Olympus FV-1000 Quick Guide

I. STARTING THE OLYMPUS FV-1000

A. Sign in to the log book and turn on the following numbered switches in this order:

1. Turn on mercury power supply (1).
2. Turn on microscope control box (2).
3. Turn on the power switch for the Argon Laser (3 A). Turn the key 90° to the right (3 B).
4. Turn on the power switch for the laser power supply (4 A). Turn the key 90° to the right (4 B).

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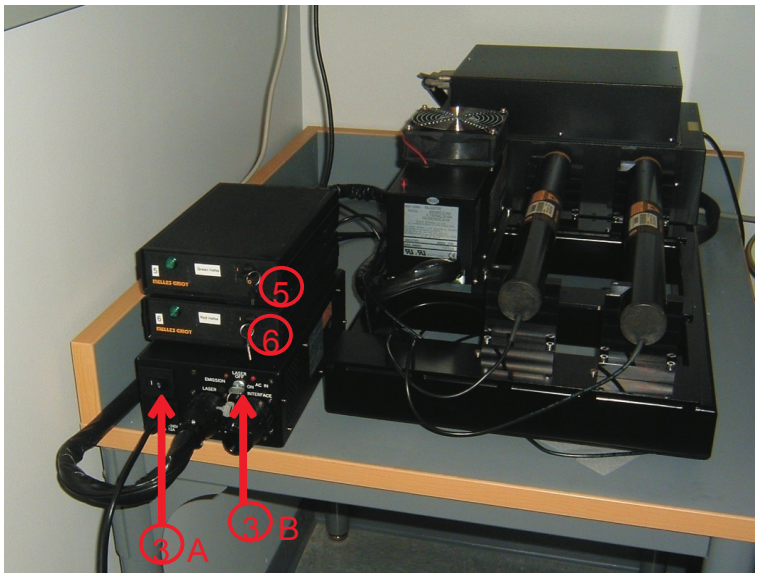
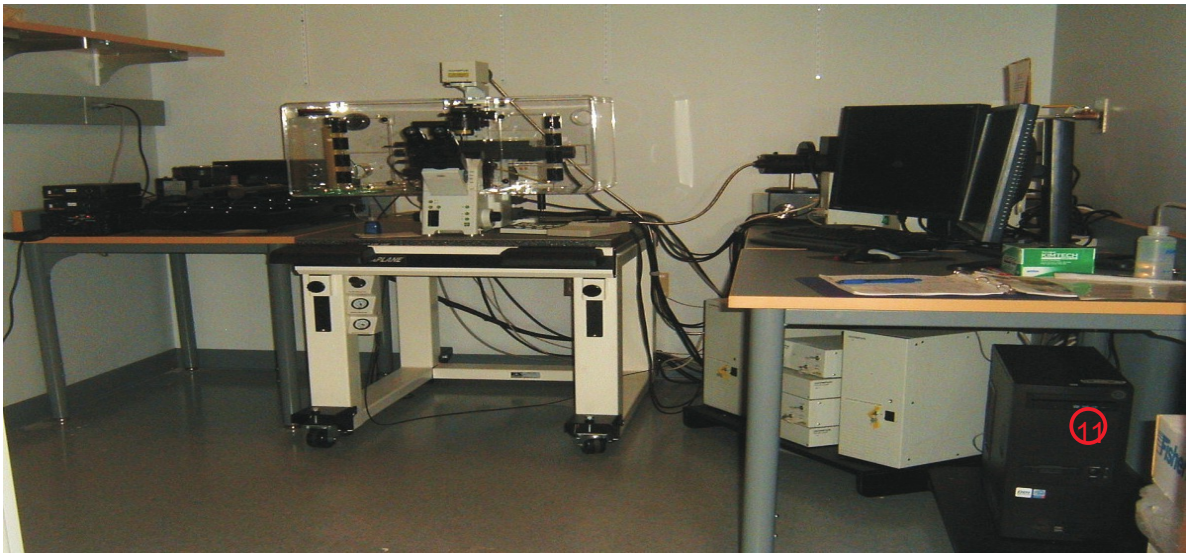
5. Turn key for the green HeNe power supply 90° to the right (5).
6. Turn key for the red HeNe power supply 90° to the right (6).
7. Turn on the power switch for the laser power supply (7 A). Turn the key 90° to the right (7 B).
8. Turn on the power switch for the Sim Controller (8). The key should always be in the “On” position.
9. Turn on the power switch for the Main Controller (9). The key should always be in the “On” position.
10. Turn on the power switch for the Prior Proscan II motorized stage controller (10).
11. Press the power button (11) on the front of the computer on the floor to the right of the microscope.





June 2, 2011

II. LOG IN AND START THE SOFTWARE

1. Enter your Vunet ID and password.

2. Click on the Fluoview software icon.

3. From the drop-down list, choose “guest” and no password, OR choose your saved setup file.

4. After the software loads, close the Stimulus Settings window if it pops up.

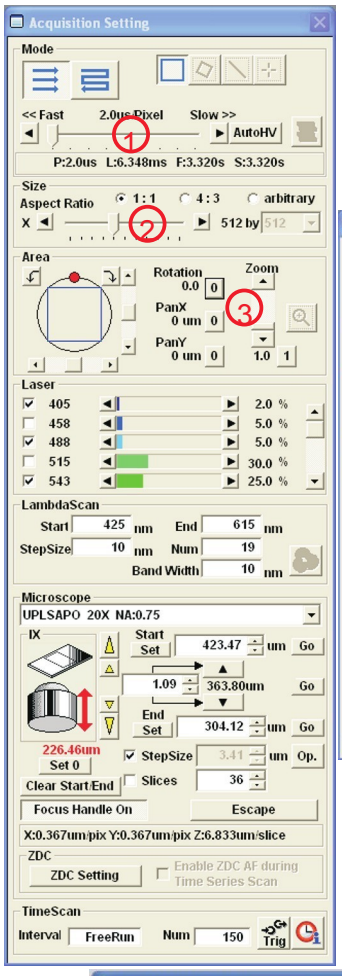
III. VIEWING THE SPECIMEN WITH TRANSMITTED LIGHT

1. Begin by resetting the defaults in the Acquisition Setting window:

A. In the Mode section, slide the scan speed slider ① to Fast.

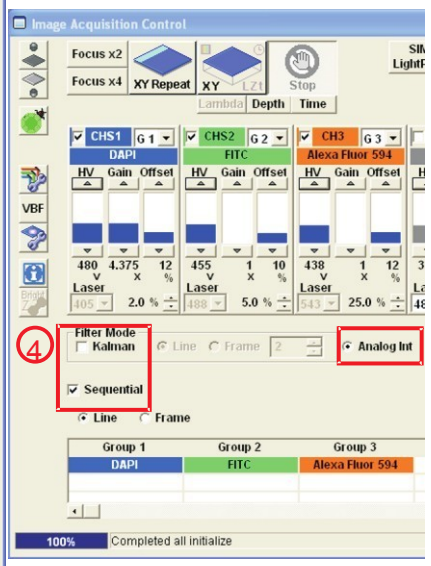
B. In Size ② choose 512 x 512 (pixel density).

