Penn Medicine
The Children's Hospital of Philadelphia®
A pediatric healthcare network
Center for Dynamic Imaging of Nervous System Function

Standard Operation Procedure (SOP)

Confocal Microscope Quick Start Guide

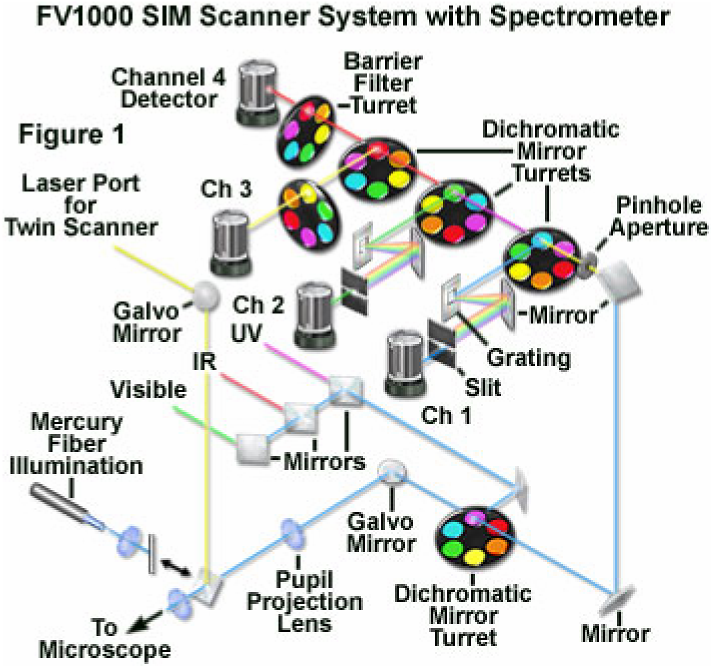
Equipment Location:	458 Stemmler Hall
Original document created:	10/23/04
Document updated:	12/10/04
Director :	Phil Haydon (pghaydon@mail.med.upenn.edu)
Safety Trainer (ST) :	Hajime Takano (htakano@mail.med.upenn.edu)
Requirement:	Read this document.
	All users need to be trained by ST.
	Basic understanding of how fluorescence microscopy works.
Safety Precaution:	Do not stare the laser beam.
Protect equipment:	**Protect equipment from solution leakage.**
	Protect objective lenses from immersion oil leakage.
	Protect PMT from over exposure.
Emergency Contact:	Hajime Takano (267-693-7384)
	Phil Haydon (610-246-6013)

Olympus Fluoview FV1000

Fluoview FV1000 unit with IX81 motorized inverted microscope

Control unit

Laser unit



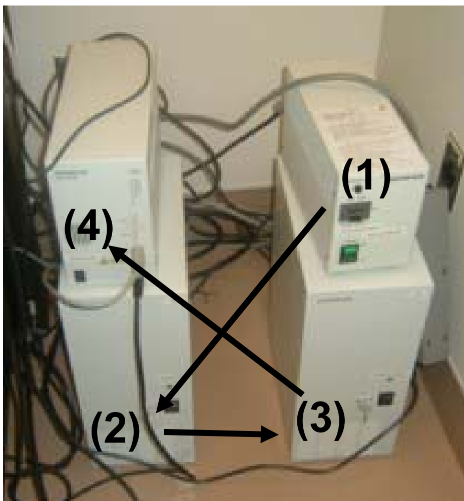
Visit Olympus website for more detail.

http://www.olympusfluoview.com/index.html

Standard Startup Procedure

1. **Check the log book. Sign in. (Name, Lab,dye, laser to be used, Time)**

2. **Turn on (1) mercury lamp, (2) main scan controller, (3) SIM scan controller, (4) microscope controller.**

