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# Olympus2 User Notes

Olympus-Fluoview1000-MPE  Software version 1.7b

R2-E233

***Important note to Olympus2 users:***

When you sign up on the calendar please indicate:

1) Which objective you will use

25x 1.05NA water immersion – special permission only

60x 1.2 NA water immersion

60x 1.42 NA oil immersion

4x 0.16 NA Air

2) If using confocal, which laser(s) you will use

Confocal  405, 458, 488, 515, 559, 635

If using multiphoton, include  excitation wavelength and b/g or g/r emission cube.

Multiphoton 710-990

***AND**, if you are using multiphoton and are using a different objective and/or wavelength than the previous user, please allow time between users for facility personnel to perform system optimization.*

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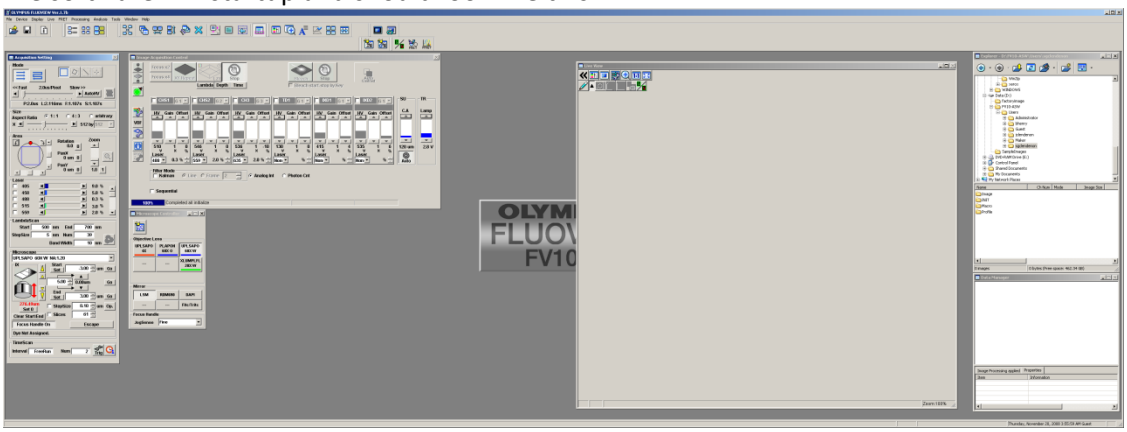
Logon to Windows XP using your UserID and password.

Double click the Olympus Fluoview software icon

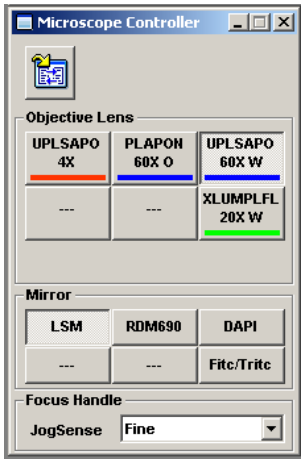
Start the Olympus software.



The software will startup and should look like this.



In the Microscope Controller window, click the correct button to tell the software which objective lens you are using.




> *In software version 1.7b, when using the 25x, click the 20x button. The next software update will include settings for the 25x objective. Keep this in mind when you apply scale bars to your image*.

Focus control can be toggled between Fine and Course in this window. Fine and Course, F/C Focus can also be toggled by pressing the bottom green button next to the focus knob on the microscope body.

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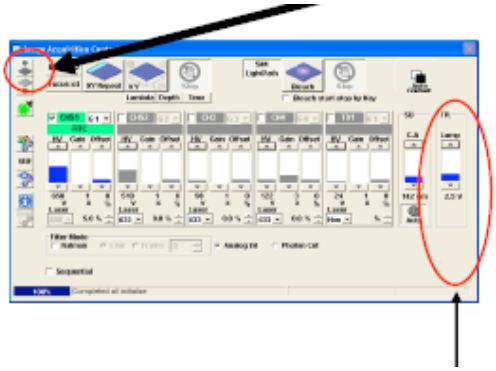
## OBSERVATION THROUGH THE OCCULARS

Find and focus sample using the oculars

Transillumination

Click the Trans Lamp button in the Image Acquisition Control window

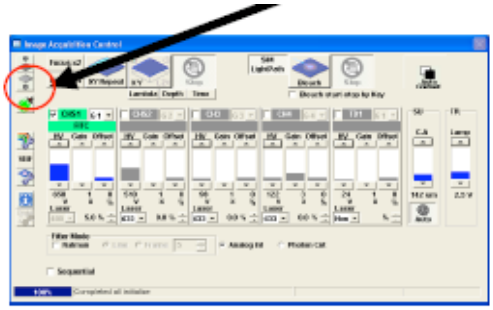


Adjust brightness of transillumination lamp with the TR Lamp control.