C. Under Area set the Rotation to 0.0 and the Zoom ③ to 1.0 .

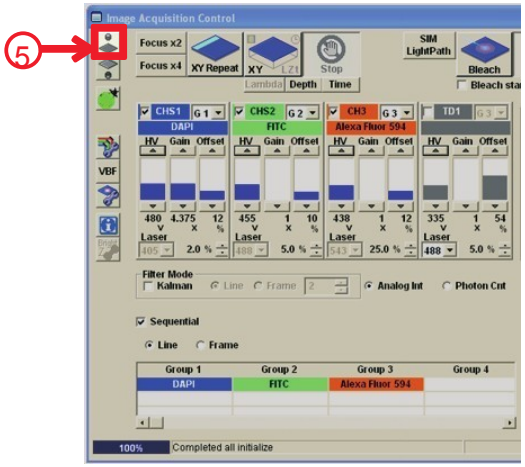


2. In the Image Acquisition Control window, make sure

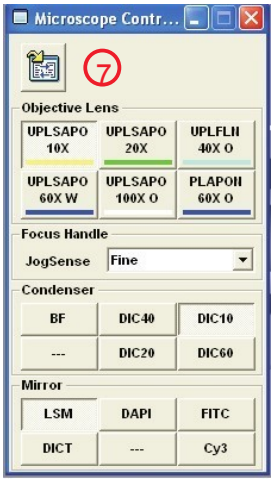
④ the Kalman box is unchecked and the Sequential box is checked. Analog Int should also be checked.



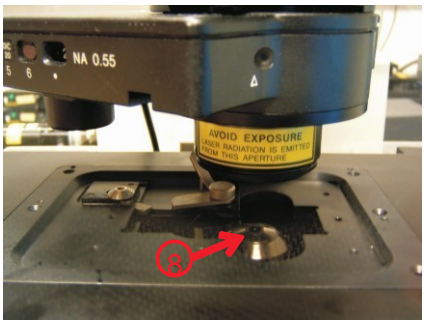
3. Select the “TransLamp ” button ⑤. This enables the microscope to be configured for conventional viewing through the eyepieces.



4. Select the objective by clicking the appropriate button on the keypad (6) or the onscreen microscope controller window (7).



5. Apply appropriate immersion fluid to the objective (8) if needed. Make sure the sample cover glass is clean and dry; do not allow oil from other microscopes to mix with the oil on this system. You may need to raise the condenser in order to more easily reach the objective.



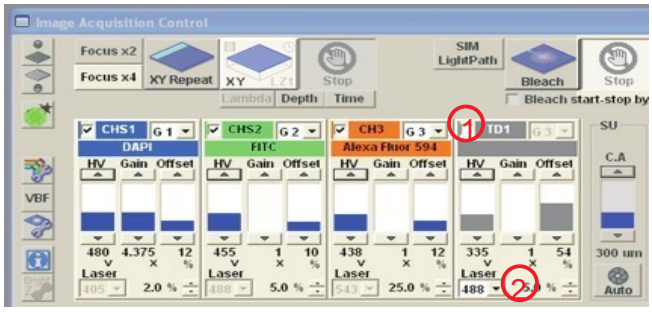
6. Mount the specimen on the stage. Remember that for inverted microscopes - those with the objectives below the stage - microscope slides must be placed upside-down.

7. Bring the sample into focus. The focus knobs (9) are for fine focusing and the up/down focus buttons (10) on the front left below the eyepieces are for coarse focusing. For fine focusing, turning the focus knobs "up" (12) (left knob counter clockwise and right knob clockwise) moves the objective "down" or away from the coverslip.

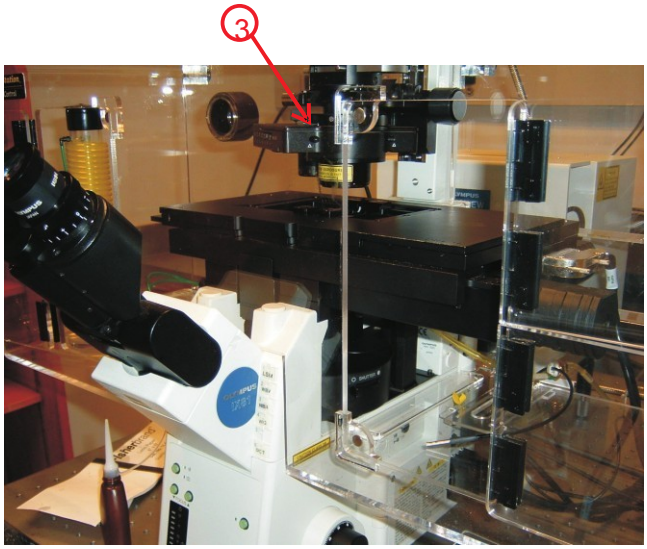
8. If you are NOT going to acquire DIC images, please skip to page 6 now.

IV. DIC

The 4th channel ① is reserved for bright field DIC (Differential Interference Contrast) images. Any laser line ② can be used; shorter wavelengths give more detail while longer wavelengths penetrate farther into thick tissue samples.



For optimal DIC images, light should be focused in the same plane with the sample. This is accomplished by adjusting the condenser ③ and is called Köhler Illumination.



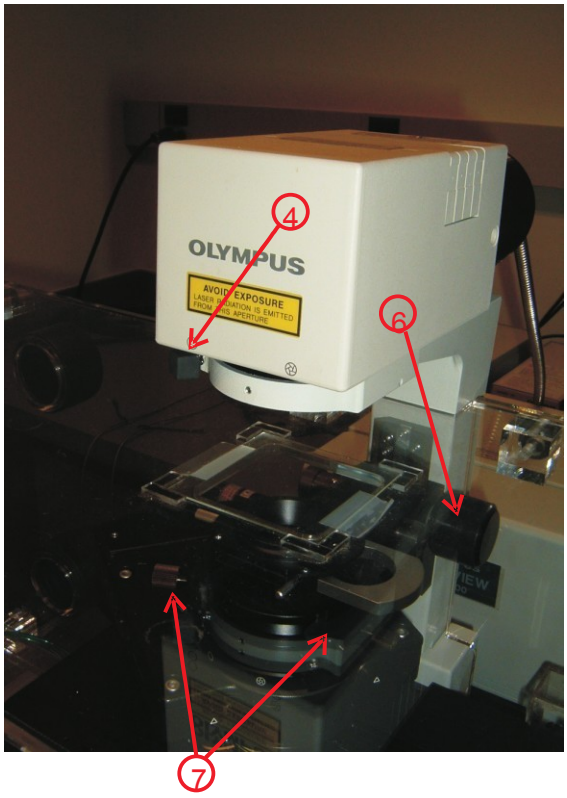
1. Focus the specimen using transmitted light.

