Microscopy Shared Resource Facility
Icahn School of Medicine at Mount Sinai

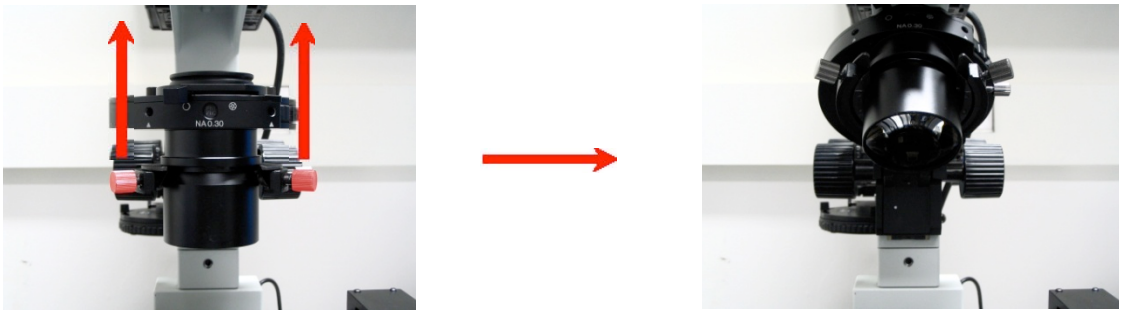
Olympus IX-70 Imaging Protocol

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 your account, and open Metamorph software.

2) Lens Cleaning

- If necessary, please lift the condenser by its centering screws (highlighted in red) as shown below:

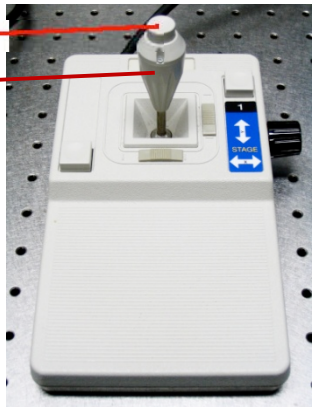


- Please clean all of the lenses (used and unused) before your session and afterwards if you use the 60x oil immersion lens. Refer to the lens cleaning poster if you need any help recalling the rules and steps.

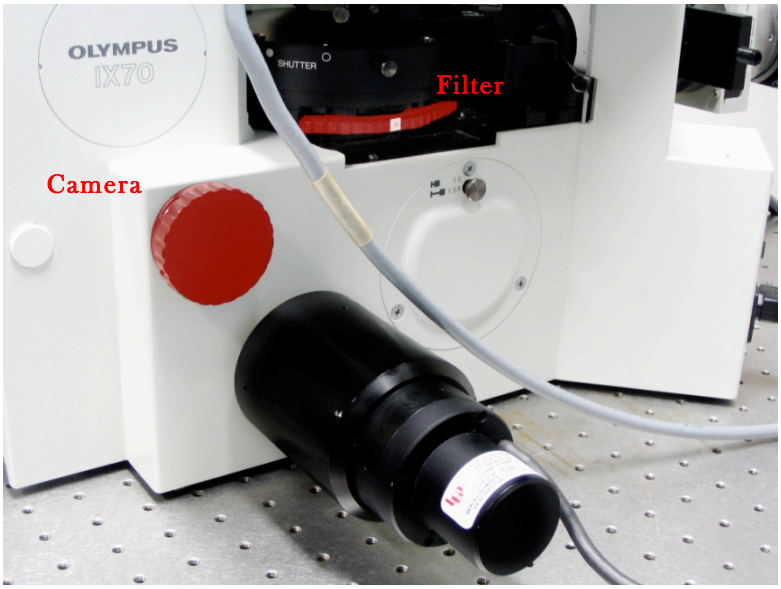
3) Microscope control and loading your sample

- **To load your slide, plate, or dish** and take care for the following issues:
  - Make sure that the stage adapter that correctly matches your sample fits securely and flush with the stage.
  - For **slides**:
    - Select and inspect each slide. If it is dirty, gently clean with a KimWipe and/or cotton swab. You should do this with all of your slides before you come.
- Use the stage movement joystick to position your specimen over the lens.