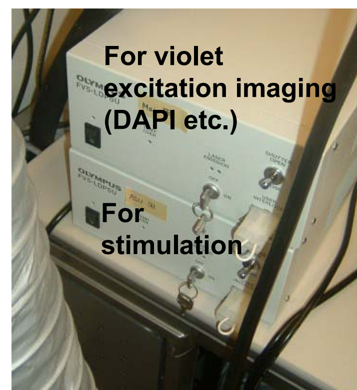


3. **Turn on necessary laser sources.**

For typical imaging (blue, green, red excitation)

Multi-line Argon Laser (457nm, 488nm, 515nm)

For special imaging



diode violet lasers (405nm)

1. Run the fluoview software. (overview)

imaging mode, scan start/stop

scan control

PMT control

laser power control

z control

t control

2. Check the sample with epi-fluorescnce / bright field with eye-piece.

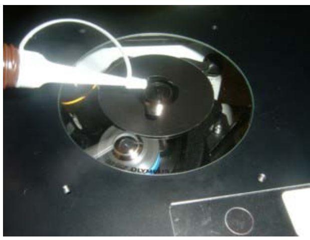
Click here for bright field imaging

Click here for epi-fluorescence

Bright field lamp intensity control

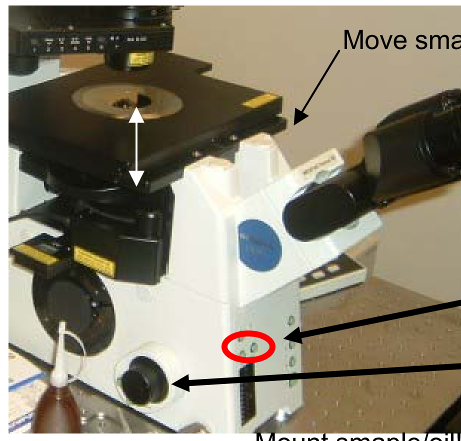
1. Setup the sample.

No oil for x20



oil for x40, x60

Changing x20 to x40 without moving sample



Move smaple stage in x,y direction with a knob

Course focus up/down

fine focus up/down

Mount smaple/oil!

Note: Objective lens moves up/down

After the experiment, or when changing x40,x60 to x20, gently dab oil with lens cleaning tissues. Do not use Kimwipes.

1. Select dyes from the list to setting up the optical configuration.

Click here for the dye list.

Double click to remove.

Double click to select.