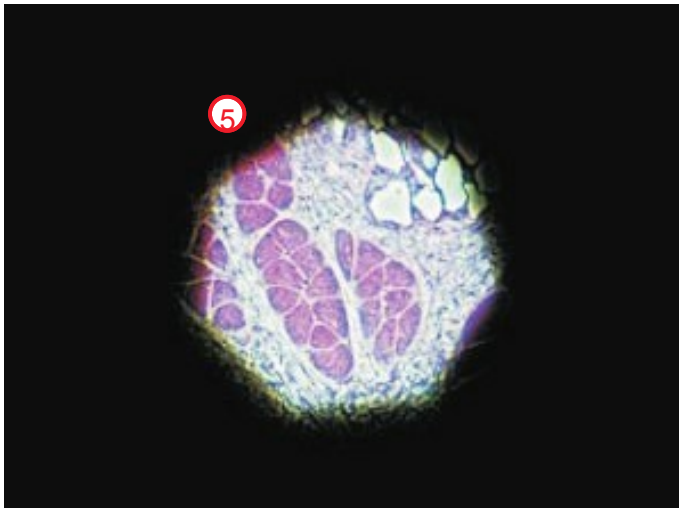
2. Close the field iris ④ until only a small spot of light remains ⑤. If you can't see a small spot of light, try raising or lowering the condenser with the adjustment knob ⑥ .

3. Adjust the condenser knob until the polygon is in focus (edges as sharp as possible.)

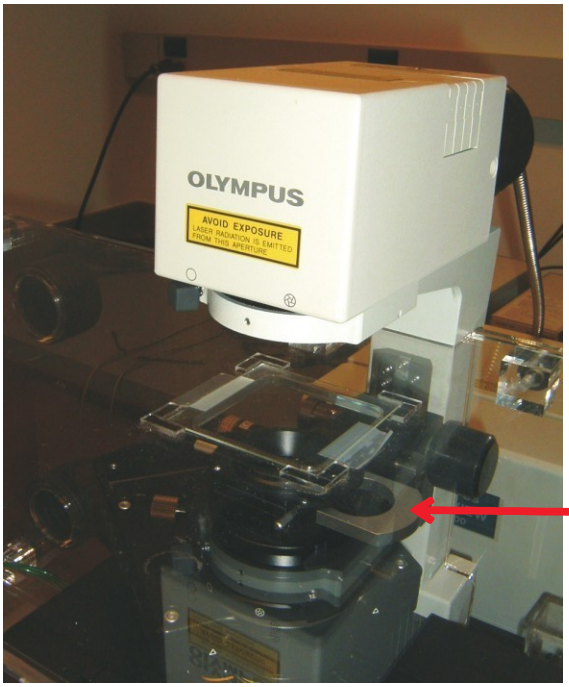
4. Turn the "rabbit ear knobs" ⑦ to center the polygon.

5. Open the field iris completely and turn off the transmitted light.





Now continue setting up the DIC.

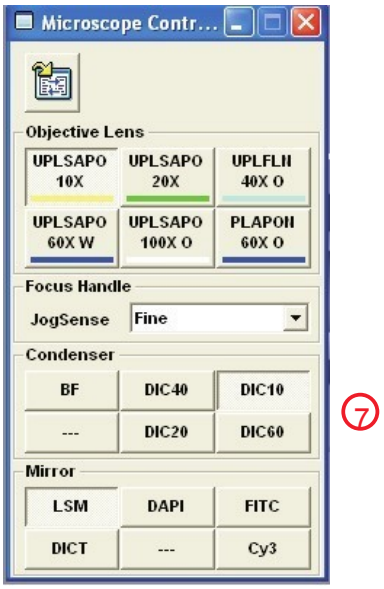


6. Push the analyzer ⑥ into the light path.

7. Ensure that the correct DIC condensor ⑦ matching your objective is selected in the Microscope Control window.

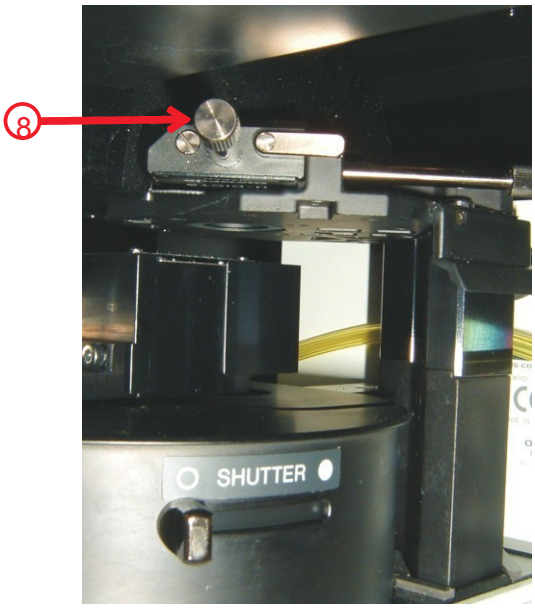
7. Turn off all channels except the DIC channel. Choose a laser line to use and check it.

8. Start xy repeat and adjust HV and laser power until an acceptable image appears.



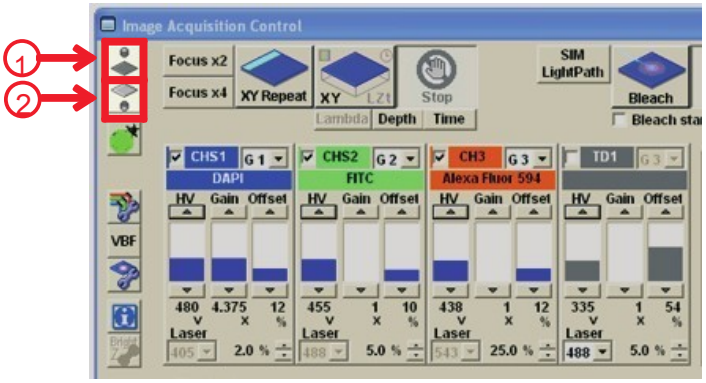
9. Fine tune the contrast with the polarizer adjuster knob ⑧.

10. Turn on the other channels and take a test image. If the DIC image is too bright or too dim, use XY repeat mode again, with all channels running, to optimize it.

