DSU Start-Up instructions

Always:

- start with the 10x objective
- properly center the stage around the current objective before changing to another objective
- when done, leave the 10x objective in standby position

To start:

1. Remove the dust cover and store neatly folded. Do not place it on the floor.
2. Sign the logbook - Record the mercury bulb hours
3. Start-up the system

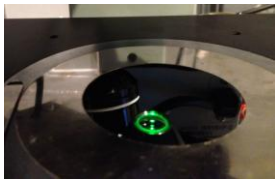
4. Turn on the mercury bulb (A)

Mercury bulb hours

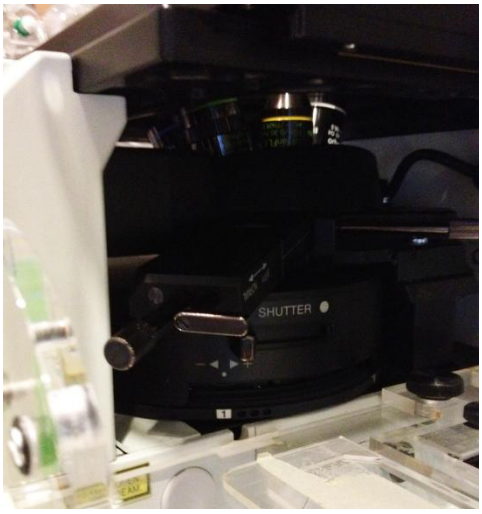
5. Turn on the 3 control boxes (B1,B2, B3)

6. Turn on the computer and monitor.
7. Double click the desired MetaMorph software icon (may take a few moments)
   a. Glass Only – is for imaging through Coverglass using the 60 or 100x Objectives
   b. Plastic Only – only engages the Long Working Distance Objectives (10x, 20x, 40x)
   c. Expert – allows you to move from Plastic Plates to Coverglass Objectives.
   d. Note: This might allow you to ram an objective into the stage. Be sure you are familiar with the system and focus your coverglass specimens at low magnification!

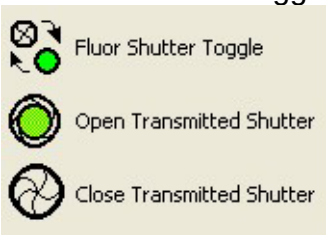
To begin, first focus your sample in the eyepieces:



1. Center the 10x Objective under the hole in the stage.
2. Center your sample on the stage and over the objective.
3. Select BF to Eyepieces or Fluorescence to Eyepieces
4. Rotate the appropriate filter cube using the manual filter turret.
    a. (Use the number code on the microscope.)



5. Hit Fluor Shutter Toggle or Open Transmitted Shutter for the appropriate light source.



6. Open the Shutter manually on the microscope if necessary.
7. If transmitted light is being used, Kohler the illumination.

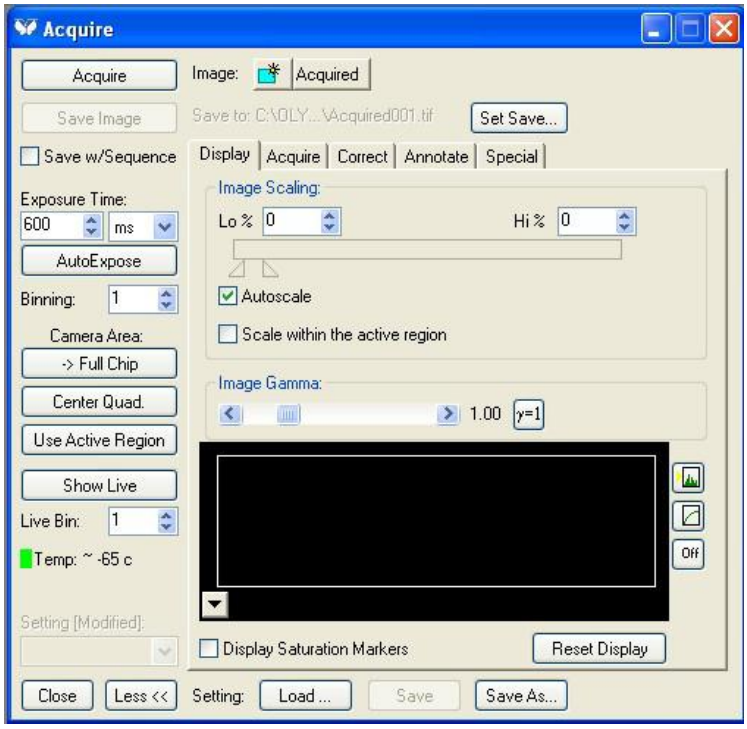
8. You should have light to the eyepieces
9. You may use the course or fine focus knob to bring your sample into focus.

