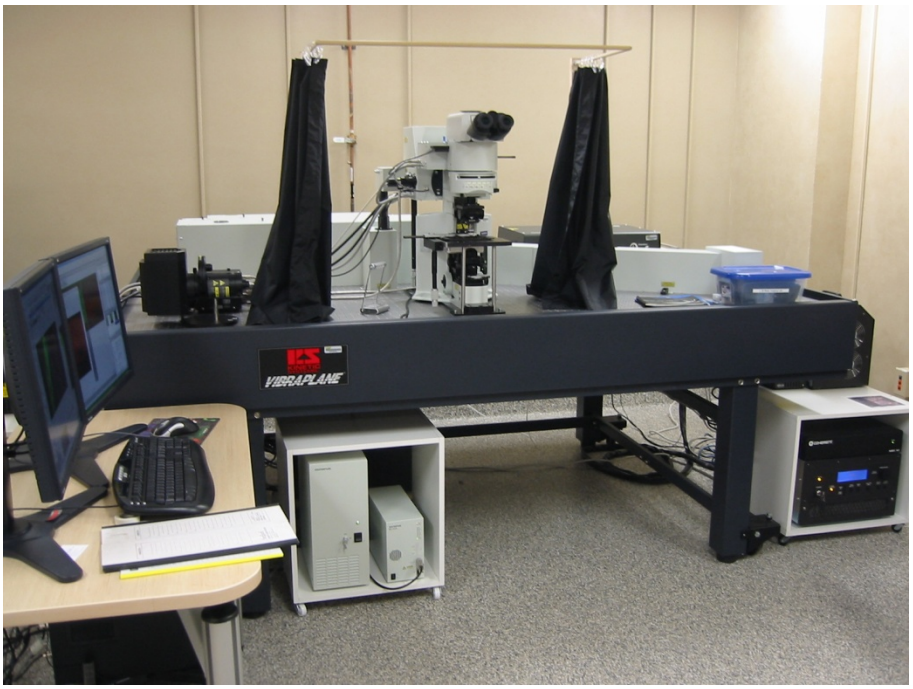
Olympus FV1000 MPE Microscope User Guide



A. System Start Up

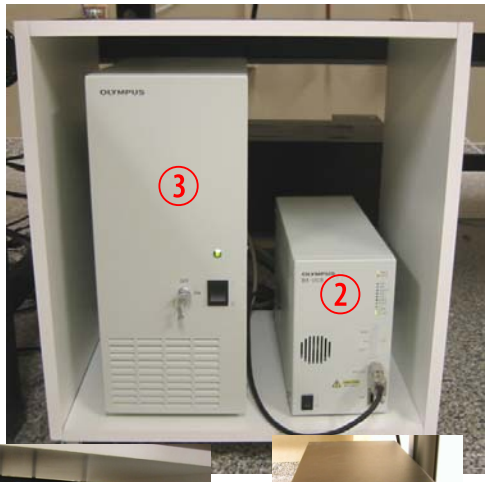
1. Sign in to the log book. Indicate the laser lines you will use; visible laser and IR laser.
2. Turn on mercury lamp power supply ①.
3. Turn on microscope controller and scanner.

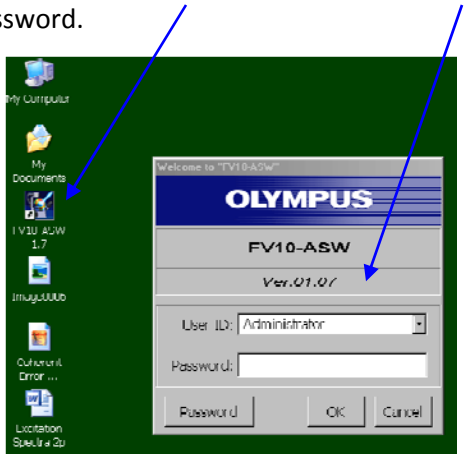
   Microscope controller- switch ON ②

   Scanner – switch ON and turn Key to ON ③.
4. Turn on laser combiners ④⑤.
5. Turn on laser power supplies