click apply

2. Check a default scan parameters

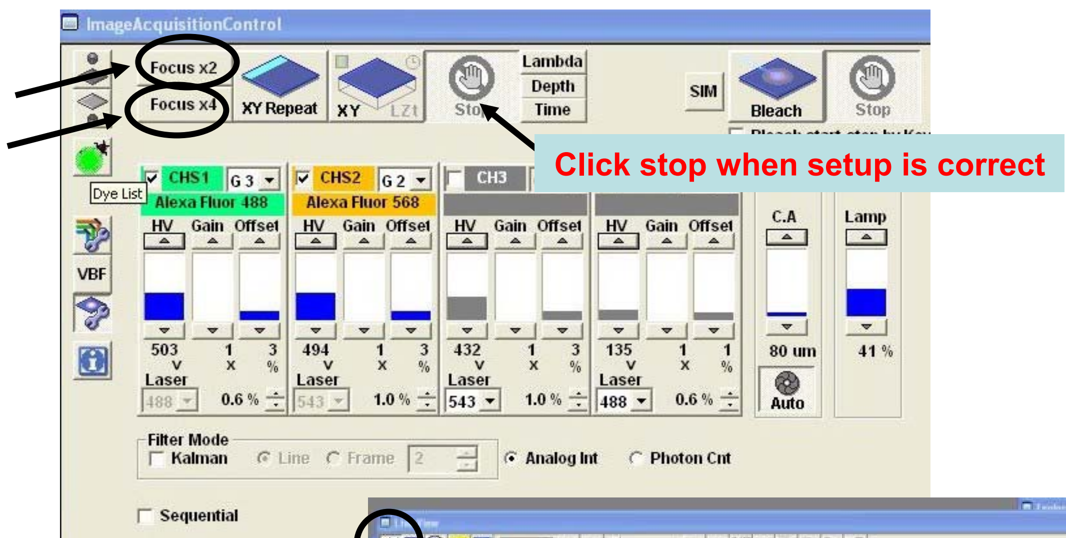
scan mode

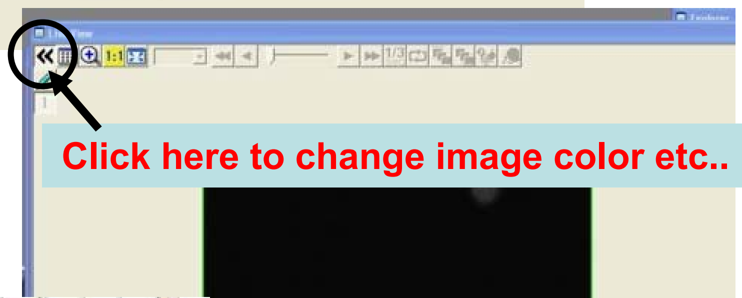
Scan speed 4μs-8μs/pixel

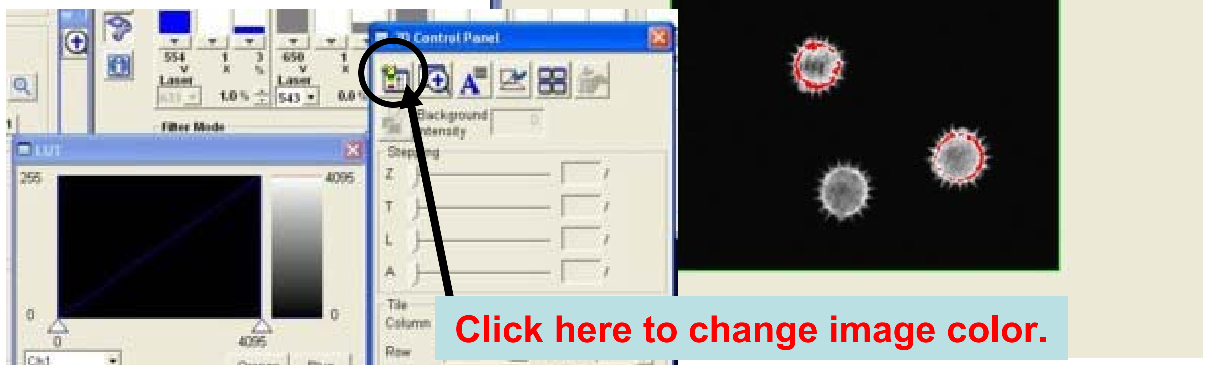
512 x 512

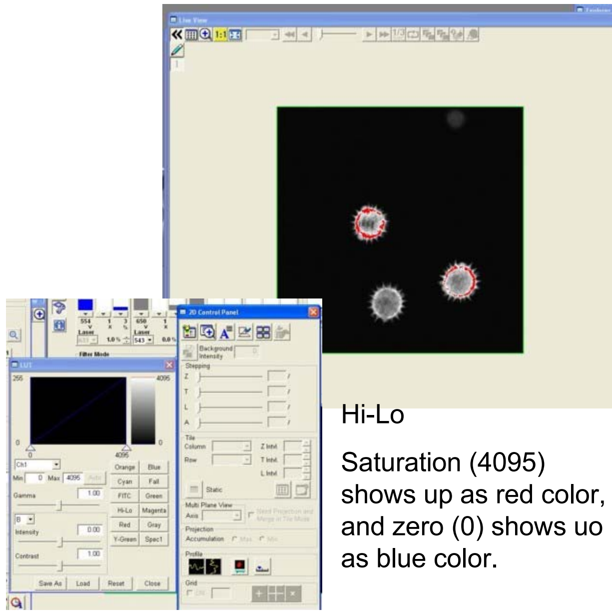
zoom 1 (no zoom)