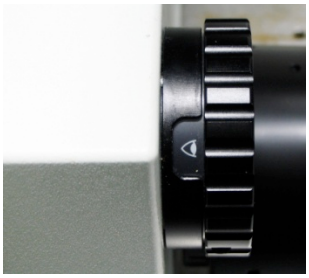
  - For faster movement, hold down button
  - Joystick for X & Y stage movement



☞ Set the microscope into viewing mode:



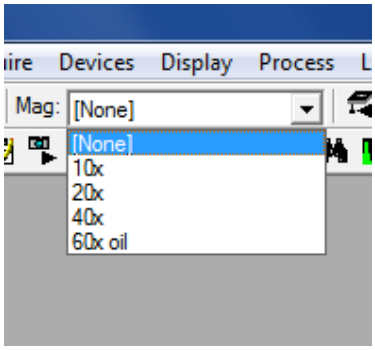
- Turn Camera dial: turn to 



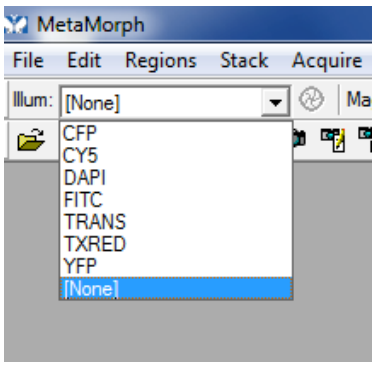
- Filter wheel: set to A

☞ Use the illumination control menu in the software to select your channel:

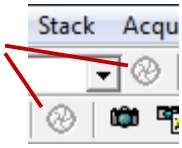
- Click the magnification drop down menu: Select your channel from this menu.



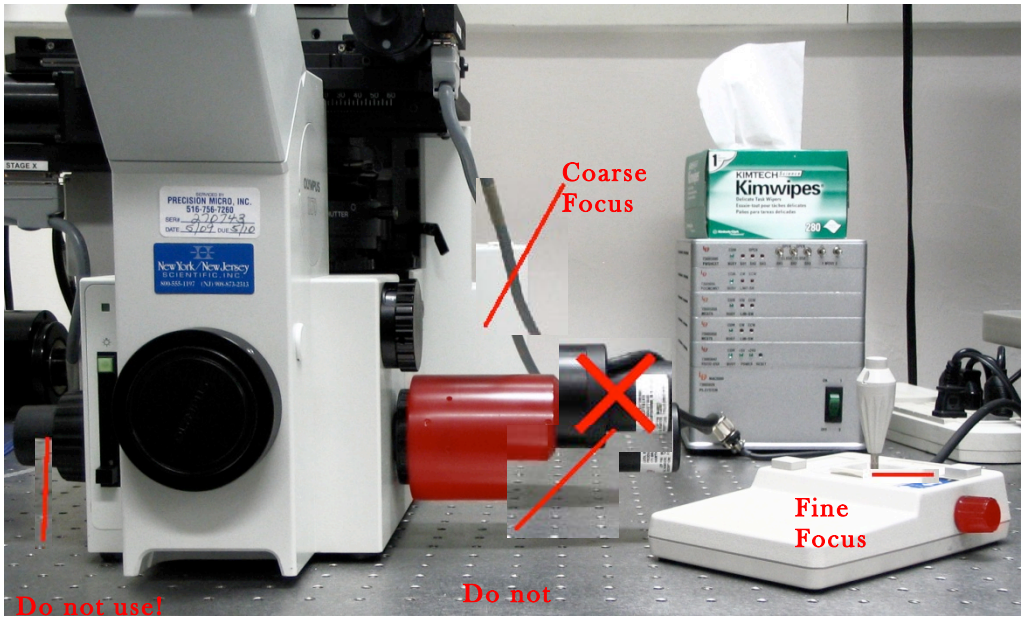
- Click the channel dropdown menu:



- Use this button to open and close the fluorescence light shutter:



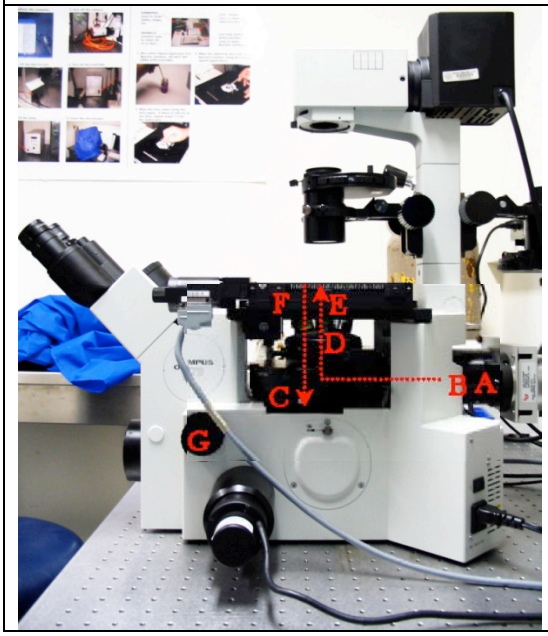
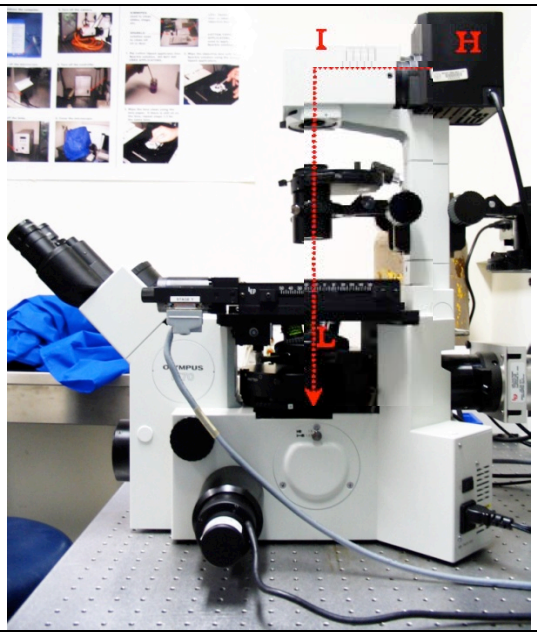
☞ Focus on your sample and center your region of interest using the following controls:



Take care not to use the focus knobs with X's.

☞ Check all of your fluorophores in your specimen. You need to have an idea of what to expect before you start your scan.

☞ Close the reflected light shutter to prevent photobleaching of your sample.

☞ Take note of the fluorescence light path: A. Fluorescence imaging light source B. Direction through microscope C. Filter D. Lens E. Stage and specimen F. Lens G. Eyepiece/Camera Dial	☞ Take note of the transmitted light path: H. Transmitted light imaging light source I. Direction through microscope J. Condenser K. Stage and Specimen L. Lens M. Filter N. Eyepiece/Camera Dial
	