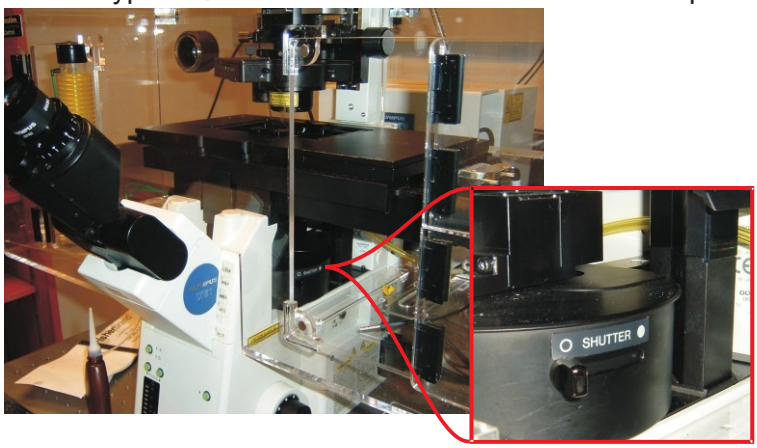


V. VIEWING THE SPECIMEN WITH FLUORESCENT LIGHT

1. Turn OFF the transmitted (white light) by clicking the TransLamp ① button.

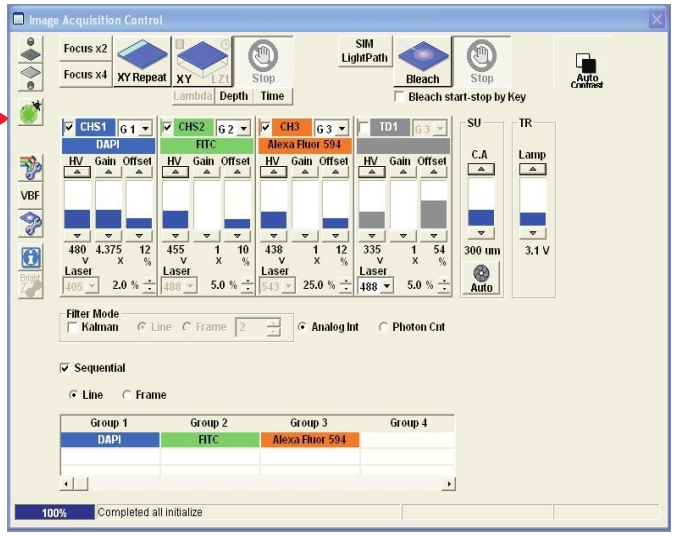


2. To view in fluorescence, click on the "EpiLamp" ② button and select the appropriate filter set using the remote control keypad. ③ Check that the manual shutter is open.



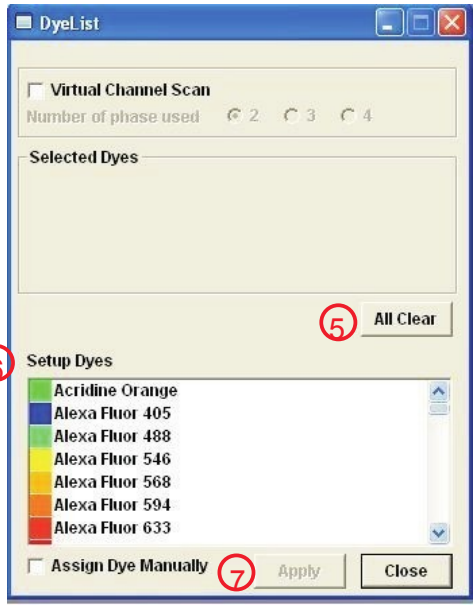
3. After the specimen is focused, the area of interest is selected, and fluorescence is confirmed, unclick the EpiLamp button.

4.Press the "Dye List " ④ button on the Image Acquisition Control window.



5. Click on "All Clear " ⑤ to clear previously selected dyes.

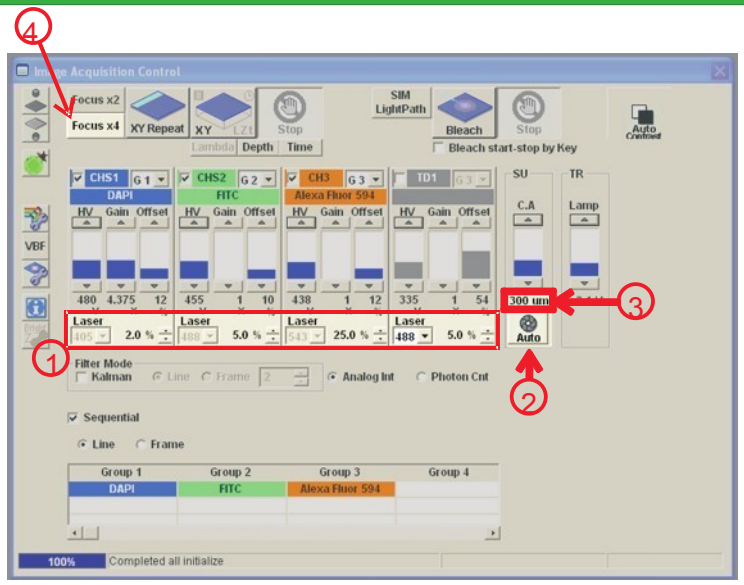
6. Under " Setup Dyes " ⑥ double click the appropriate dyes for your sample. Once all the dyes have been selected, click "Apply " ⑦. Close window.



VI. IMAGE OPTIMIZATION

1. When you looked at your sample with reflected light through the oculars, you saw how bright or dim the fluorescence was. Use that knowledge to adjust the laser strength for each channel before scanning.

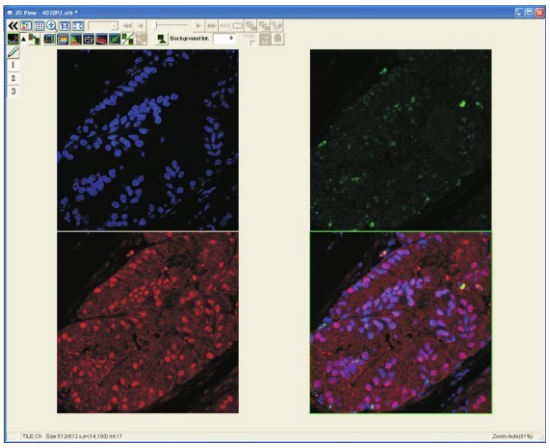
Set the Argon laser to 1-5% and the HeNe lasers to 10-25% (1). (These are starting guidelines; your samples may require a higher setting, but never turn the excitation level lower than 1%.)



2. Under the pinhole adjustment labeled SU and C.A. click on the "Auto" (2) button which sets the pinhole to 1 Airy unit. The pinhole diameter (3) will change. Unclick the auto button. Highlight the pinhole diameter and type in double the number. Note that the auto pinhole diameter is different for each objective and is dependent on the chosen dyes. SEE NOTE ON RIGHT SIDE OF PAGE FOR MORE INFO ON PINHOLES.

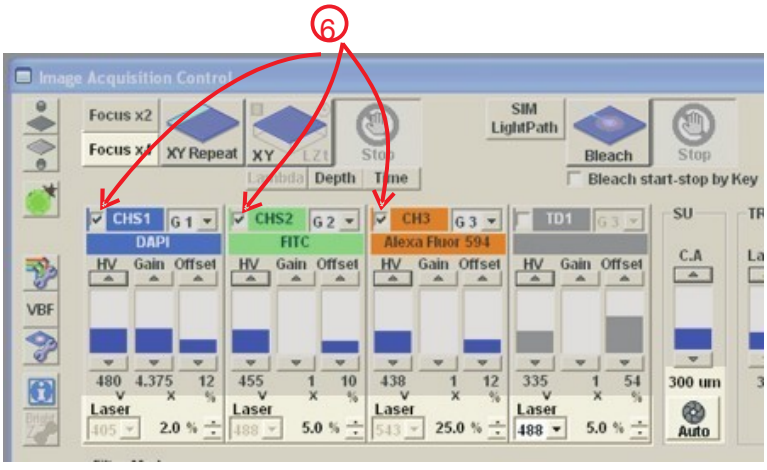
The pinhole value determines the thickness of sample from which light is gathered at one time. Higher pinhole values= more light but also more bleaching. A larger pinhole means that z-sections and projections will have less definition because each slice will be thicker. A smaller pinhole will produce a nicer looking projection, but it requires a stronger signal or higher HV and laser settings.