To change objectives

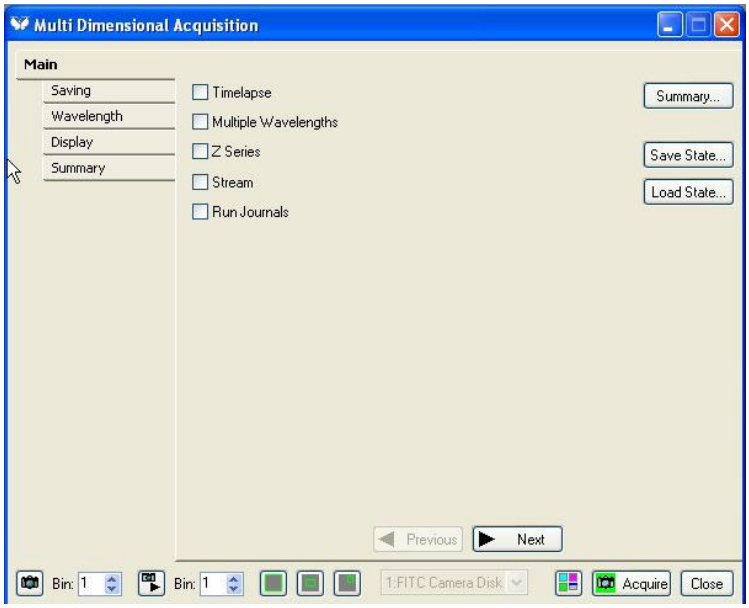
1. Toggle the shutter closed (whenever not viewing your sample, close the shutter to protect your sample from photobleaching)
2. Select the desired objective from the pull-down menu.
3. Open the shutter.
4. Re-focus your sample (as before) through the eyepieces.
5. If transmitted light is being used, Kohler the illumination.
6. Toggle the shutter closed.

Imaging Instructions

1. Rotate the manual filter turret to position #1 (DSU)
2. Click Open Camera Control

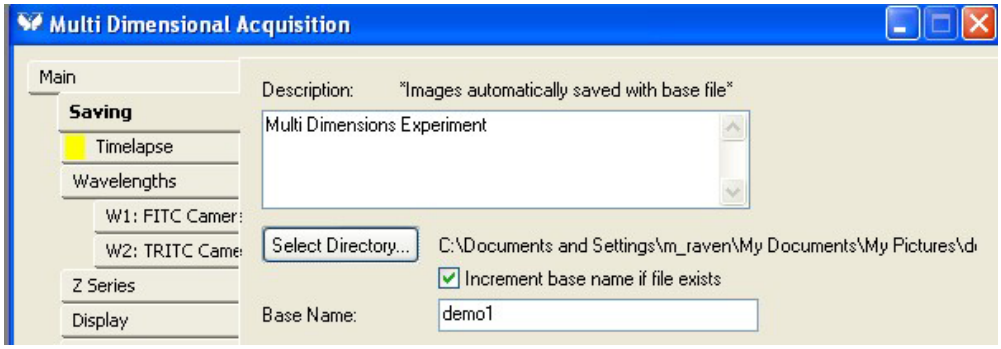


3. Click Experiment Manager

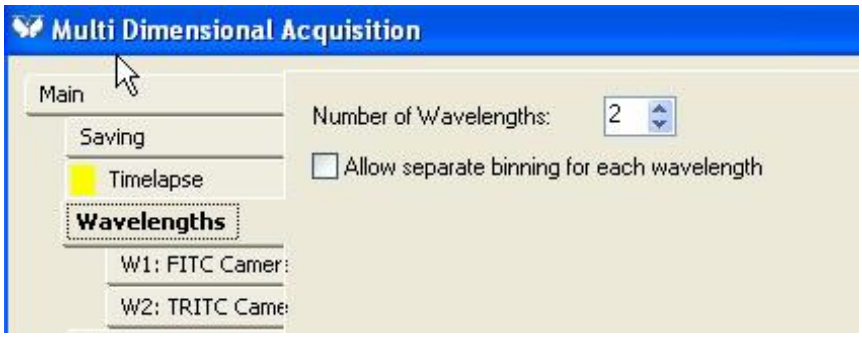


Work in the Experiment Manager:

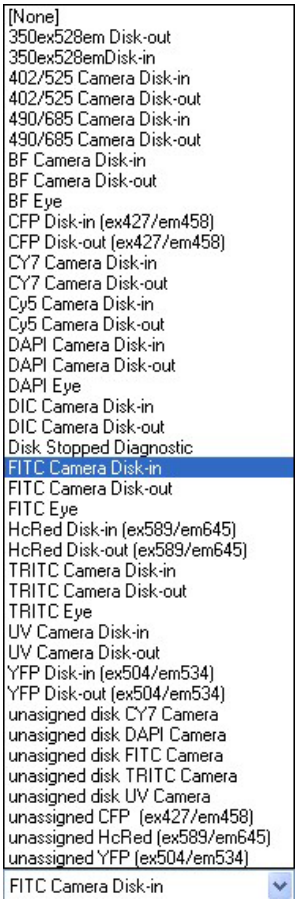
1. Select the Options that Apply
2. Work through the left-hand tabs under Main to set the acquisition parameters
3. Saving
    a. Add a description if desire
    b. Select the directory for the files
    c. Provide a base name



