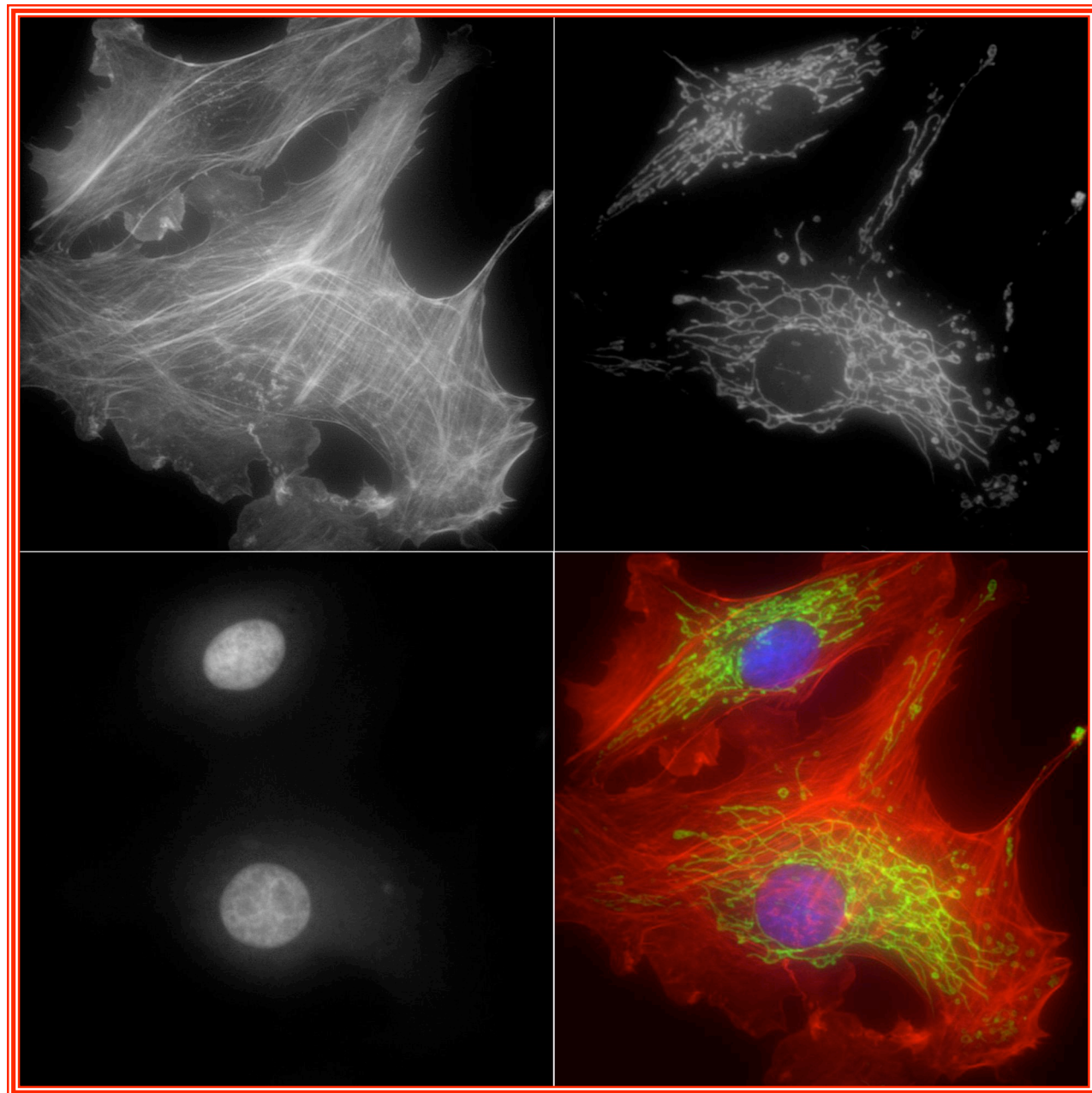


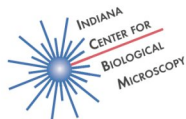
**Widefield-NikonEclipseTE200-ORCA**  
**Nikon Eclipse TE200 Inverted Microscope**  
**with Hamamatsu 1394 Orca-ER Cooled CCD Camera**  
**and Micromanager Software**



September 2007

Check website for most current User Guide

<http://www.nephrology.iupui.edu/Imaging/Guides/WF-NikonEclipseTE200-ORCA.pdf>



This system is equipped with the highly sensitive Hamamatsu Orca-ER camera which is ideal for detection of fluorescence in live samples and in samples that have low signal levels. Illumination is provided by a 100 W Mercury Arc lamp. This high performance widefield microscope system is also equipped with Nomarski Differential Interference Contrast optics. The inverted microscope stand accommodates slides or culture dishes. Best results are obtained using thin samples, such as well spread single cells.