Click Trans Lamp button again to turn off

Epifluorescence

Click the EPI Lamp button in the Image Acquisition Control window



In the Microscope Controller Window, choose either DAPI or Fitc/Tritc.

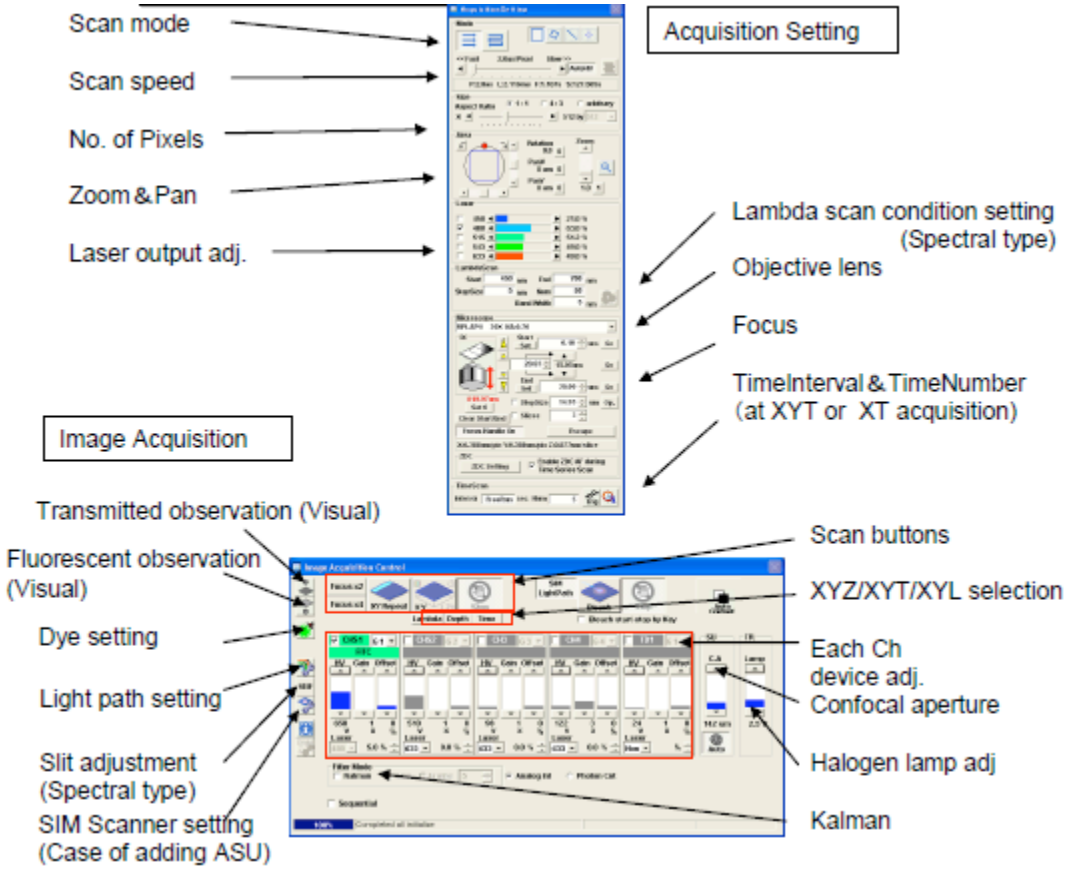
Set mechanical shutter to Open position

Open o

Closed •

Click EPI Lamp button again to turn off

## CONFOCAL IMAGE ACQUISITION




***1) Acquisition Setting Window***

• Mode - Scan Mode  - Oneway should always be selected

• Mode – Normal  – rectangular image – used most often. Other available modes are irregular shapes, line scans and point scans.