4. Wavelength
    a. Select the number of wavelengths (transmitted light is one)

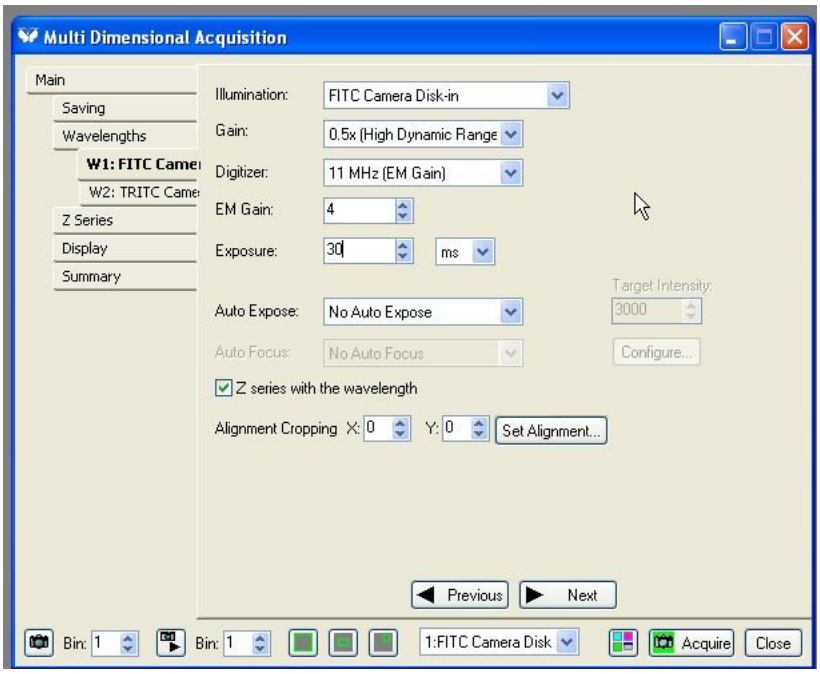


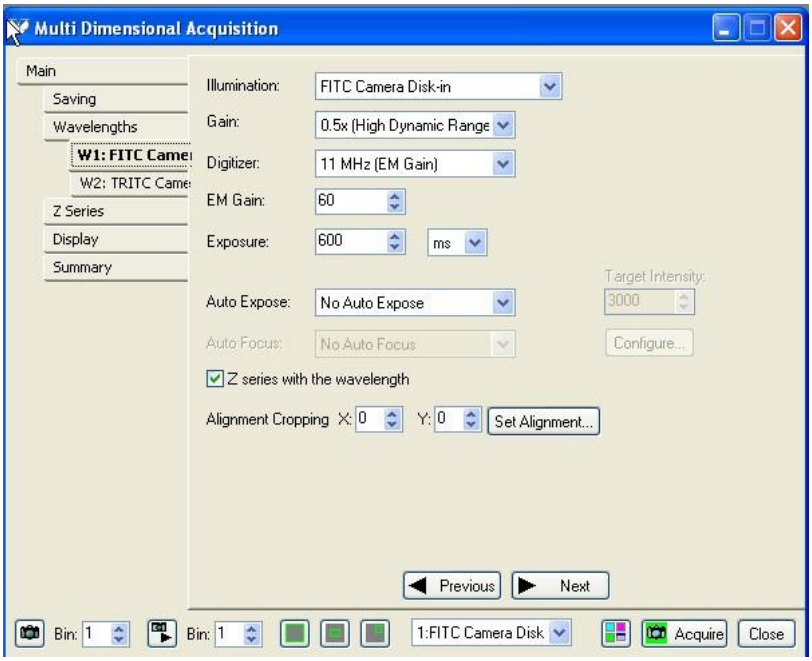
    b. Select the wavelengths
        i. Disk-out is regular epifluorescence
        ii. Disk-in is DSU confocal (much less light but less out of focus light)



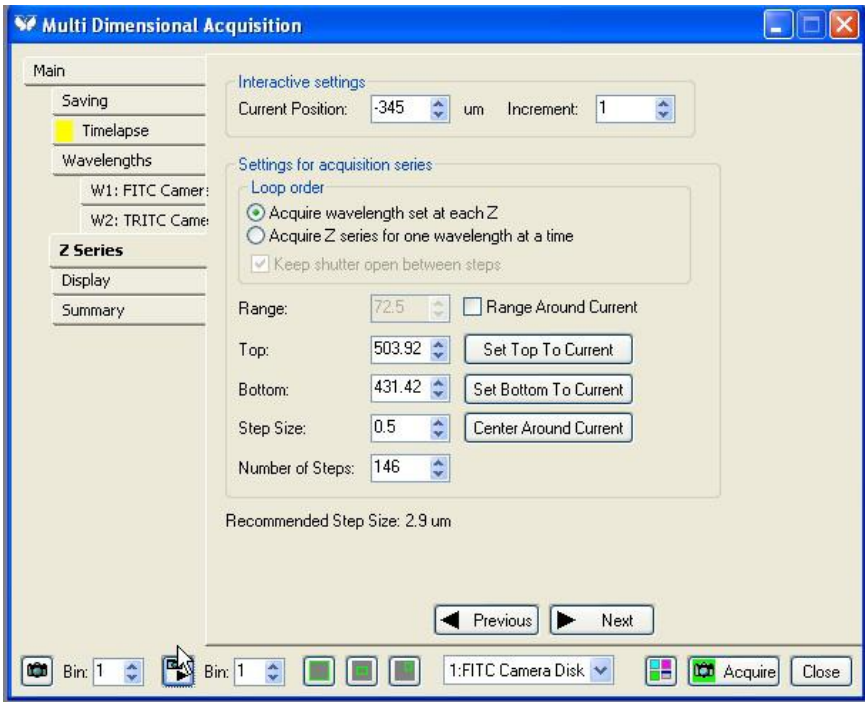
c. Adjust the Capture Parameters for each wavelength
   i. Gain for Fluorescence Usually 0.5x
   ii. Digitizer 11 MHz
   iii. EM Gain 4-1200
   iv. Exposure >30 ms