Stage warmers are available if needed for live imaging. If needed, please sign up for the stage warmer when you make your microscope reservation.

Objectives currently available:

40x Air ELWD Plan Fluor 0.60NA DIC

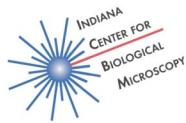
40x Oil S Fluor 1.3NA

60x Oil Plan Apo 1.4NA DIC (this objective is not kept on the scope and must be checked out)

Excitation/emission filters currently available for imaging:

	Excitation	Emission
CFP	436/10	470/30
DAPI	360/40	457/50
FITC	490/20	528/38
RHOD	555/28	617/73
CY5	640/20	685/40

Z-series acquisition is not available at this time. YFP is not available at this time.



## **System Startup** (*see illustrations starting on page 7*)

Turn on Mercury Arc Lamp (first on – last off)

Turn on Red Rocker Switch (it supplies power to these devices)

- Hamamatsu ORCA control box

- Tungsten light

- Uniblitz shutter controllers

- HP-Vectra PC - controls stage, EX/EM filters, NC filters

- Nanomotion 2 device controller

Turn on HP-Vectra PC (*under table, open large metal door*)

- Lower objective and remove stage insert

- Initialize stage, following instructions on Vectra PC screen

Log in on Dell Optiplex PC

- Start ImageJ-Micromanager

- Startup configuration: Choose config then press OK.

- Use this config: MMConfig\_ORCA\_Uniblitz2\_TimeSeries

## **Imaging setup**

Dichroic mirror

- Located under objective turret. Select correct mirror using slider.

Neutral density filters

- Change using ND buttons on keypad

- Current filter is displayed on the Vectra PC screen

Excitation/Emission filters

- Change using EX buttons on keypad

- Current filter is displayed on the Vectra PC screen

Camera settings

- Exposure time –

- for DIC 5-10 ms

- for fluorescence, begin with 100 ms and increase as needed (max 2000 ms)

- Use short exposure times to find cells and focus, then increase exposure time to use more of the dynamic range of the camera for the final image.

- Pixel type

- choose 16 bit (camera is 12 bit, but images are saved in 16 bit format)

- Binning

- Binning = 1 image is 1024x1024 pixels

- Binning = 2 image is 512x512 pixels

- Binning = 4 image is 256x256 pixels

- Use binning of 2 or 4 if you have a dim sample or if you want to minimize exposure time for a live sample.

- Gain – leave at 0

- Auto shutter – on

## **Viewing through eyepiece**

- Send image to ocular by setting lever on right side of scope toward the user.

- Under configuration presents, click on the blank box beside the box labeled Channel.



From dropdown menu choose desired mode, Brightfield or Fluorescence. For brightfield illumination it may also be necessary to press the square button on the left of the scope body.

Press “Live” to open shutter. Press “Stop” to close shutter.

Find cells through eyepiece.

Align Koeler illumination if you plan to use DIC

(If you don’t know how, please ask for help. This simple step will greatly improve your DIC images.)

Do not switch between brightfield and fluorescence illumination without first pressing “Stop”. The software may stop responding correctly if you do this and no longer correctly control the shutters. The solution if you do make this error is to exit then restart the software.

## **Image Acquisition**

Send image to camera by setting lever on right side of scope away from the user.

Choose desired mode, Brightfield or Fluorescence.

Press “Live” to find and focus.

Use short exposure time to reduce lag while focusing and reduce photobleaching.

If image was in focus in eyepiece, rotate fine focus slowly counterclockwise to find focus for camera.

Press “Stop” when you have field in focus.

Look at the Min and Max pixel intensity.

Using a CCD camera for image acquisition, there are two main imaging parameters that you can change, intensity of excitation light (via changing neutral density filters) and exposure time. If your sample is still too dim, you can also try binning.