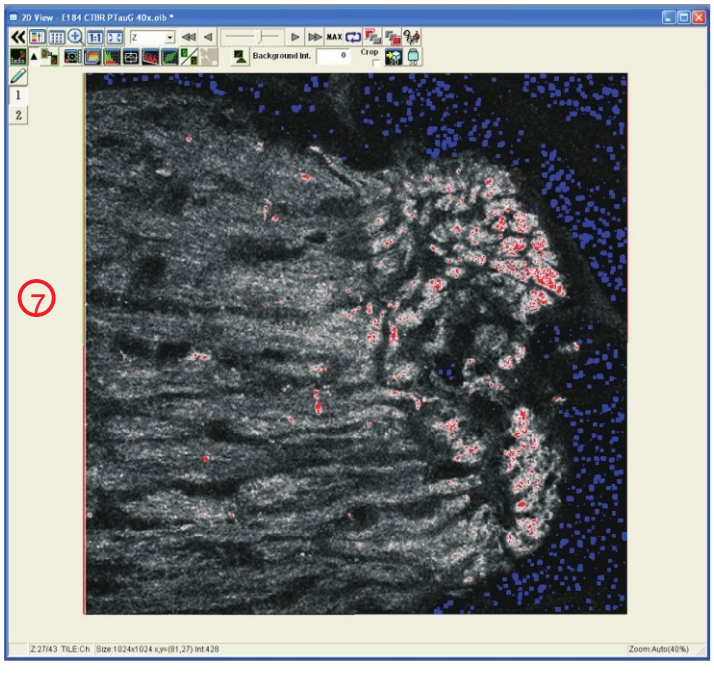
3. Click Focus x4 . (4) Animage (5) will appear. This is a continuous scan of your sample done rapidly at low resolution. To stop it, hit the red STOP button.



4. If you are unhappy with the intensity of an image in one or more channels, you can adjust the HV (brightness) and Offset (black level) for each channel, one at a time, as follows:

A. Turn off all but one channel by clicking in the box to the left of the channel label (6) . Click Focus x4 , then press CNTRL + H on the keyboard. Now the image employs a specialized look-up table (7) in which blue pixels represent a zerointensity value or true black, and red pixels represent an off-scale intensityor white (detector is saturated).





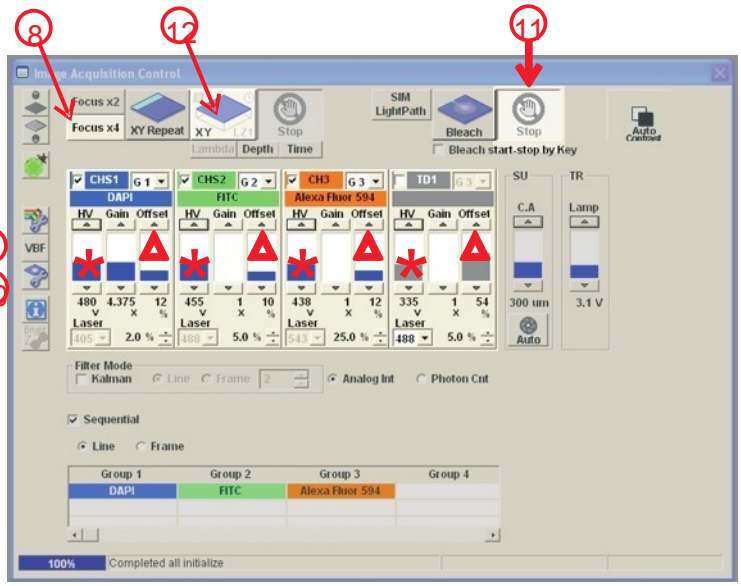
B. You should optimize all settings within the brightest focal plane of your specimen. To do this, click "Focus x4" (8) and focus up and down through your sample. Find the focal plane where pixel saturation (red pixels) is greatest.

C. To maximize your signal-to noise-ratio, adjust your settings so that your image contains a few red pixels and a few blue pixels.

D. To make the image brighter or dimmer by increasing or decreasing thesaturation (depicted by red pixels,) adjust "HV" (9) *.

E. To adjust the black level (background) thereby increasing or decreasing the amount of blue pixels, adjust " Offset " (10) ▲. Offset is set most accurately by temporarily turning off the laser for that channel.

F. Once you have optimized one channel, click "Stop" (11). Turn off the current channel by switching to the next desired channel; repeat steps A – E except for DIC channel. For DIC optimizing, turn to Appendix 2 and follow instructions 6 through 10.

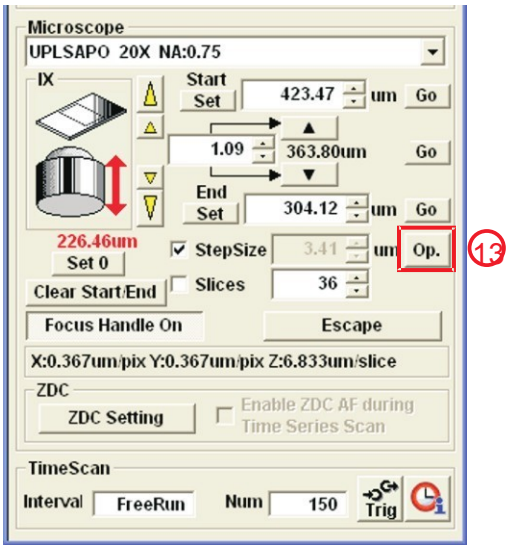


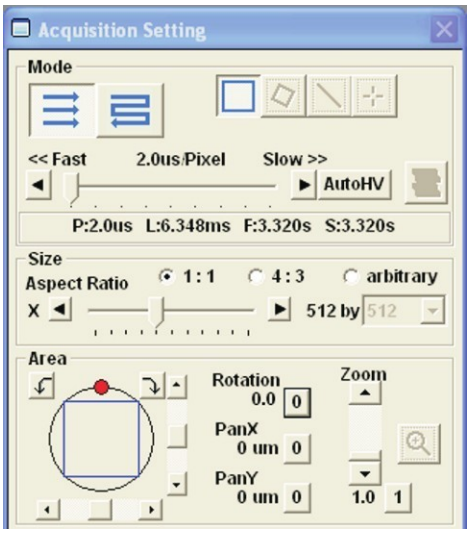
5. To see what the image looks like at this point in optimization, turn on all the channels, and click the "XY" button (12). This shows a single scan that can then be saved.

6. Save images as you normally save any Windows file. The default file format, .oib or .oif, can only be opened by Olympus software. Use the export function in the file menu to save images in other formats. We recommend saving images directly to your CISRstore folder which appears as a separate drive in the folder list. Any data saved on the hard disk of the microscope computer will be deleted after 30 days.