In general, you are adjusting the EM Gain And Exposure to capture images in the full 16 bit range

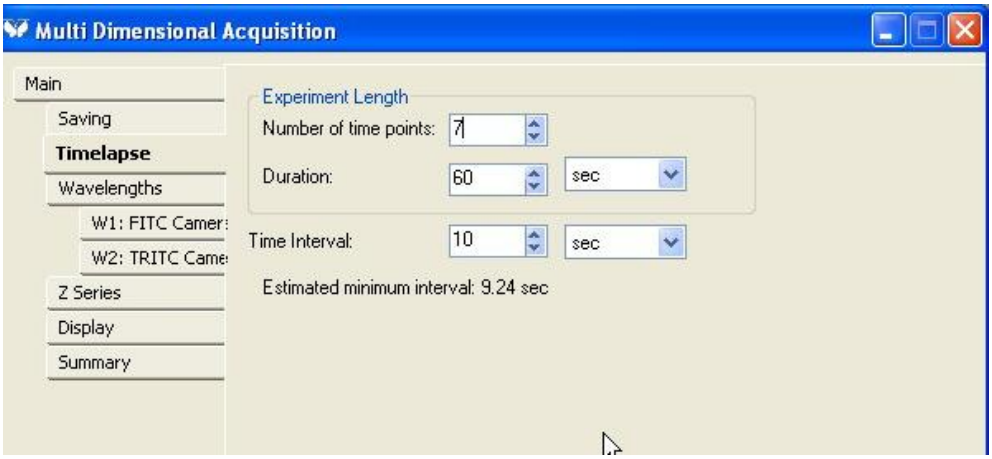




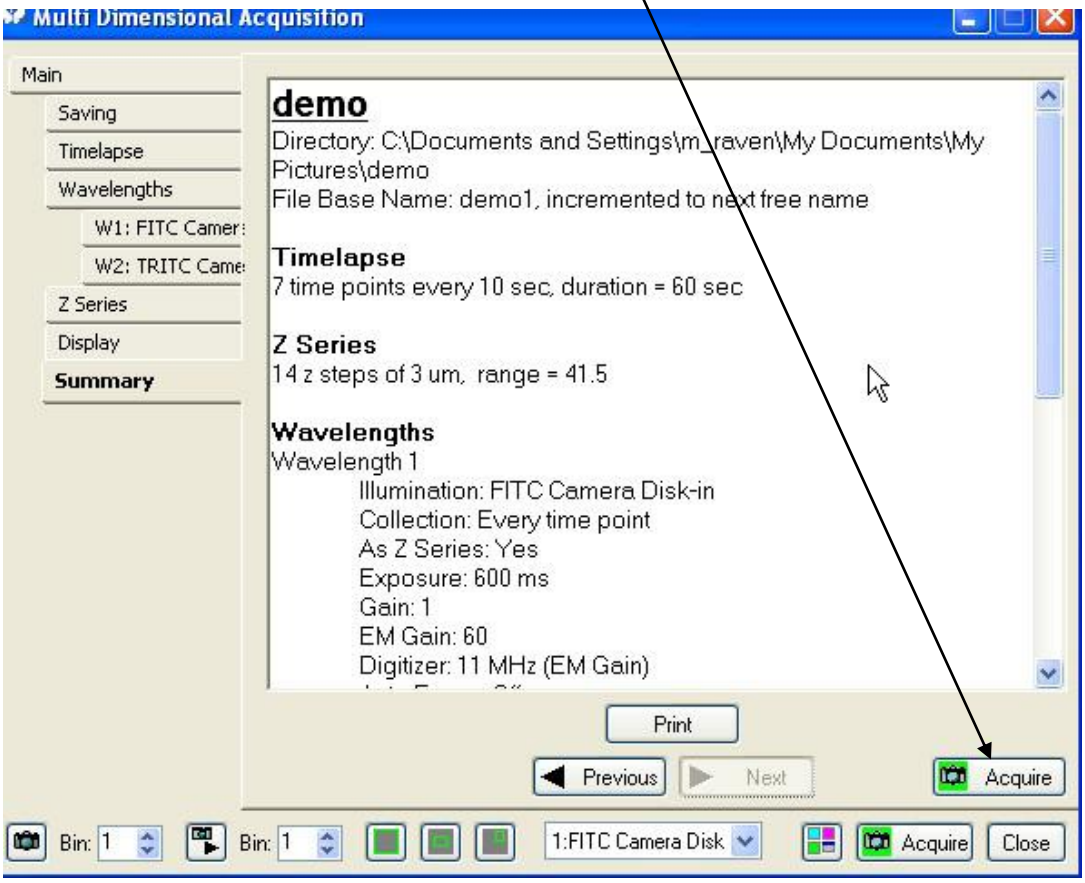
5. Z-Series
    a. Set Top (highest objective position)
    b. Set bottom (lowest objective position
    c. Set step size
    d. The number of steps is determined and constrained by Top, Bottom and Step Size