Change neutral density filter and/or exposure time to use more of the dynamic range of the camera.. The CCD camera on this system is 12 bit, so you have 4096 grey levels to work within. Avoid saturation (ie: grey levels  $\geq 4096$ ).

This camera has a maximum exposure time of 2000 ms.

Press “Snap” to acquire image.

Press “Save As” button at the bottom of the image acquisition window.

Save in Users/YourFolder

Repeat for additional channels.

## **Time Lapse Image Acquisition**

The system can perform time lapse image collection in a single plane. Acquisition can include both brightfield and one fluorescence channel. The current setup does not support time lapse of more than one fluorescence channel at a time.

Begin by determining optimal imaging parameters in single image mode (exposure time and neutral density filter).

Press “Acquisition” to open the Multi-dimensional Acquisition dialogue box.

In the Channel group box press “New”. A configuration will appear. Choose Brightfield or Fluorescence. Repeat if you want to use both brightfield and fluorescence. The

color for the channel is red by default. Double click on the rectangular red area and then choose the desired color in the color swatch box. Choose white for brightfield.

Set the exposure time in the multidimensional Acquisition dialogue box to the optimal time for your sample.

Set the exposure time by highlighting the time within the channel configuration and typing in the new exposure time.

Uncheck the use slice settings box. Slices (Z) is not yet active. The stage z-motor is not currently linked to the acquisition software.

Uncheck the save files to acquisition directory box. Set the directory path to your User folder. Set the name prefix. A new folder with this prefix will automatically be made within your user folder for each time lapse sequence. **Give each sequence a new prefix or your previous sequence may be overwritten.**

In the Frames (time) box, set the desired number of repetitions and the interval. At interval equals zero, acquisition rates in frames per second are shown in the table below.

Press “Acquire!” to begin the time lapse acquisition. Press the X in the red Stop Sign icon at the bottom of the time lapse acquisition window to abort acquisition.

Review the time lapse sequence by pressing the play/stop icon at the bottom of the acquisition window.

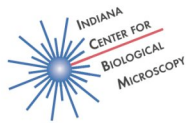
Press the floppy disc icon at the bottom left of the time lapse acquisition window to save the time lapse sequence. Save in your User folder. Time lapse is saved as a series of tif images.

Image size	Exposure time	Frames per second
1024x1024 pixels	10 ms	4.5
	30 ms	4.4
	50 ms	3.9
	100 ms	3.4
512x512 pixels	10 ms	8.0
	30 ms	7.15
	50 ms	6.32
	100 ms	5.0
256x256 pixels	10 ms	6.25
	30 ms	5.80
	50 ms	5.3
	100 ms	4.37

This table gives approximate acquisition speed in frames per second for a range of exposure times with and without binning when interval is set to zero. Acquisition is fastest at 512x512, binning = 2.

## Image Archiving

At the end of your imaging session, go to in-neph-toast. Move your images from your user folder on in-neph-Orca (the PC running the MicroManager software) to your user folder on in-neph-toast. In-neph-toast has the fastest CD and DVD burner in the facility and is dedicated for CD and DVD burning only. Burn 2 copies. Blank CDs and DVDs are cheaper than the time and materials needed to repeat your experiment. After you have spot checked whether the files on your CDs or DVDs are readable, PLEASE DELETE THEM from both in-neph-Orca and in-



neph-toast. This is your data and you are responsible for its storage and preservation. The ICBM does not make backups of user data and does not provide long term storage of user data.

## **System Shutdown**

Remove all samples and if an oil objective was used, clean oil from objective. If you do not know how to do this safely, ask for help.

Press ESC key to exit program on HP Vectra PC, then choose File:Exit.