• Scan Speed - Choose scan speed
For live imaging use fastest speed
For fixed cells/tissue, slower scans produce a less noisy image.

• Size – Choose Number of Pixels
Most users choose 512x512 as a reasonable tradeoff between resolution and time needed for acquisition. Imaging small structures like endosomes may require 1024x1024 and/or zoom.

After choosing your dyes (later in this guide) you can then click the info button in 

the Image Acquisition window to see the Optical Resolution and the Pixel Size for your chosen configuration.

Area - Zoom and Pan

Choose zoom. Reset by clicking 1
Rotate structure of interest in image by dragging red dot. Click 0 next to rotation to reset.
At zooms >1 drag blue box around – pan - to refine area of interest. Click 0's next to PanX and PanY to reset

• Laser Output – set laser output

Lambda Scan – advanced feature. Useful to determine emission peak of autofluorescence or uncharacterized dye. Ask for assistance.

• Microscope – Objective – displays objective that was chosen in the Microscope Controller window.

Microscope – Focus controls will be used to set up z-scans

• Microscope – Focus Handle On should be pressed

Microscope – x,y,z in microns at the bottom of the microscope panel gives the optical resolution of the currently chosen setup, not the pixel size.

Time Scan – set Interval and Number for time series. You can use this if your time series will fit in the computer's RAM. If your time series will require greater memory resources, you will need to use the Time Controller under the Device menu.

***Image Acquisition Control Window***

1)Trans Lamp and EPI Lamp buttons off

2)Click Dye List button.  Dye List window will appear. Click All Clear. For confocal, click Single Photon. Double click desired dyes from list.

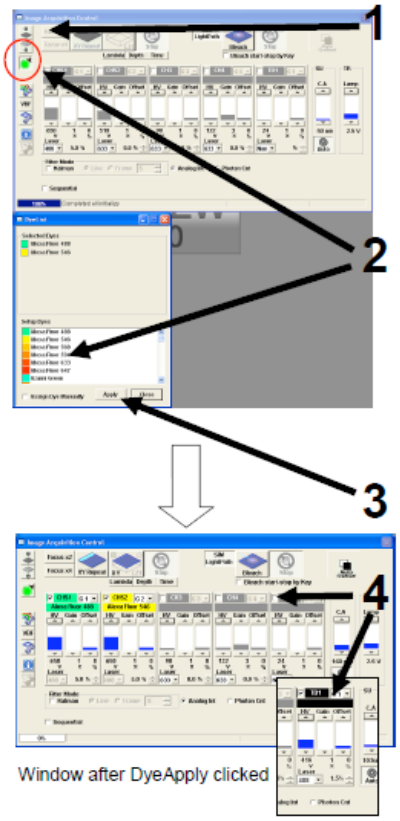
3) Click Apply.

The correct optics for the dyes that you have chosen will move into place.

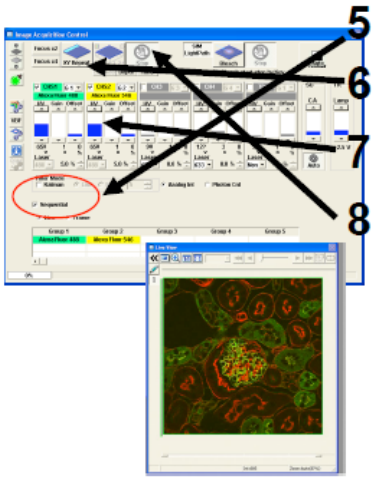
Check to see that the Auto button is pressed for the confocal aperture.


4) If also collecting a transmission image, check TD1.

Illumination wavelength for TD1 - choose the same as CHS1.




Window after DyeApply clicked




5) Check sequential. Select Line.
With sequential checked, live scanning will only display one channel at a time.

6) Click X,Y Repeat to begin scanning.


7) <u>While scanning, adjust laser power, hv gain, gain, and offset (black level) for each channel using Hi-Lo LUT to attain best quality image.</u>

↑ laser power will ↑ brightness, but also bleaching. Keep as low as possible.
↑ hv gain will ↑ brightness, but also noise. Keep below 700 if possible.
Adjust laser and hv gain so that there are a minimum number of red (saturated) pixels.
Adjust offset to have a minimum number of blue pixels (black level = 0 where blue).
Keep middle gain (not hv gain) slider set to 1.

