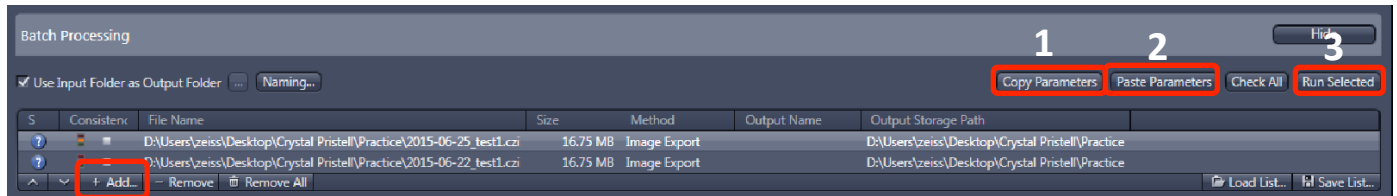
- Enter  to Batch Export your data

#### ☞ 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 “Original 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”

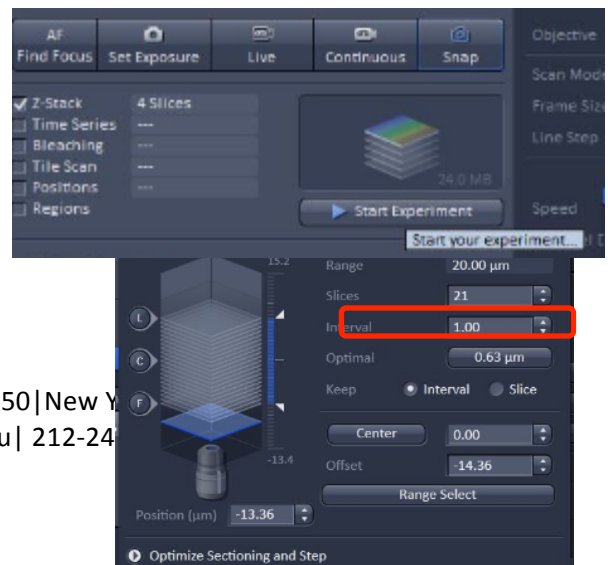
## 7) System Shutdown

- ☞ 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.

## SECOND SESSION

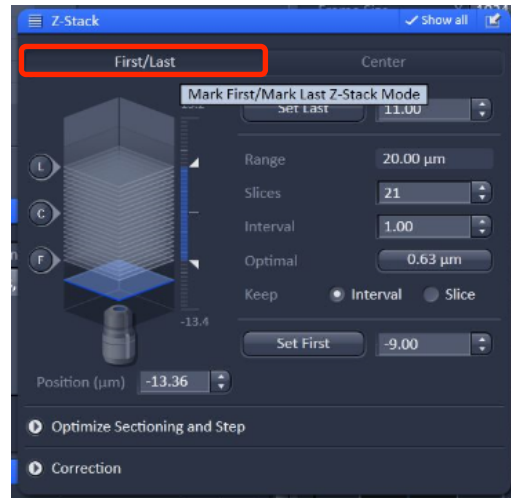
### 8) Your 2<sup>nd</sup> session and beyond: Taking a Z-stack & adding a DIC channel

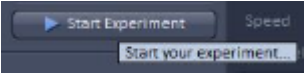
- ☞ Taking a Z-stack
  - When you come in for your second session, proceed with the system startup and cleaning the lenses. Load your specimen and remember to focus from low to high power.
  - Turn on your relevant lasers and load your scan settings.



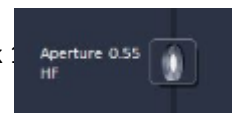
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- Set you gain for the brightest focal plane of each channel and click STOP in the Acquisition tab.
- In the Multidimensional functions options, check Z-series. A new window will open in the control panel under the Multidimensional function windows.
- Enable only the channel that has the signal which will determine your stack limits.
- Click “Live” to preview your scan.
- There are 2 ways to setup your z-stack:
  - Centered:
    - (a) Find the brightest plane of your signal
    - (b) Set the number of slices in your stack (for example, if you desire 6 slices, 2 slices will be taken above your center, and 2 will be taken below and 1 will be at the designated center.)
    - (c) Set the interval in between each slice.
  - First and last:
    - (a) Focus toward the top (towards yourself) and click [first icon]
    - (b) Focus towards the bottom (away from yourself) and click [last icon]
    - (c) Set the interval in between each slice.
  - \* Note: If you need to perform post processing techniques like deconvolution or 3D reconstruction, click “Optimal Interval”