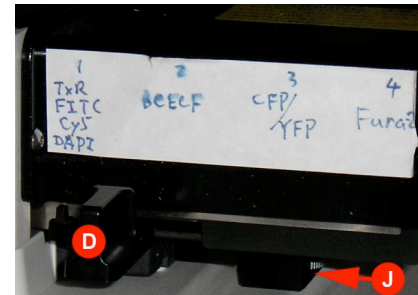
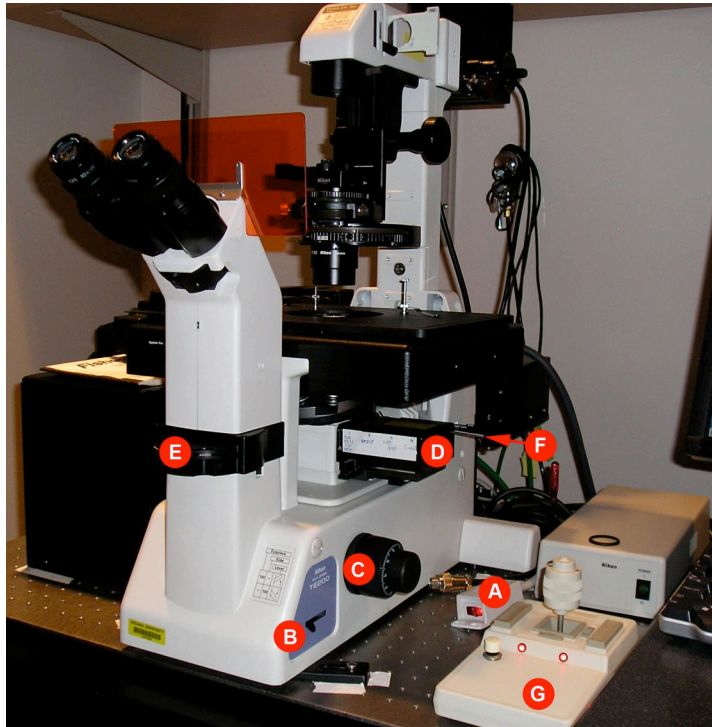
Turn off Red Rocker Switch

Turn off Mercury Arc lamp

Place dust cover on microscope, taking care not to bump or move any microscope adjustments.



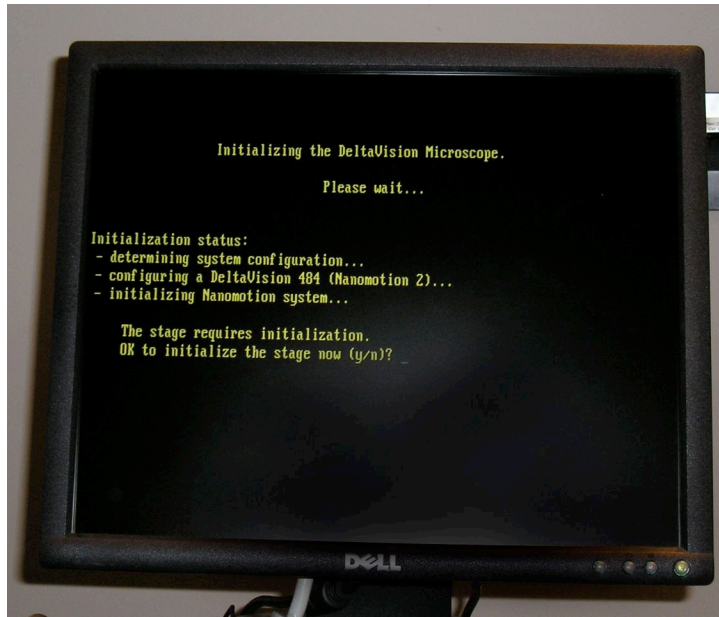
## Appendix – Microscope System and Screenshots Nikon Eclipse TE200 Inverted Microscope



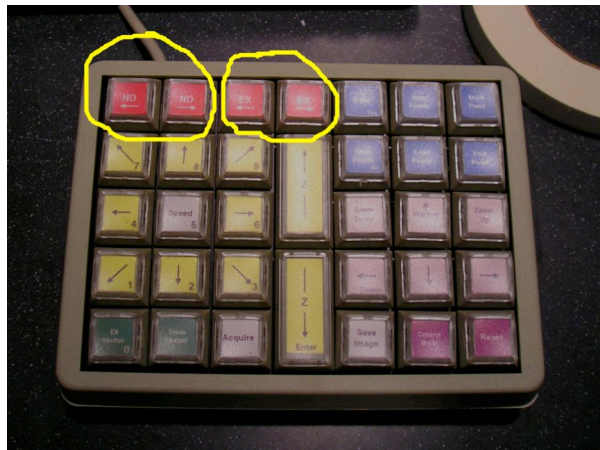
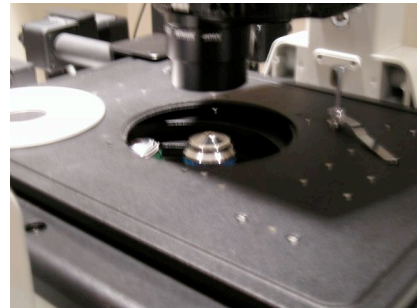
- A) Red Rocker Switch: turns most of system on/off
- B) Switch between sending image to eyepiece (toward user) or to camera (away from user).
- C) Focus – raise, lower objective
- D) Dichroic slider mechanism.
- E) Emission filters for eyepiece.
- F) Manual block for Mercury illumination path. Push left – path open. Pull right – path closed.
- G) Joystick – control x,y,z position of stage
- H) On button for HP-Vectra PC
- I) UniBlitz shutter control box (one is shown, there are two)
- J) Analyzer for DIC imaging. In position shown. May pull to out position for fluorescence. Do not remove!