To further reduce noise in the image:
Use a slower scan speed.
Advantage – crisp low noise image. Disadvantage – Slow.
Kalman Average – specified number of images is averaged
Advantage – averaging reduces noise
Disadvantage – Averaging may result in a dimmer image due to bleaching and is slow

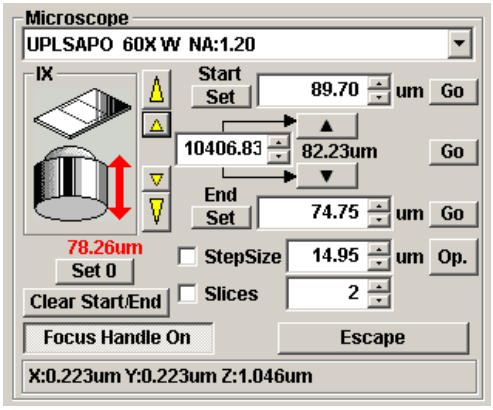
8) Click Stop to stop scanning.


9) Click XY button to acquire a *single plane image*. See next page for z-scan.


10) Save Image.
Choose File/Save As
Or Right click mouse while over image and choose Save As

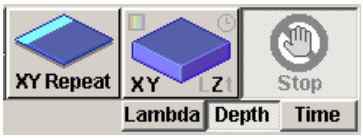
The default image location is:
D:\FV10-ASW\Users***YOUR-USERNAME***\Image\

## Z-SCAN -acquiring an image volume




Find focal plane of interest.
In the Acquisition Setting window - Microscope,
Click Clear Start/End and click Set 0
Click XY Repeat to begin scanning.
Click up buttons or turn focus knob to find top of sample – click Start Set
Click down buttons or turn focus knob to find bottom of sample – Click End Set
Enter a Step Size and check the Step Size box.

In the Image Acquisition Control window
Click Depth




Click the XY Z button and the defined stack will be imaged.

When acquisition is complete, Click Series Done.


Save Image.
Choose File/Save As
Or Right click mouse while over image and choose Save As

The default image location is:
D:\FV10-ASW\Users***YOUR-USERNAME***\Image\

***Q: How do I choose a step size?***
**A: It depends on the sample.**

Some starting points are:
½ the optical resolution in z (see info panel)
or
z = the x,y pixel size

But, depending on what you are trying to visualize, you may be able to take z-steps 2x this or more.

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# MULTIPHOTON IMAGE ACQUISITION – non-descanned detectors (external)

***Image Acquisition Control Window***

1)Trans Lamp and EPI Lamp buttons off

2)Click Dye List button.  Dye List window will appear. Click All Clear then click Two Photon. Do not choose dyes here.