1. **Check the focus, sample position, laser power, PMT voltage, offset, gain , color table, with focus scan mode (fast scan).**



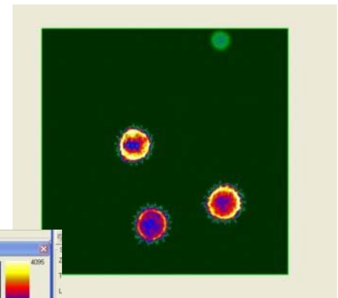






Hi-Lo

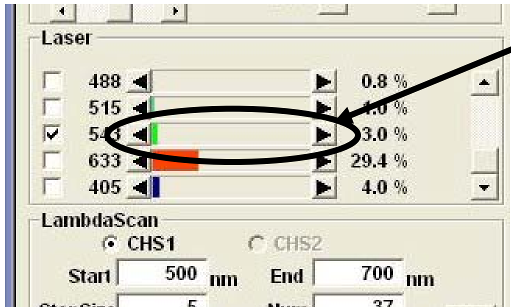
Saturation (4095) shows up as red color, and zero (0) shows uo as blue color.



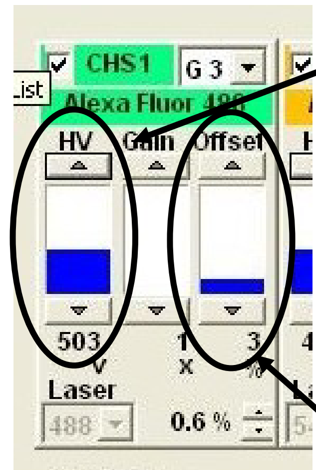
Spec1

Saturation (4095) shows up as white color, and zero (0) shows up as blackcolor.

It is important to pay attention to the signal level. In general, signal should not be saturated. It should not be too dark, but dark image can be processed to a higher intensity.



To change the laser intensity, click arrows or put the cursor on the color bar and use mouse action key.



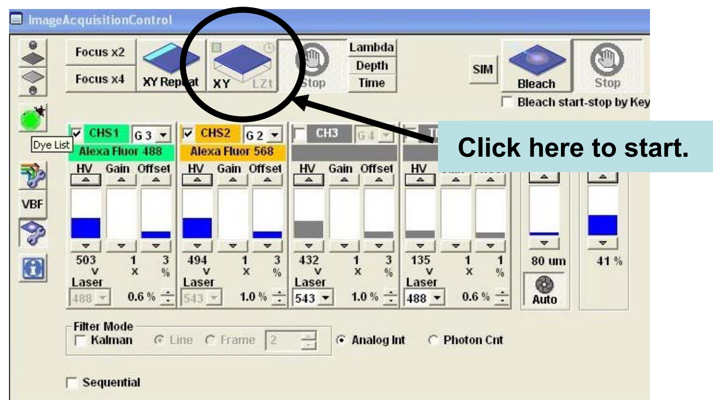
To change the PMT high voltage, click arrows or put the cursor on the color bar and use mouse action key.

For a starter, stay with 600-680. If it is too bright even when the laser intensity is minimum, decrease upto ~500.