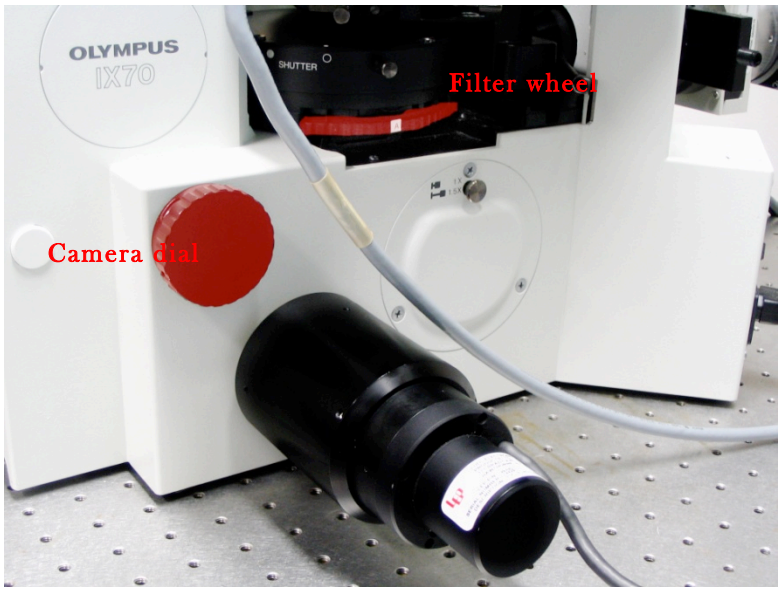
4) ***Your first image***

☞ Set the microscope into camera mode:

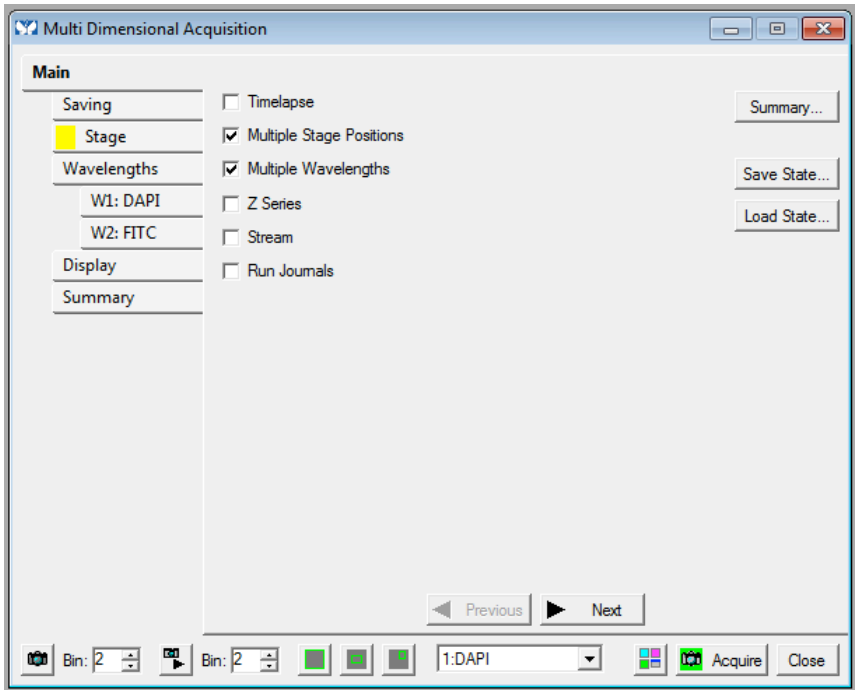
- Camera dial: turn to 

- Set the filter wheel to the position that best matches your experiment:

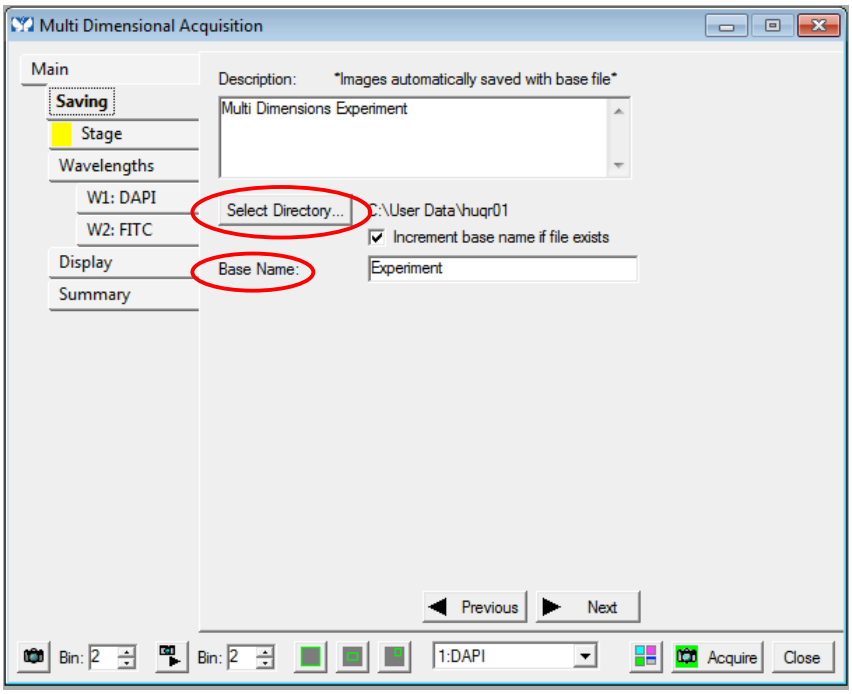
  B. DAPI, GFP, TxRed, Trans
  C. CFP, YFP
  D. GFP, TxRed, Cy5