6. Timelapse
    a. The estimated minimum interval is determined by the imaging conditions (e.g. the time required to image the wavelengths (exposure), change filters and image multiple Z planes.
    b. Time Interval should be greater than the estimated minimum interval.
    c. Duration is the total experiment time
    d. Number of time points is constrained by the Duration and Time Interval.



7. Display and Summary
    a. Set the Display to default or custom
    b. Check the summary data and confirm all the settings are prepared.
    c. Click Acquire and the acquisition will begin



8. Select Save State to Save the settings for a future experiment. Save the state file to your My Documents Folder

Köhler illumination – Inverted Microscopes

In order to get the best image possible from brightfield, phase contrast, differential interference contrast, or polarization optical setups with the light microscope, it is crucial that the light path be set up properly. If the light path is set up properly, you will have the advantages of an evenly illuminated field, a bright image without glare and minimum heating of the specimen.

The following instructions apply to any microscope, upright or inverted which is equipped for transmitted light bright field illumination. Focusing of the field diaphragm as discussed here should be done for phase and differential interference optics as well.

1. **Light source**: Switch on the light source and make sure that light is coming through the field diaphragm. It may help to place a piece of paper over the field stop to see the light.

2. **Condenser lens:** Place the condenser lens all the way down toward the stage.

3. **Objective Lens:** Place your specimen on the stage and turn the nosepiece (which holds the objective lenses) to the 10X power objective lens. Make sure the top piece is in place.

4. **Other Optics:** Be certain Phase or other optics (fluorescent filter cubes) are out of the light path.

5. **Focus:** Now bring your specimen into focus with the coarse and fine focusing knobs. The best way to do this is to rack the lens as close possible to the specimen watching the objective lens all the time (and NOT looking into the oculars) to make sure that the lens does not run into the slide. Then rack the lens away from the stage (or vice versa) while looking through the oculars to bring the specimen into focus (details are as sharp as they can be).

6. **Field Diaphragm:**
    a. Close down the field diaphragm to the smallest spot
    b. Use the condenser focusing knob to bring the spot into focus such that you can clearly see the leaves of the diaphragm
    c. Use the centering knobs to bring the field into rough centering.
    d. Open up the diaphragm to almost fill the field. This is the best time to do the final critical centering.
    e. Open the diaphragm to just fill the field.

7. **Condenser Diaphragm:** controls the cone of light. Plays resolution versus contrast. Full open results in flaring, closing it down improves contrast and if closed too much you see the fringe.
    a. Remove the oculars and look down the viewing tube. Open the condenser diaphragm all the way up. Close it down about 3/4.
    b. Put the oculars back in.

8. Your specimen should be properly illuminated and should give you a great image. If it does not, check to make sure your lenses and other optical components are clean. Then, recheck to see that you have followed each step properly.

Repeat these steps for different objective lenses.

DSU Shut-Down instructions

A. Wipe the oil off and oil objectives you used and return the 10x objective (use the software) to standby position
B. Confirm that Nobody is using the system within an hour or less. Leave the system on if they are.
C. Save what you need to the \\microscopy-nas1 or transfer but other methods via the internet or USB.
D. Close the software
E. Shut down
    a. the computer and monitor (C)
    b. 3 boxes (B1, B2, B3)
    c. LASTLY the mercury bulb (A)
F. Record the final mercury bulb time and finish signing the log book
G. Cover the A-B with the dust cover