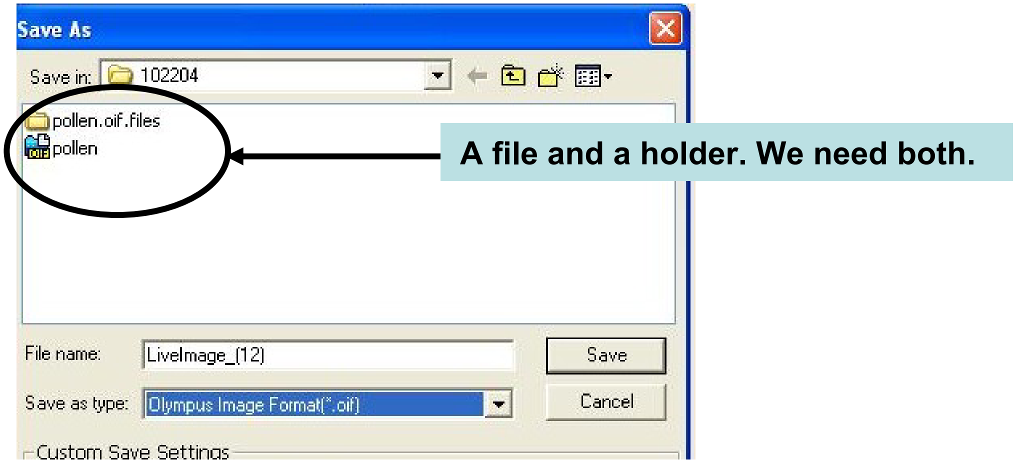
For dim sample, typically do not go over 780. Increase gain or increase laser intensity.

Increase offset when background is high. Remember offset can correct with mapping post acquisition.

1. **Capture an image. Check the image.**

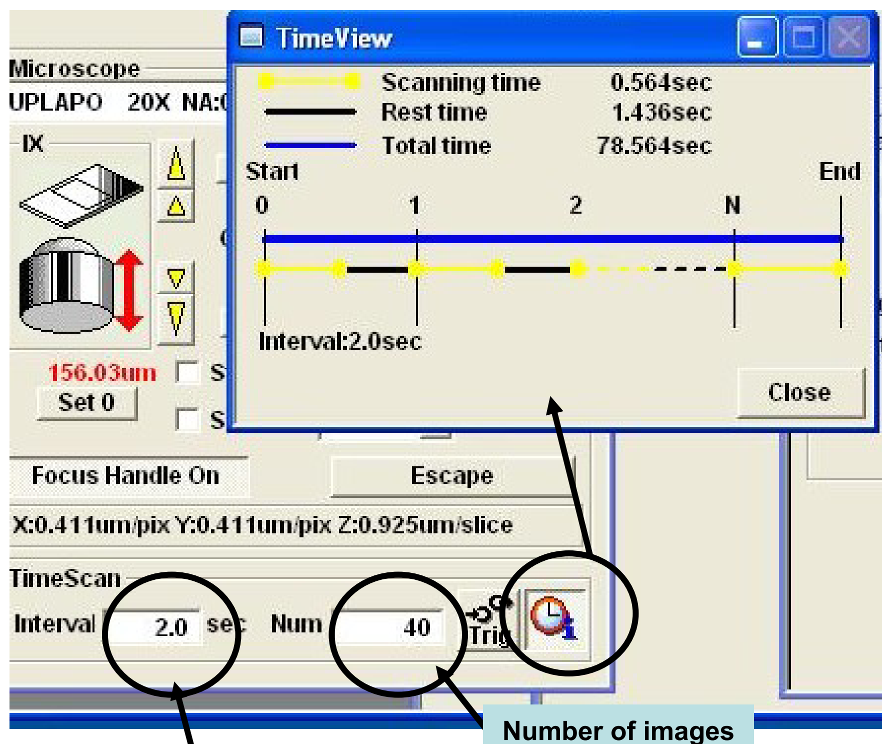


2. **Save the image as "Olympus Image Format (*.oif)" from file menu. Note that the image is saved as a file and a holder containing several files. Do not misplace them.**



T-scan (time series) mode.

- Setup parameters (cycle time, number of images), and check from Time-View window.