7. At this point, you should consider whether your optical slice thickness, i.e. the z dimension resolution, needs to be changed. This dimension is directly related to the pinhole diameter: smaller pinhole means thinner optical slice and less light reaching the detector. To see your slice thickness, at the bottom of the Acquisition Setting window hit the "op" button (13). Change the pinhole as discussed previously, and then hit "op" again to refresh the new optical slice thickness. Anytime the pinhole size is changed, all channels should be re-optimized.

8. Further optimization can be done in the Acquisition Setting window where the size (resolution) (14), area, and rotation (15) of the image can be changed. Additional optimization depends upon the nature of your sample and should be discussed with one of the CISR staff.





VII: Z-sectioning

1. In the Image Acquisition window, click the depth button (1).

2. Choose the channel that is most appropriate for determining the z dimension size you want and turn the other channels off.

3. Start focus x4 and find the beginning of your z stack, ie. the focal plane nearest the cover slip. Hit the set button (2) just under start at the bottom of the Acquisition Settings window.

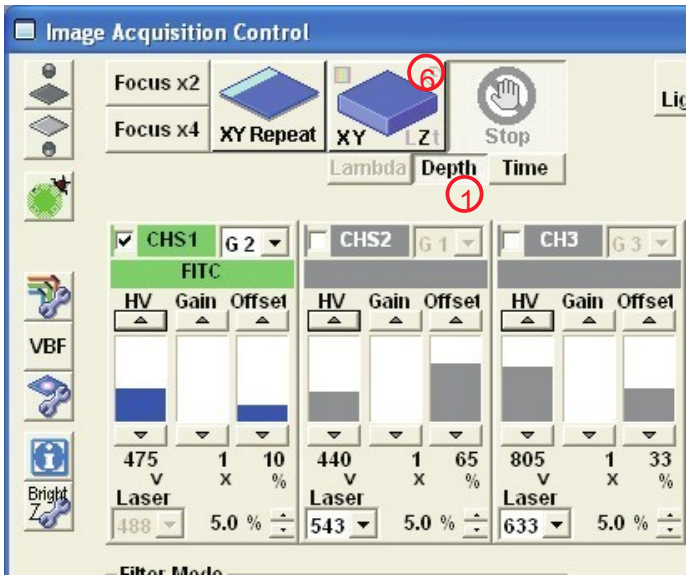
4. Find the last optical section to be included and hit the set button (3) just under end. The number of optical slices is now shown (4).

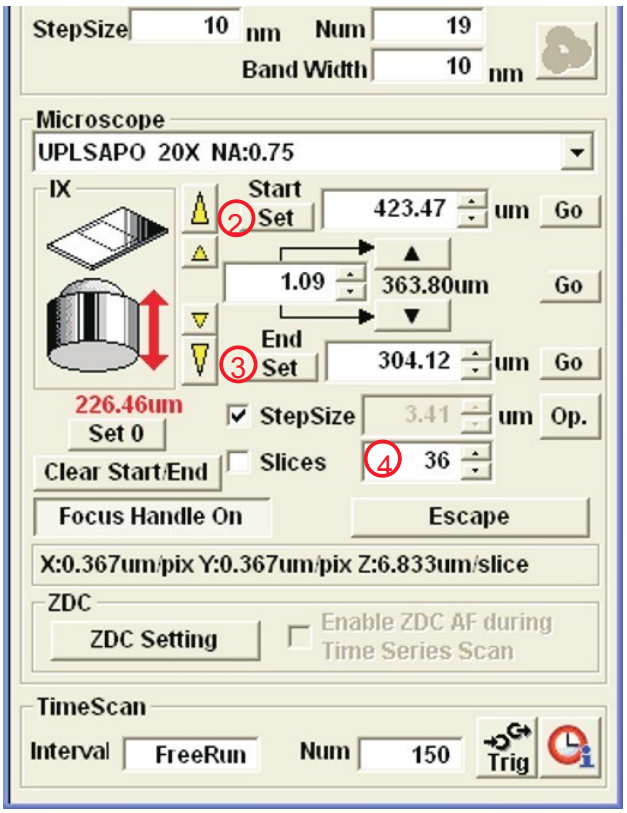
5. Turn on all channels. To see how long acquisition will take, look in themode settings portion of the Acquisition Settings window (5).

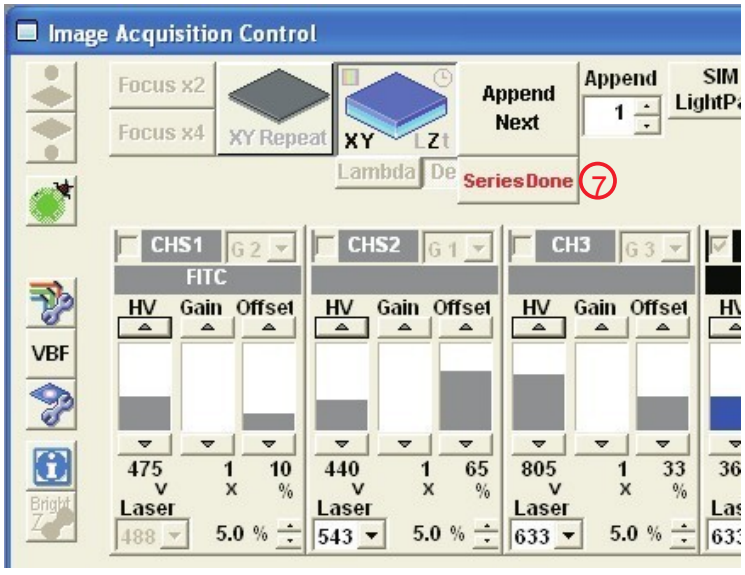
6. Start the image acquisition by hitting the xyz button (6) in the Image Acquisition window.

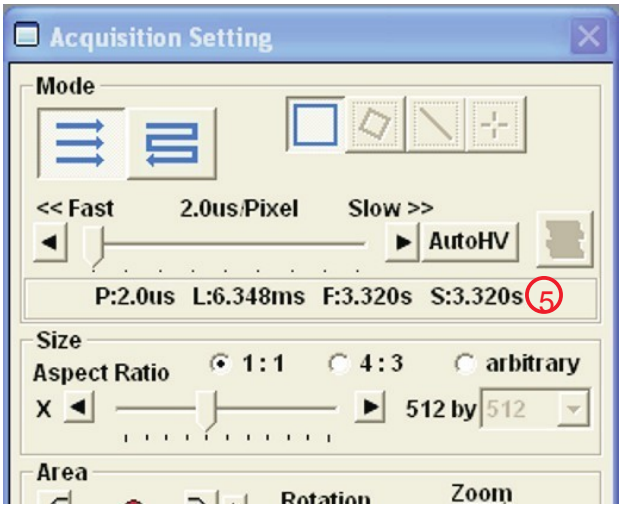
7. When all images are complete, the series done button (7) appears under the stop button in the Image Acquisition window. If satisfied with the series as captured, press this button to end acquisition. Or append the number of optical slices and continue capturing more images.