To look at your data use the Review Multi Dimensional Data Set Utility

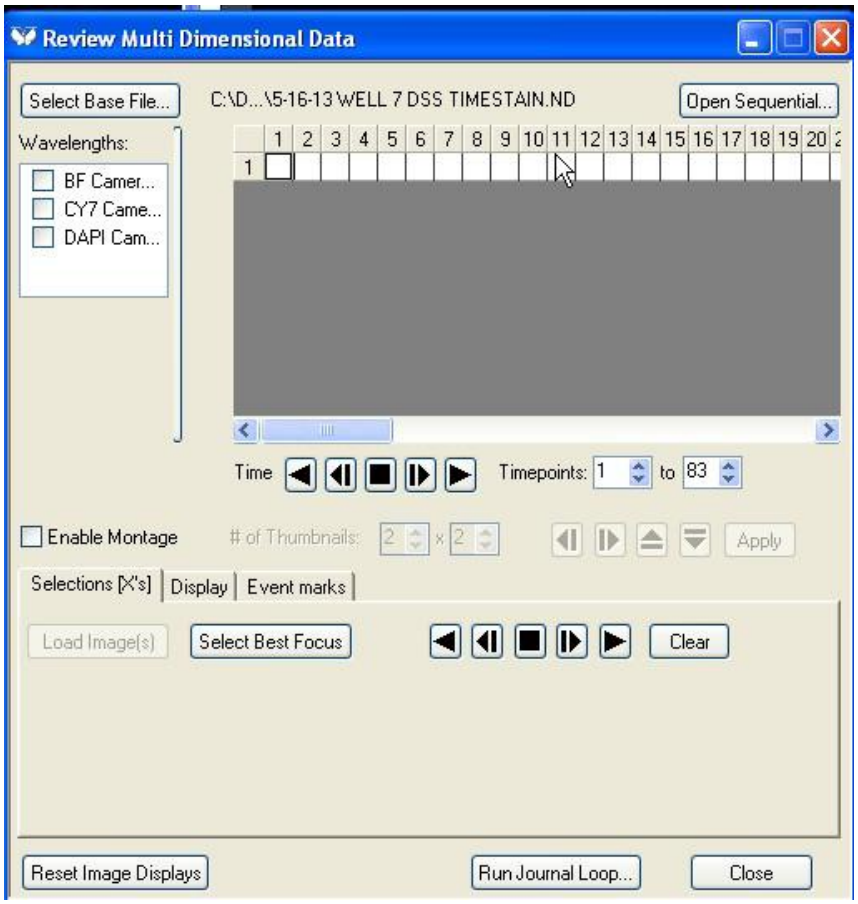
Apps

Review Multi Dimensional Data

Window Opens

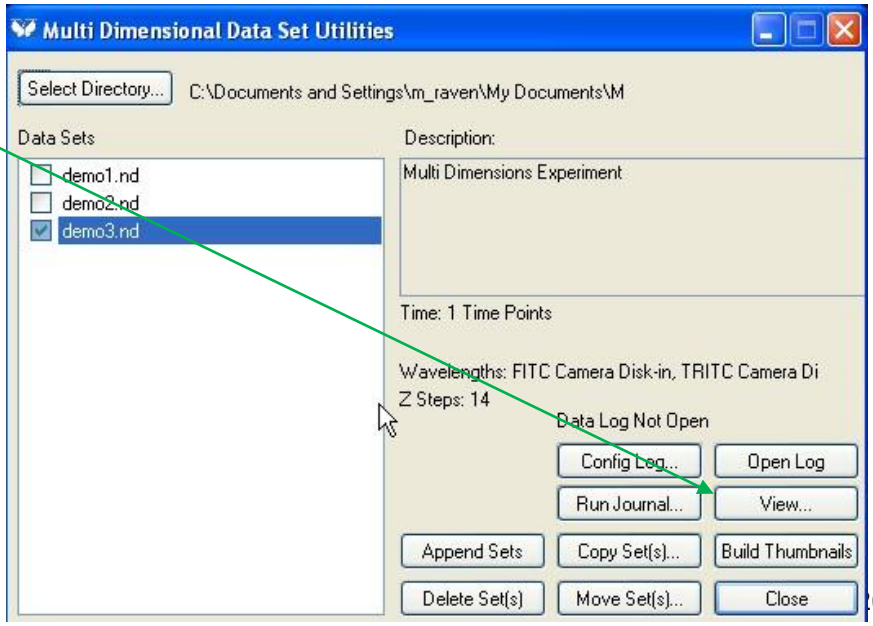
Select Base File

Find your folder



Select the Desired Base File

Click View

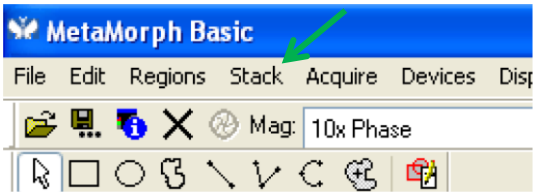


Other MetaMorph Operations

Removing Planes

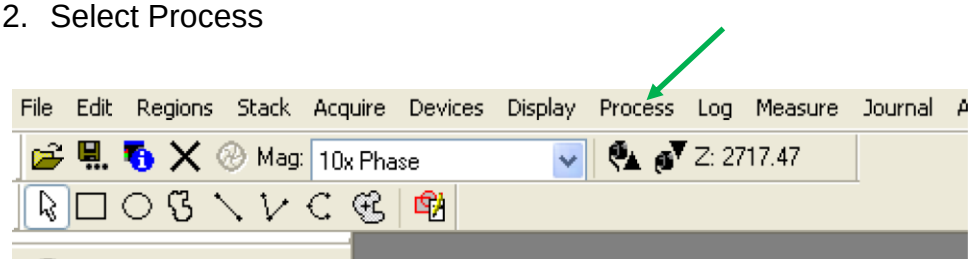
To remove out-of-focus planes:

1. Open stack and determine which planes to start and end with
2. Select Stack
3. Select Remove Plane

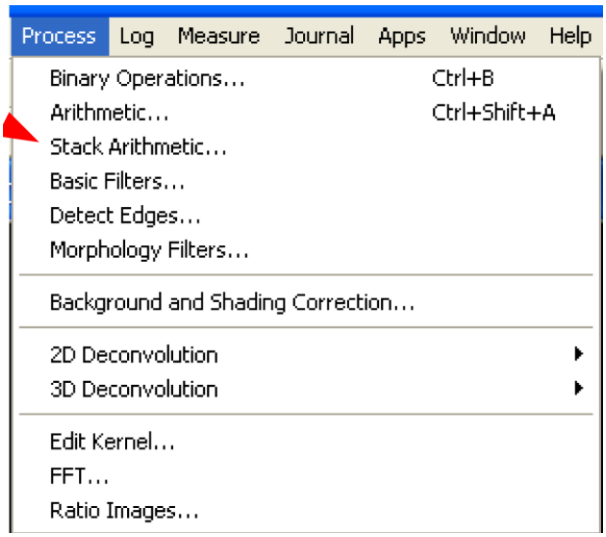


Projection Images:

1. Open a stack file
2. Select Process

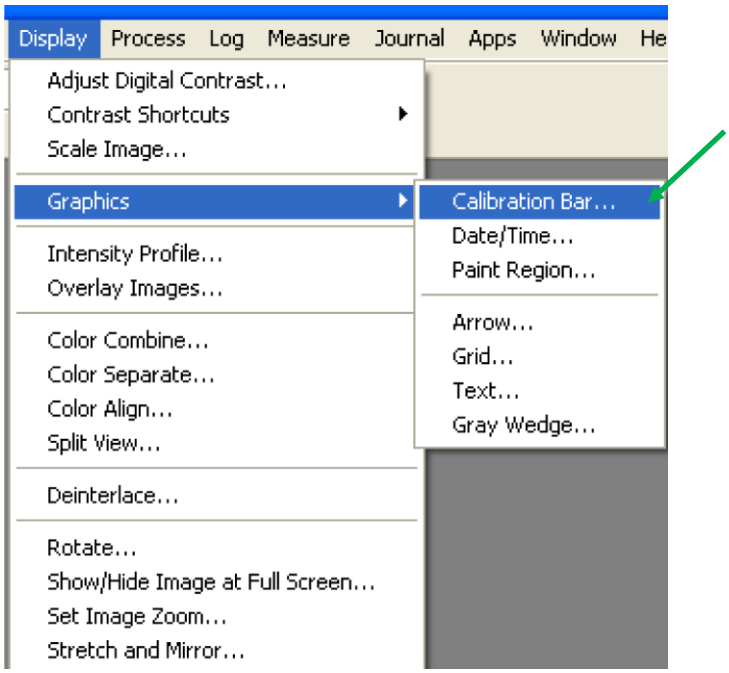


3. Select Stack Arithmetic



4. Select a method of combination – Average and Maximum are common

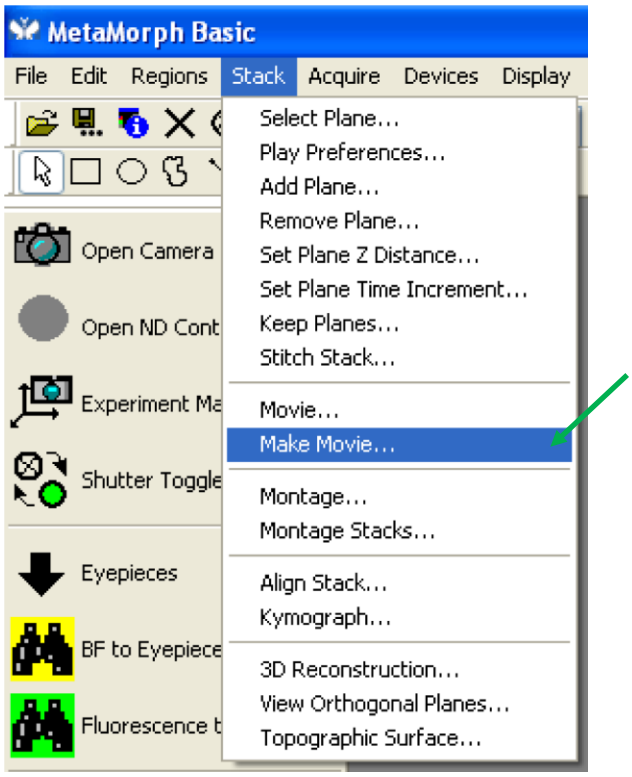
To create a scale bar:



To create a movie:

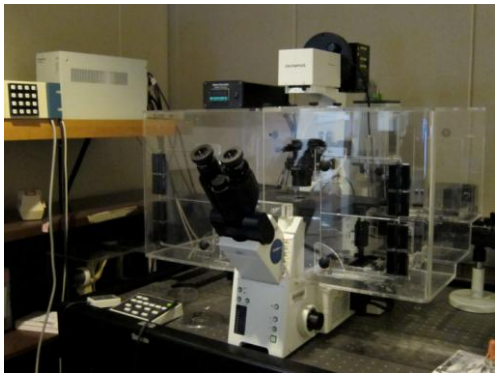
Select Stack

Make Movie



Olympus DSU (Spinning Disk Confocal)

The DSU in this facility is on an inverted microscope base with a manual X, Y stage. The system is configured with phase optics and mercury arc lamp illumination. MetaMorph software controls the camera and microscope allowing for long-term imaging. The microscope is enclosed to allow warming to 37°C and has humidified 5% CO2 availble. The stage and samples can be cooled below the ambient temperature in the room (25°C) by special arrangement.