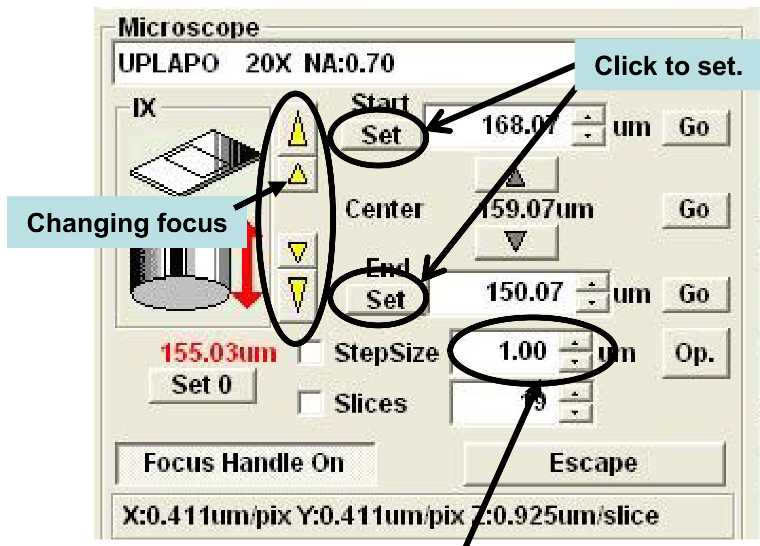


- Click "time" and start.

- "Series Done" shows up. Click to accept.
- Save the series as "Olympus Image Format (*.oif)" from file menu. Note that the images are saved as a file and a folder containing several files including individual TIF files.

Z-scan mode.

- Change focus either by the arrows on the software or by the focus handle on the scope and set the starting position and the ending position.

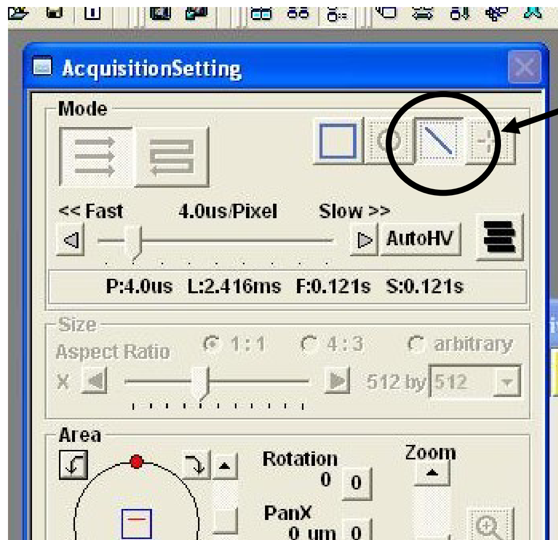


- Set step size. Typically 0.5 ~ 1μm.
- Select "depth".

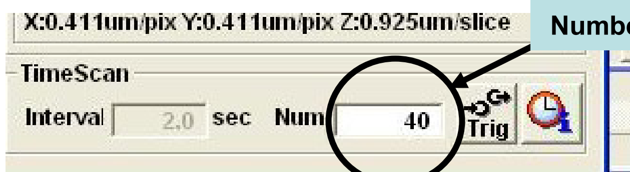
- "Series Done" shows up. Click to accept.
- Save the series as "Olympus Image Format (*.oif)" from file menu. Note that the images are saved as a file and a holder containing several files including individual TIF files.

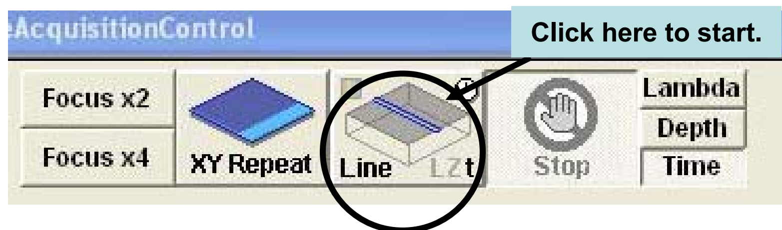
Line scan mode.

- Select line and draw a line on the live view image. Set the number of scans.



Select line and draw a line on the live view image.