8. Save the images as you would a single picture.



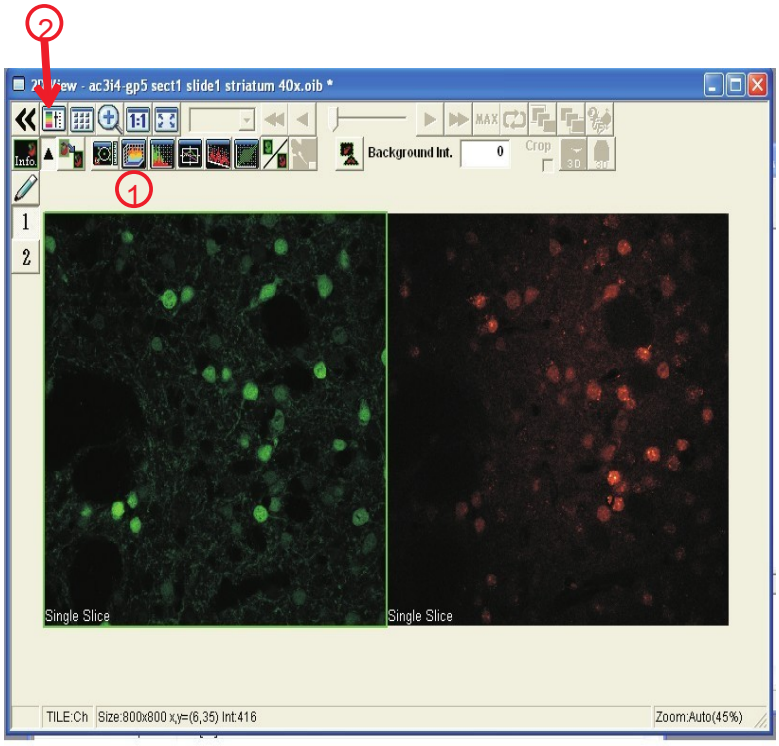






VIII. IMAGE MANIPULATION

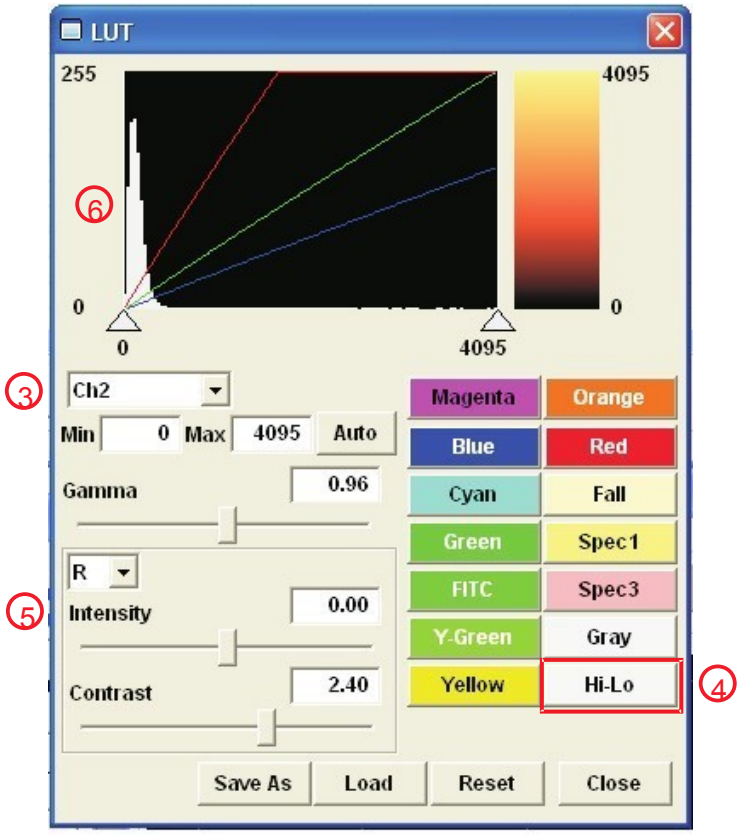
1. Use the icons/buttons along the top and left side of the image to change what you see on the screen without changing the underlying data file (1). Many of the icons are self-explanatory; just place the cursor over the button and wait for an explanatory note to appear.



2. Some buttons open menus or boxes. Commonly used buttons include:

A. The LUT menu (2) opens controls for changing contrast, intensity and gamma for each channel. Choose the channel of interestin the drop-down list (3). Change the color ofa channel by clicking a colored box, or choose one of the special palettes, such asthe Hi-Lo (4) for saturation intensity. Note that the Intensity (5) slider affects only one RGB color at a time: use the drop-down list to switch colors.

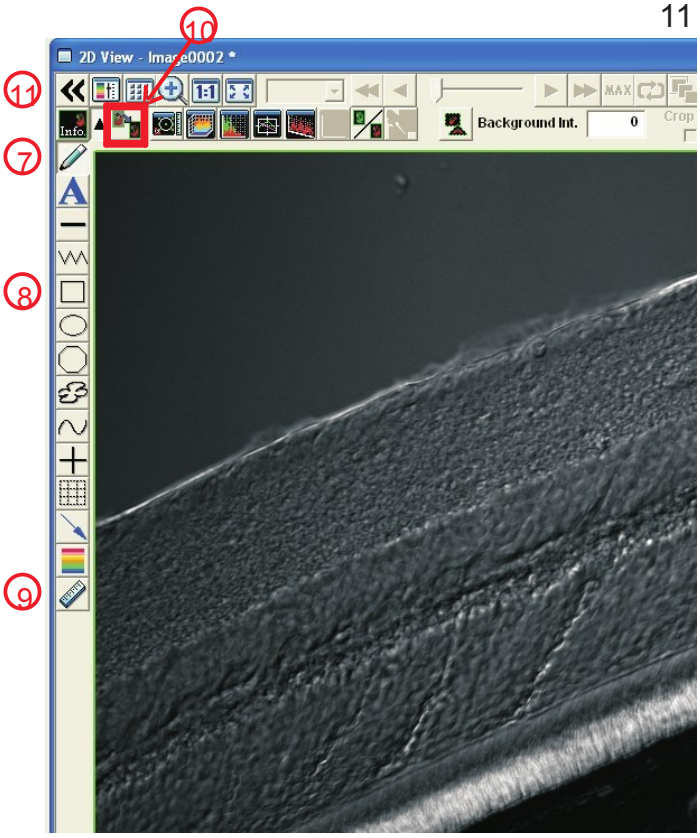
Use the LUT histogram (6) to gain an appreciation for how each slider changes the image. Remember that any changes that you want to keep must be saved as a separate file.