☞ Use the Multi Dimensional Acquisition Menu:

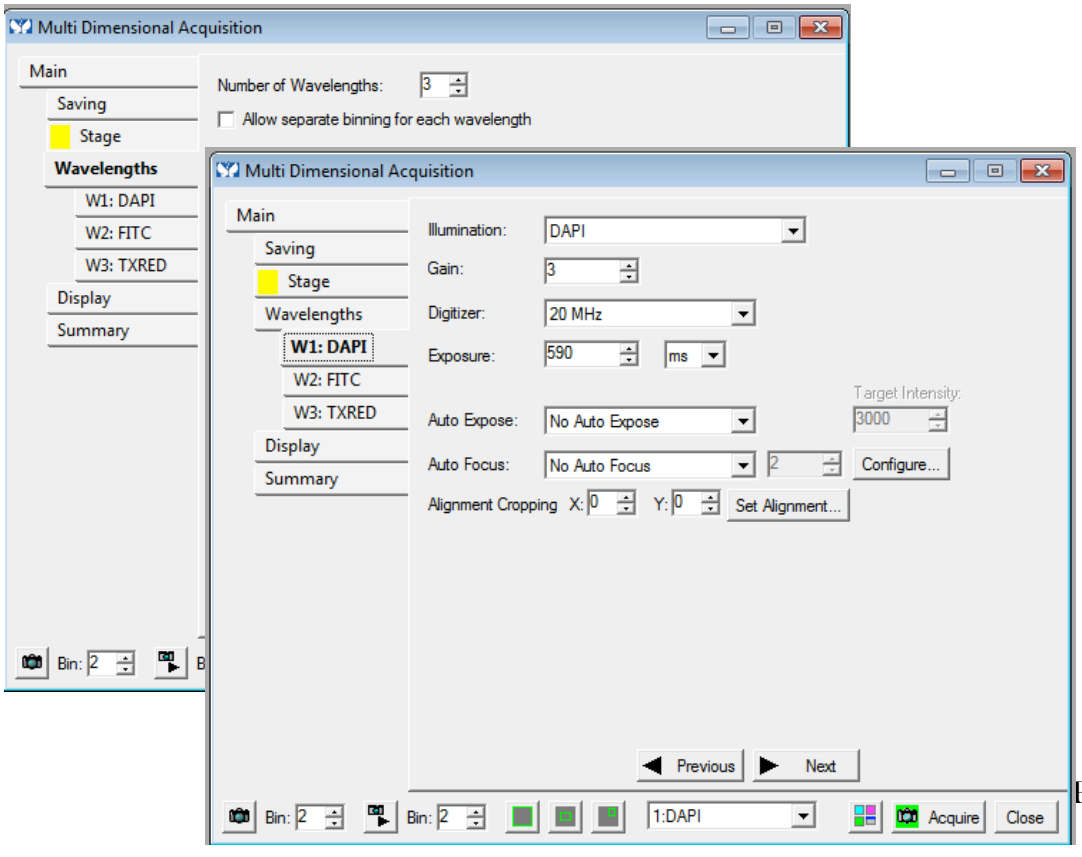


1. Click the **Saving** tab within the window.

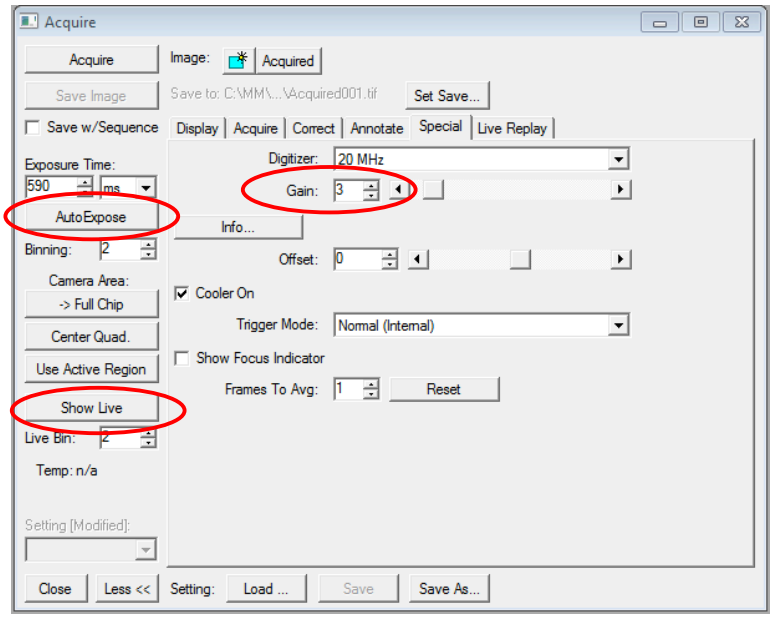


   a. Select you data folder in the Select Directory
   b. Create a base name

2. Click **Wavelengths** to set the number of wavelengths; set each wavelength illumination

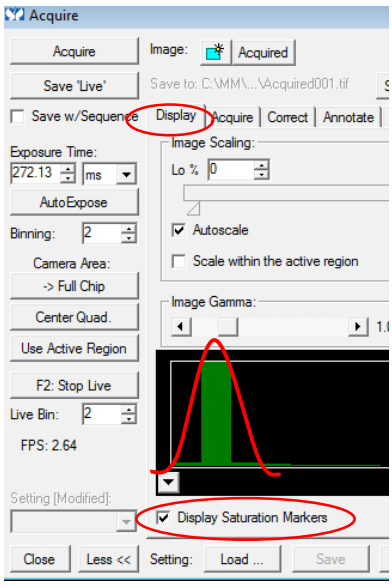


☞ Use the Acquire window to acquire your images:



- Make sure the gain is above 1; you can use a recommended starting point of 3

1. Select a channel from **Wavelengths**
2. Click **Show Live** to view the image.
3. Click **AutoExpose** to calculate an exposure time or calculate your own exposure time; note remember to check **Display Saturation Markers** in the **Display tab** to see the full range of intensity.
   a. Red pixels = saturation; Blue green pixels= black
   b. Use the histogram to help you find the best exposure:



4. Click **F2: Stop Live** after calculating the exposure.

- Repeat the above steps for each channel.

5) Returning to the microscope

To return to the microscope to find another field, and set the camera dial and filter wheel for viewing. Focus and center another region of interest.

You also can perform the following operations after returning to the microscope:

Using the oil immersion lens

If you need to switch to the 60x oil immersion lens, first use the course focus to drop the lenses.

Apply one drop of oil to the center of the lens.

Carefully raise the lenses and stop when you see oil contact between the lens and slide.

Focus & center your specimen. Please remember to use the fine focus control!

6) Multichannel Imaging

7) System Shutdown

Back up all your data.

If you used the 60x oil lens, please 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 the shutdown poster steps.