Objectives	**Mag/N.A.**		
UPlanAFLN	10x/0.30	Ph1	∞/-/FN 26.5
LUCPlanFLN	20x/0.45	Ph1	∞/0-2 FN22
LUCPlanFLN	40x/0.60	Ph2	∞/0-2 FN22
UPlanFl	40x/0.75	DIC	∞/0.17/
PlanApoN	60x/1.42	Oil	∞/0.17/FN26.5
UPlanSApo	100x/1.4	Oil	∞/0.17/FN26.5

Camera

Hamamatsu ImagEM CCD Camera (C9100-13)

The ImagEM camera is a back-thinned electron multiplier CCD camera with a readout rate of 32 frames per second at full spatial resolution and 16 bit digitization.

Imaging Filters

Switching between sets (Sedat, Pinkel, Cy5, and Brightfield) isn't fast because the sets use different dichroic mirrors and moving those is slow but within in sets is fast.

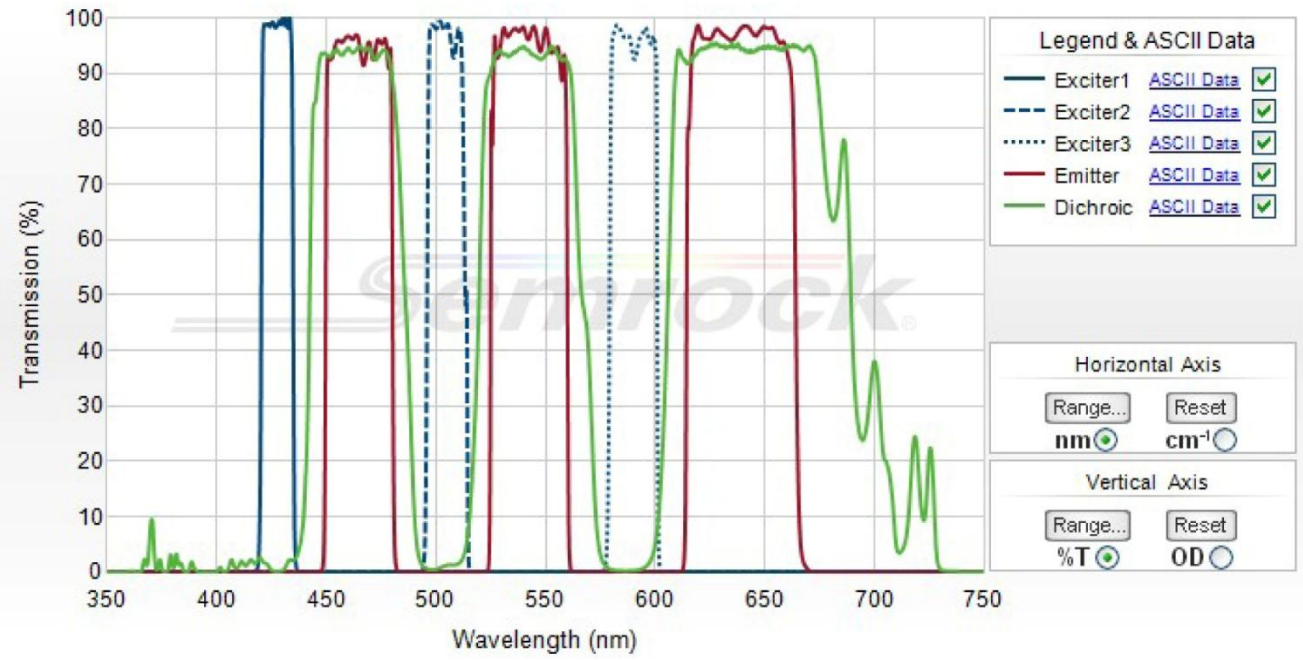
89000 Sedat Quad Filter Set

Excitation		**Beam splitter**		**Emission**	
AT350/50x	EX	89100bs	BS	ET455/50m	EM
ET402/15x	EX			ET525/36m	EM
ET490/20x	EX			ET605/52m	EM
ET555/25x	EX			ET705/72m	EM
ET645/30x	EX				

Cy5 Cube

Semrock Pinkel Set for CFP/YFP/HcRed

Excitation	**Beamsplitter (triple band**	**Triple Band Emission Filter**
427/10	diachronic)	**Center** wavelength (band)
504/12	444/521/608	464 (31)/542(35)/639(50)
589/12		


Transmission (%)
100
90
80
70
60
50
40
30
20
10
0
350
400
450
500
550
600
650
700
750
Wavelength (nm)
Legend & ASCII Data
Exciter1 ASCII Data
Exciter2 ASCII Data
Exciter3 ASCII Data
Emitter ASCII Data
Dichroic ASCII Data
Horizontal Axis
Range...
Reset
nm
cm-1
Vertical Axis
Range...
Reset
%T
OD