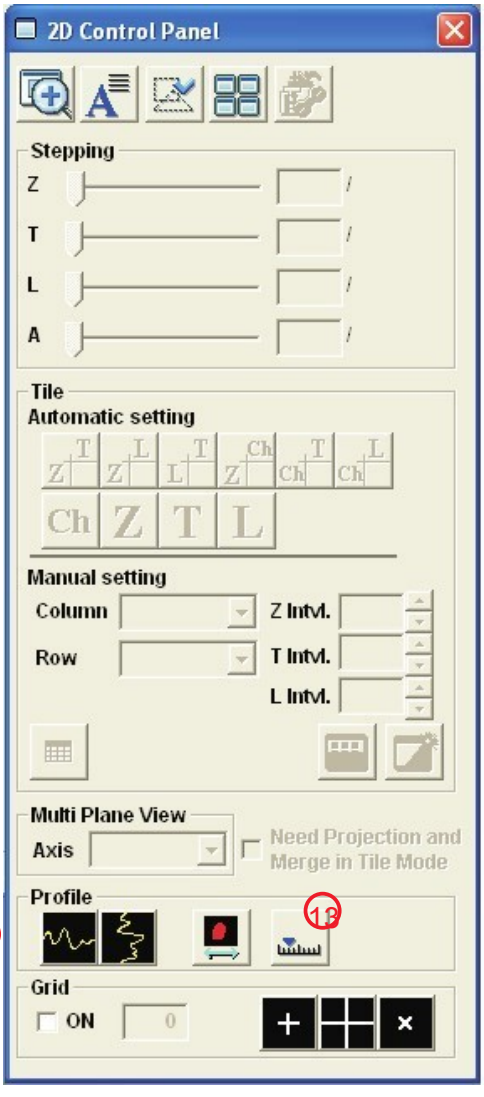


B. The pencil icon (7) opens options to draw regions on the image for cropping (8) and adding overlays such as arrows. Add a scale bar (9) or create an ROI (10) for correcting background.

C. The 2-D control panel (11) can be used to view specialty applications such as tiling, and manual settings of rows and columns for displaying z-stack images.

D.The profile (12) buttons are used to show intensity values for each color along a movable vertical or horizontal line. The scale bar (13) button adds a scale for the intensity profile.



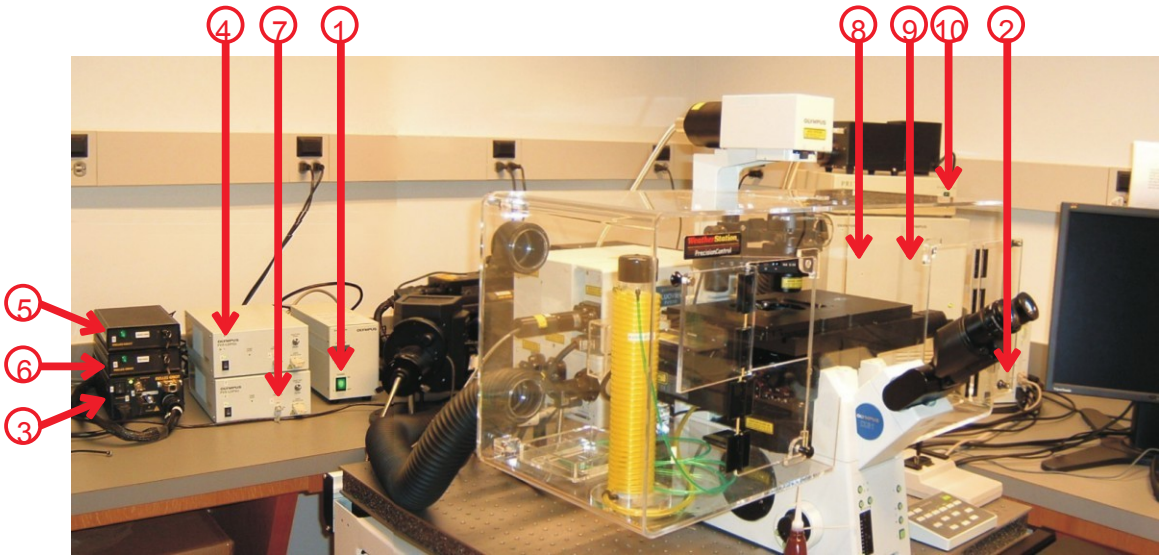


Acknowledgment:

Our funding depends upon your citing the Cell Imaging Shared Resource when you publish data obtained with equipment or services from CISR. This would include images from the microscopes or training in the use of software, . The minimum acceptable acknowledgment should read:

"Experiments/Data analysis/presentation [include what you use]" were performed in part through the use of the VUMC Cell Imaging Shared Resource, (supported by NIH grants CA68485, DK20593, DK58404, HD15052, and Dk59637).

Please let us know when you publish and, if possible, send us a reprint of the paper. This is how we justify our existence.



IX. FV-1000 SHUT DOWN PROCEDURE

1.Wipe off the oil from any oil objectives you may have used during your session.

2. Close and exit the FV-1000 software.

3. Transfer any data you may have saved on the microscope computer.

4. Check the calendar to see if someone is coming in within ½ hour of your end time. If so, leave the entire system on. If not, follow the remaining steps for proper shut down of the system.

5. Because the argon laser requires a cooling-off period, turn it off first by turning the key (3) B 90° to the left.

6. Shut down the computer by clicking Start/Shut Down in Windows.

7. Turn off the power switch for the Prior Proscan II motorized stage controller. (10)

8.Turn off the power switch for the Main controller (9). The key should always be in the “On” position.

9.Turn off the power switch for the Sim controller (8) . The key should always be in the “On” position.

10. Turn the key off, 90° to the left, for the laser power supply (labeled 7B).Turn off the power switch (labeled 7A.)

11. Turn off the key for the red HeNe power supply 90 degrees to the left (labeled 6).

12. Turn off the key, 90 degrees to the left, for the green HeNe power supply (labeled 5).

13. Turn the key off, 90° to the left, for the laser power supply (labeled 4B). Turn off the power switch (labeled 4A).

14. The Argon laser should already have been turned off. If not done previously, turn the key off, 90 degrees to the left (Labeled 3B)WAIT 2 to 3 MINUTES FOR THE FAN TO COOL THE LASER BEFORE PROCEEDING TO STEP 15.(You can do steps 16-18 while waiting.)

15. Turn off the power switch for the Argon laser (Labeled 3A).

16. Turn off the microscope control box (Labeled 2).

17. Turn off the mercury power supply (Labeled 1).

18. Sign out of the log book.

Protocol for Access to 10434 MRB IV
Cell Imaging Shared Resource

Room Access

- You must use your VU ID card to unlock the main door.
- Your VU ID card must be authorized to open the door (see authorization below.)
- Upon entry the door must be closed and locked – DO NOT prop the door open.
- Do not lend your card to anyone for entry – you will be responsible for loss or damage.
- If your card is lost or stolen, report it immediately to a CISR staff member (see contact below.)
- You will be photographed by a security camera upon entering the room.

Authorization

- Authorization of your card will require registration and proof of training.

Useful Links

- CISR's homepage: http://cisrweb.mc.vanderbilt.edu/CISR/
- Molecular Expressions – FSU's encyclopedic microscopy site http://micro.magnet.fsu.edu/primer/index.html

Free software to open your images

Please contact one of the staff in the CISR about free browser software needed to open your FV1000 images and export them as .tiff or other file types.

Contact Information

Jenny Schafer 3-3750, jenny.c.schafer@vanderbilt.edu
Sean Schaffer 6-3706, sean.schaffer@vanderbilt.edu
Bob Matthews 343-3750, robert.matthews@vanderbilt.edu