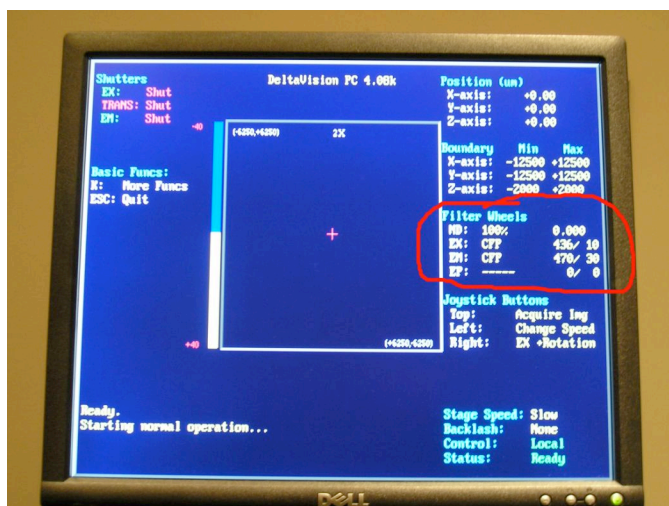
## HP Vectra PC – controls excitation/emission and stage motion



To initialize the stage type “y”.  
Be sure to remove the stage insert and put the objective into its lowest position before initializing the stage.



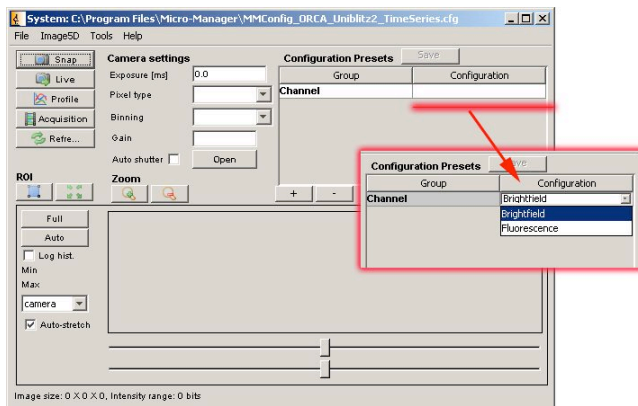
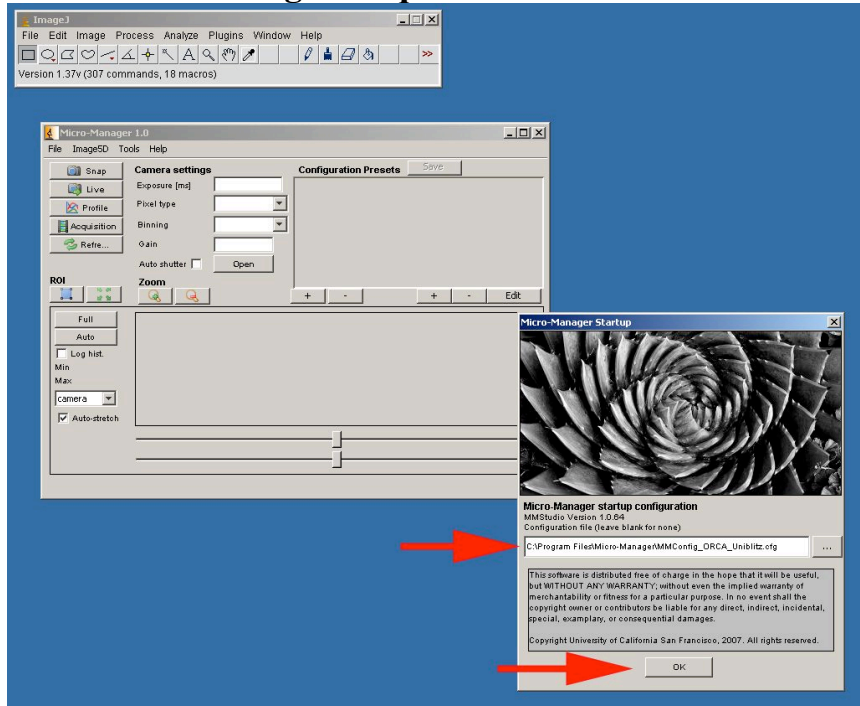
Use keypad controls to change  
Neutral density filters – ND  
Excitation/emission filters - EX