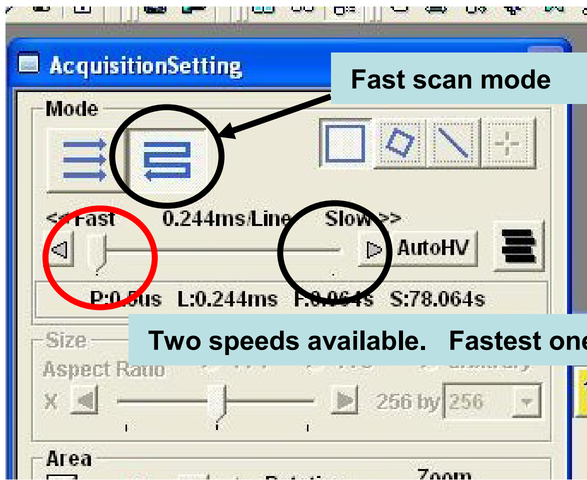




- Save the image as “Olympus Image Format (*.oif)” from file menu. Note that the images are saved as a file and a folder containing several files including individual TIF files.

Fast scan mode.

- Select line and draw a line on the live view image. Set the number of lines.

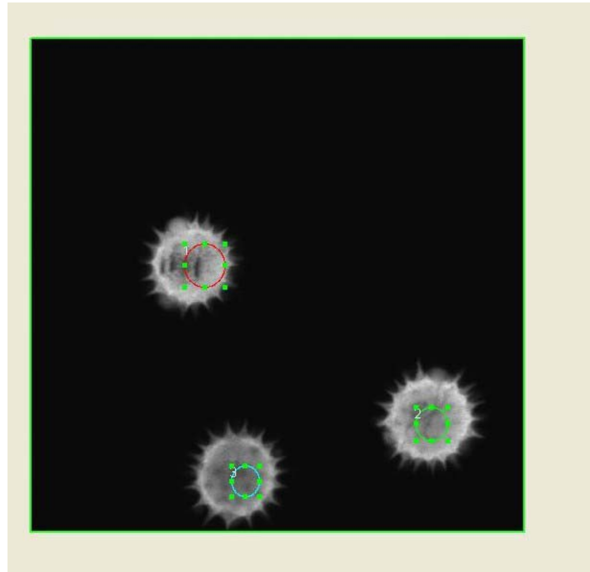


Maximum 64ms /image.

Analysis example

In time series (or z-series), plot intensity of selected regions.

- Mark regions of interest. (Note: Make sure all regions are selected.)



- Select “analysis” from main menu.

then, select “series”.

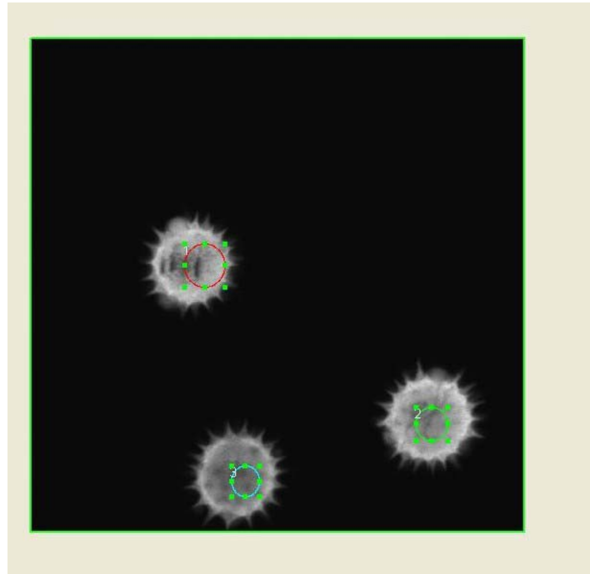
- Select “series” from submenu.

Save graph as an image or save data as text or excel.