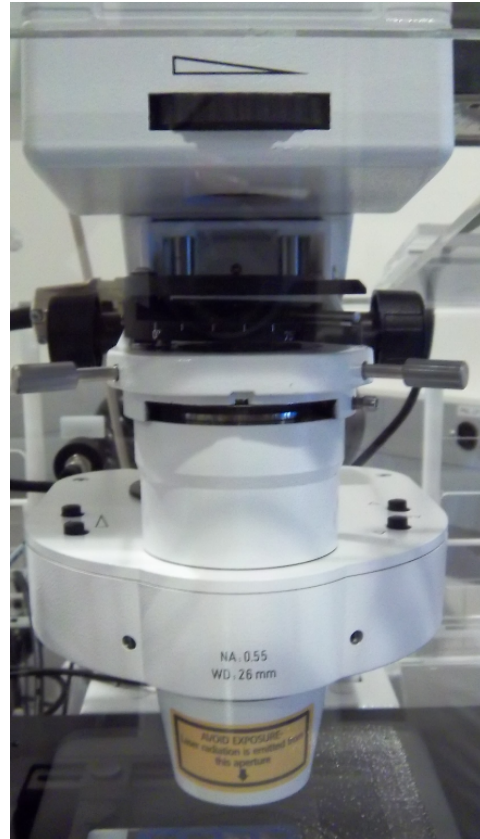
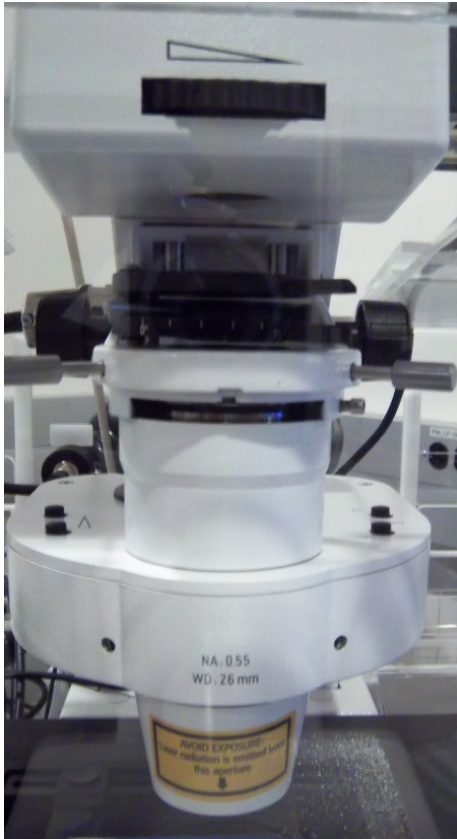
- Click Stop
- Enable all channels.
- Click  to start acquiring your stack. Since this process is vibration sensitive, please remember to keep your hands off the microscope table. While the system scan your stack, you can click gallery to see all your acquiring slices. After acquisition, you can use the [play icon] button to scroll through your sections. To retake a single image, user must unclick “Z-series” to disable stack acquisition.

☞ Adding a DIC channel





- Within the Locate tab, in addition to selecting BF option you must also select the appropriate DIC filter using the aperture window. (Appropriate filter for lenses are denoted below)
- To add a DIC channel, please note the typical microscope setup to achieve this type of brightfield illumination below.
- You will have to configure the system while switching between your viewing modes.



- Turn on the halogen light with the TL illumination shutter.
- You can adjust the brightness with the [iris picture] base of the microscope. Set the top polarizer to 0°:
- Set the condenser prism in the Locate tab menu. Match the setting in the condenser aperture field to the DIC designation on your current lens. (10x and 20x- DIC II; 40x and 63x - DIC III)
- Turn off the halogen light with the TL illumination shutter.
- Set the top polarizer to 75°
- Push the bottom polarizer back to its original position.
- Enable the TPMT detector.
- In the live view, adjust the channel gain as usual. You can slightly lower the gain to reduce saturation in the overlay with the fluorescence channels.

- If desired, adjust the lens prism located at the base of your lens.
- Pull the bottom polarizer out one click.
- Focus your sample and set up Kohler illumination; refer to the poster if necessary.
- Proceed with your session as normal.