Current filter positions are shown on the HP Vectra PC screen.



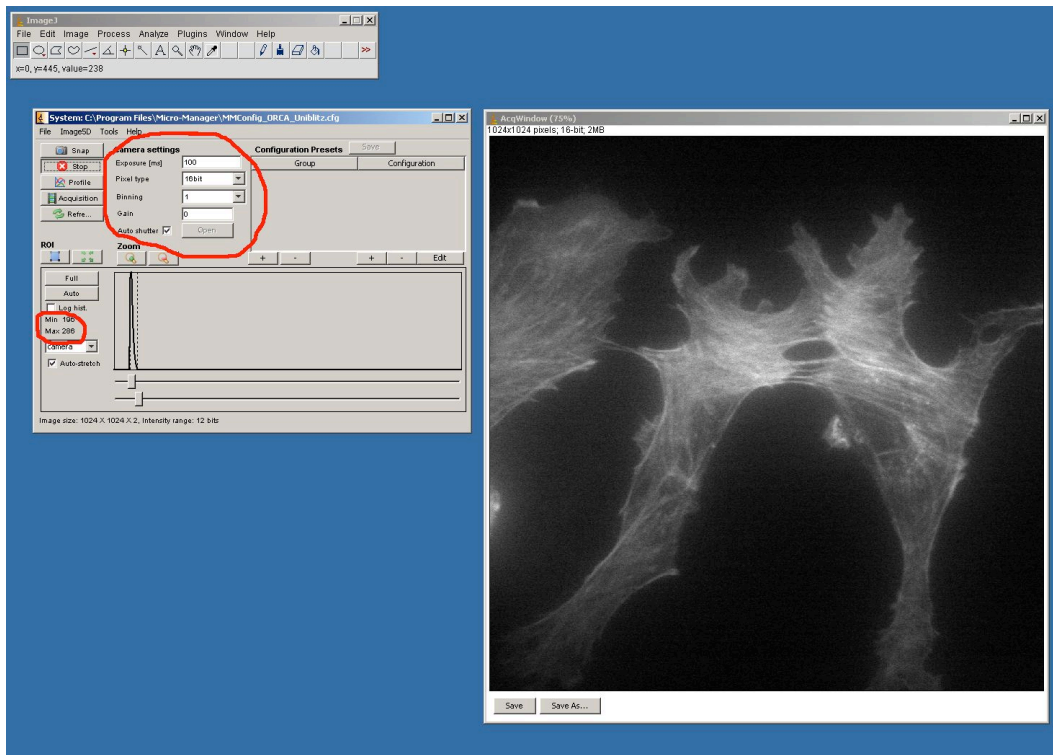
## Micro-Manager Acquisition Software – controls camera and shutters