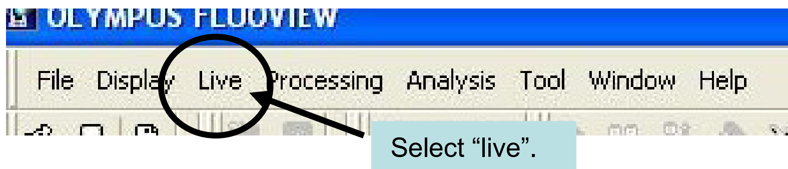
Ask ST for the details.

Live analysis

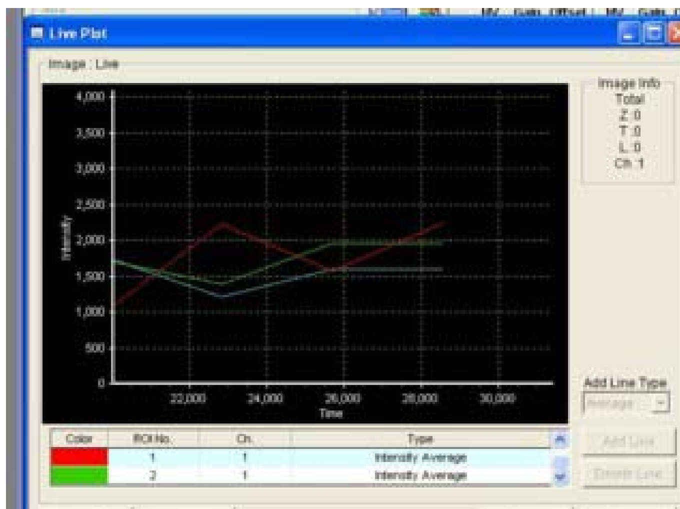
- In time series (or z-series), monitor intensity of selected regions in live.
- Take an image and mark regions of interest. (Note: Make sure all regions are selected.)



- Select "live" from main menu.



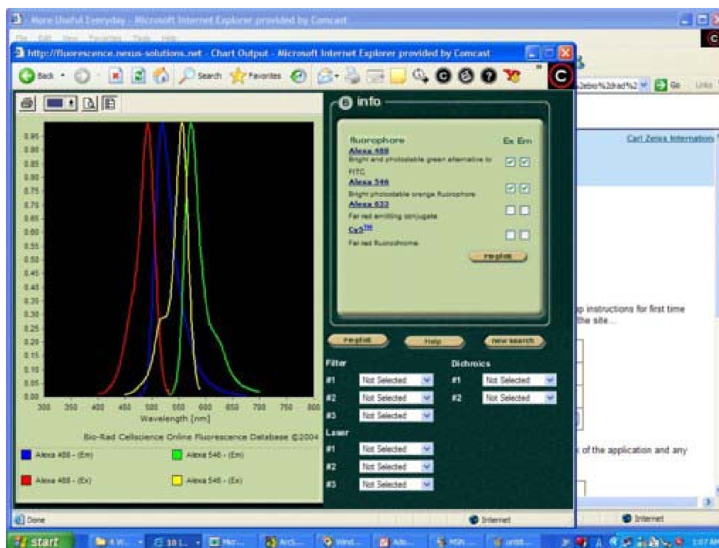
- Live plot shows up. Start time series.



Appendix

1. **Identify dyes you are using.**
2. **Check the excitation spectra and emission spectra of the dye.**

    Useful link: http://cellscience.bio-rad.com/fluorescence/fluorophoreDatab.htm



3. **Identify laser sources you are going to use.**

Exmaple

lasers in Fluoview 1000	Ex.	Em.	Example
Multi-line Argon Laser (457nm, 488nm, 515nm) , 30mW	blue	green	FITC, Alexa 488, Fluo-3, GFP
HeNe Laser (543nm), 1mW	green	red	Rhodamine, Alexa 546,
HeNe Laser (633nm), 10mW	red	near IR	Cy5, Alexa 633
diode violet lasers (405nm), 25mW	violet	blue	DAPI