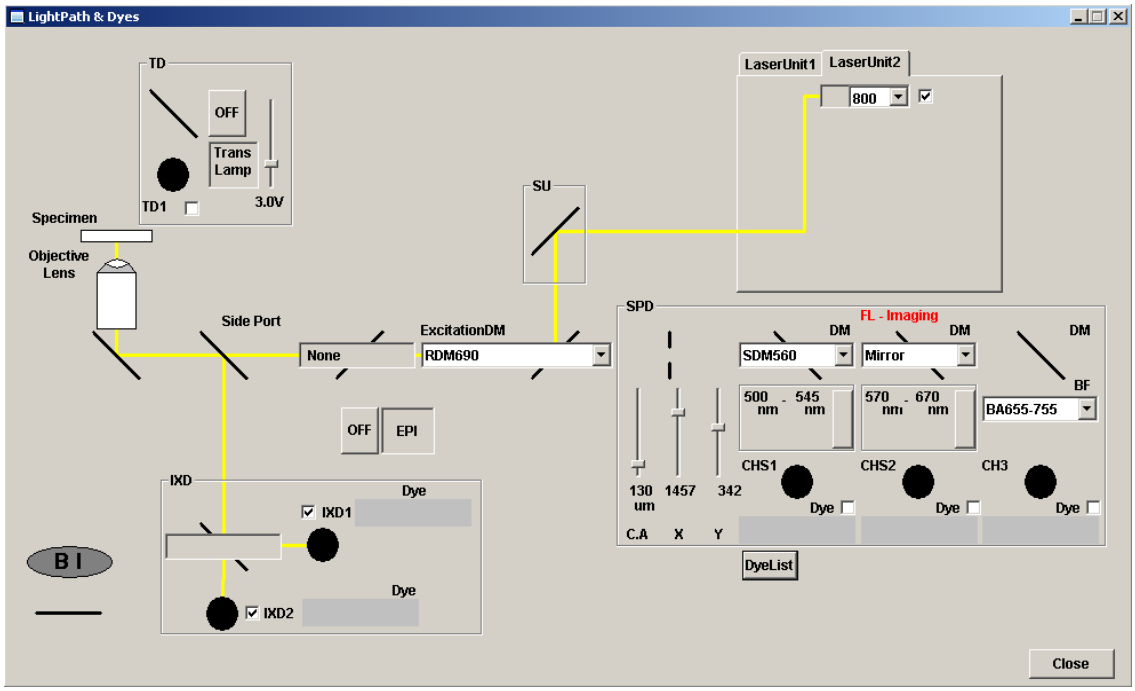
3) If also collecting a transmission image, check TD1.
Illumination wavelength for TD1 - choose the same as IXD1.

4)Click the Light Path and Dyes button. 

***Light Path and Dyes Window***

Choose Exitation DM (dichroic mirror) – RDM690
Under Laser Unit 1 – Uncheck visible lasers
Under Laser Unit2 – Check IR laser
Under IXD – Check box for IXD1 and/or IXD2

The emission wavelengths collected are defined by the cube in place in the external detector box. Your choices are Blue/Green or Green Red. Indicate which cube you need when you sign up on the calendar.



Appendix A from Olympus Fluoview Ver.1.7b QuickStart Guide

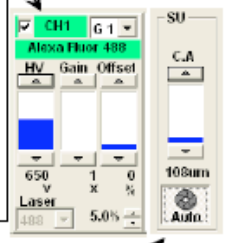


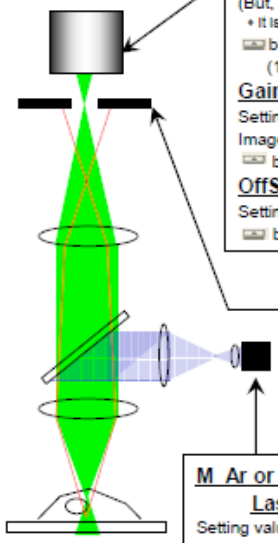
# Appendix A Relationship between confocal principle and tuning mechanism

**HV: Sensitivity adjustment of detector (Voltage)**
Setting value ↑ > Sensitivity ↑ > Image Brightness ↑
(But, noises are getting noticeable.)
• It is recommended that the voltage is set at about 700V or less.
button: changes by 1V step.
(1V in case of transmitted image)

**Gain adjustment (x)**
Setting value ↑ > Image Brightness ↑
Image gets brighter with setting value.
button: changes by x0.001

**OffSet adjustment (%)**
Setting value ↑ > Image Brightness ↓
button: changes by 1%



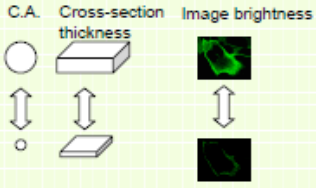



**C.A.: Confocal aperture adjustment (μm)**
Setting value ↑ > C.A. Size ↑ > Image Brightness ↑
(But, optical cross-section is getting thicker).
• It is recommended that C.A. is used with Auto.)
When C.A. should return to default setting after move,
s Auto again.
button: changes by 1μm.

**M Ar or HeNeG, HeNeR: Laser output adjustment (%)**
Setting value ↑ > Output strength ↑ > Brightness ↑
(But, fluorescent discoloring is getting larger.)
• Use with a value as small as possible recommended.
button; changes by 0.1%


■ Memo ■

Relationship between confocal aperture and optical cross-section, image brightness




Relationship between laser output/ detector sensitivity and image brightness