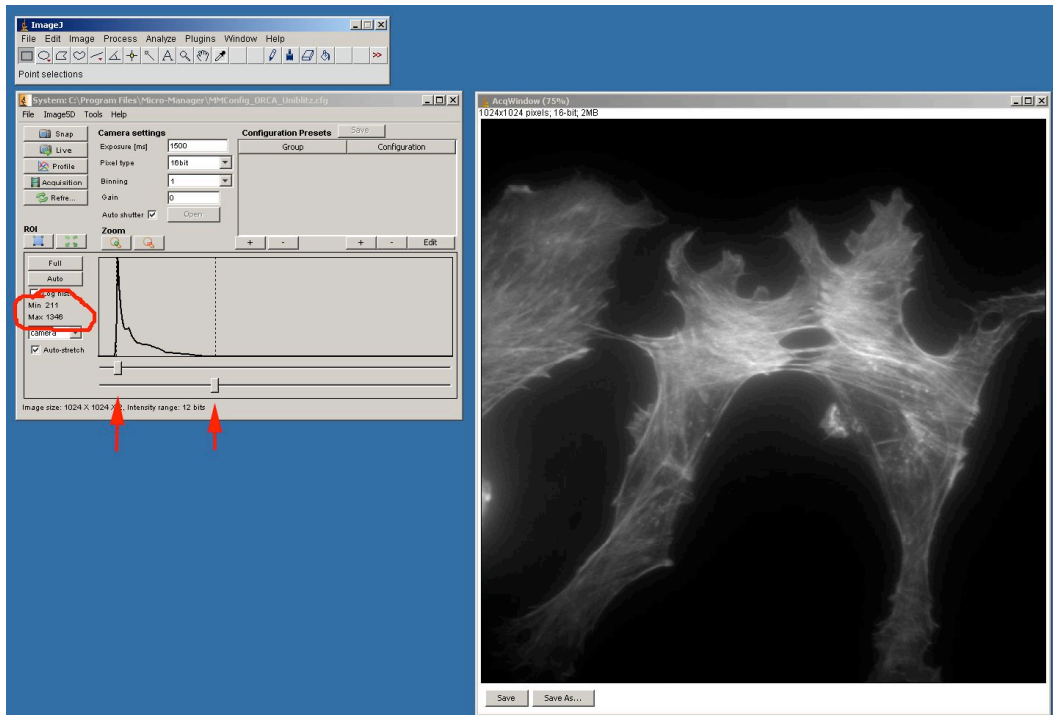
Choose Brightfield for tungsten light.  
Choose Fluorescence for Mercury Arc light.

Press “Live” to open shutter.  
Press “Stop” to close shutter.

Shutter should be closed when changing between Brightfield and Fluorescence settings.

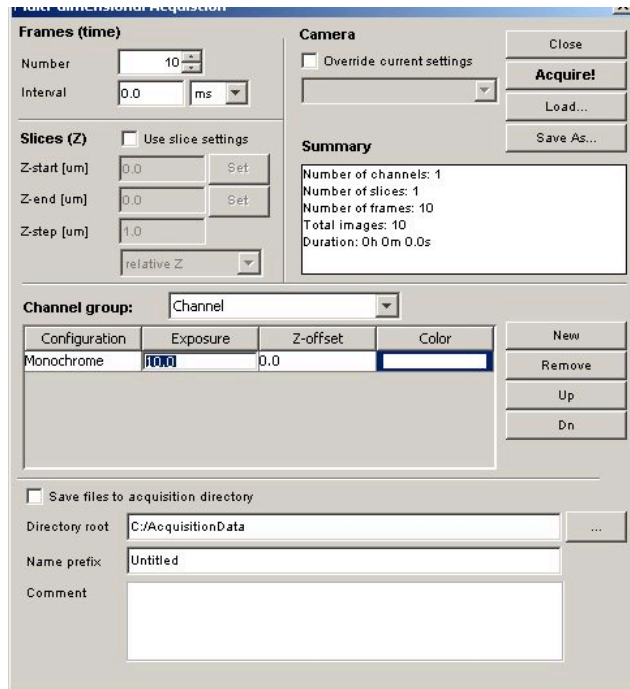


Use short exposure time for finding field and focusing.



Use longer exposure time for final image acquisition. Note that more dynamic range (greylevels) has been used.

## Multi-dimensional Acquisition dialogue box.



**Frames (time)**  
 Number: 10  
 Interval: 0.0 ms

**Slices (Z)**  
☐ Use slice settings  
 Z-start [um]: 0.0 Set  
 Z-end [um]: 0.0 Set  
 Z-step [um]: 1.0  
 relative Z

**Camera**  
☐ Override current settings  
 Close  
 Acquire!  
 Load...  
 Save As...

**Summary**  
 Number of channels: 1  
 Number of slices: 1  
 Number of frames: 10  
 Total images: 10  
 Duration: 0h 0m 0.0s

**Channel group:** Channel

Configuration	Exposure	Z-offset	Color
Monochrome	1000	0.0	

New  
 Remove  
 Up  
 Dn

☐ Save files to acquisition directory  
 Directory root: C:/AcquisitionData  
 Name prefix: Untitled  
 Comment:

## Time lapse acquisition window