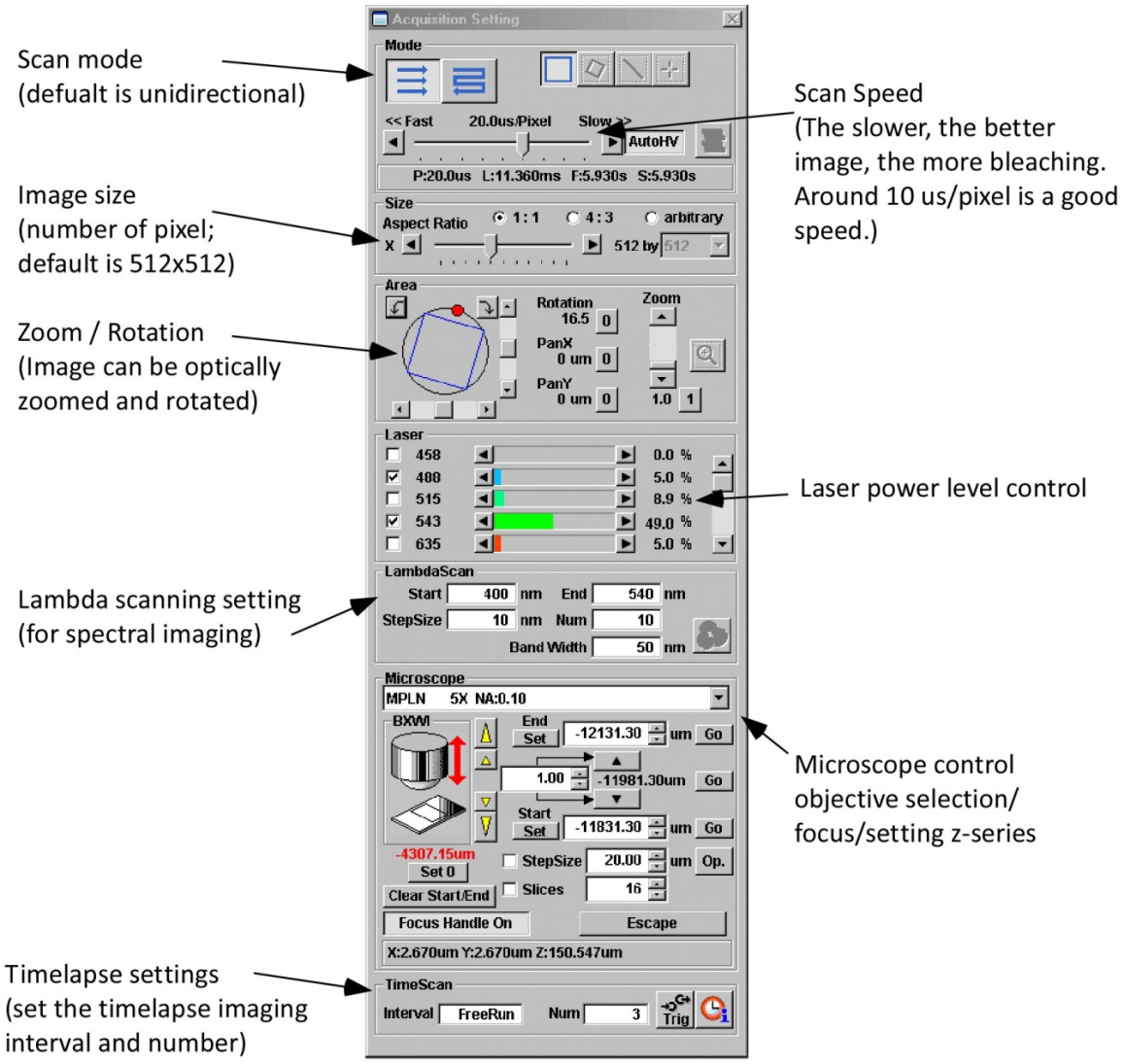
   Multi-Ar ⑥: Switch to ON and turn Key to ON.

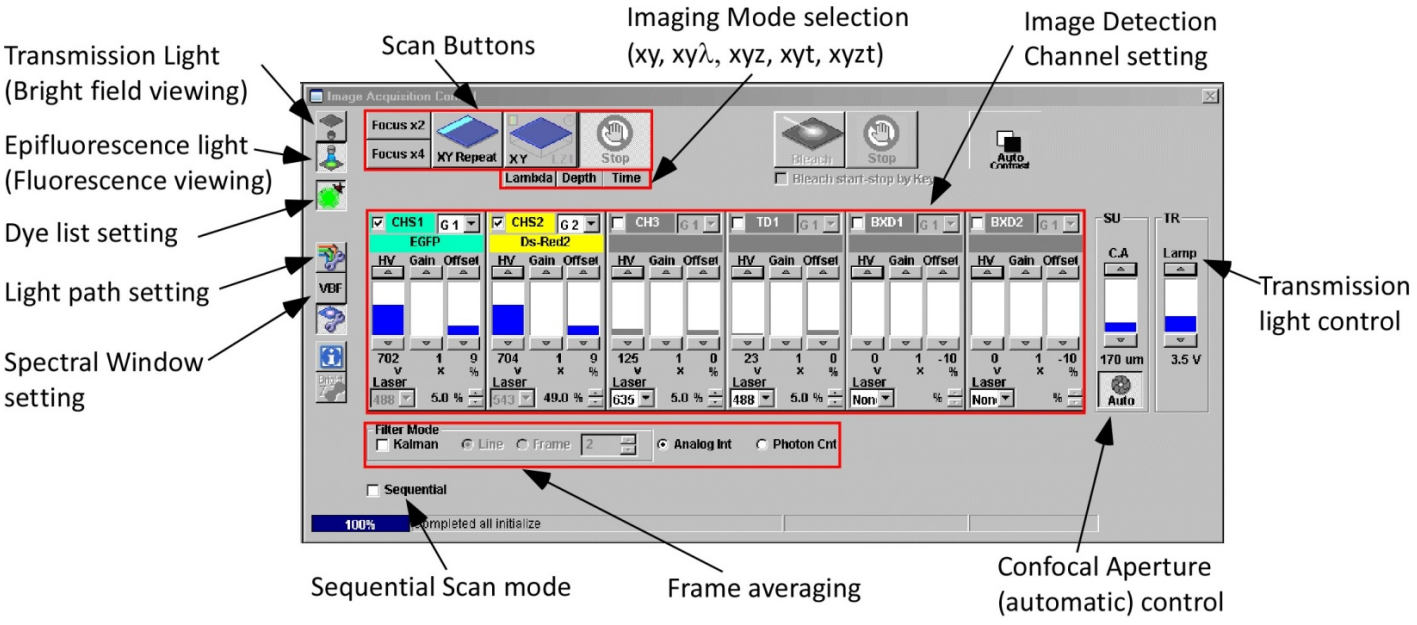
   HeNe ⑦: Turn Key to ON.
6. Turn on computer ⑧ if it is not on.
7. Enter user name/password to log on to Windows XP.
8. Start the FV10-ASW program and enter User ID and password.

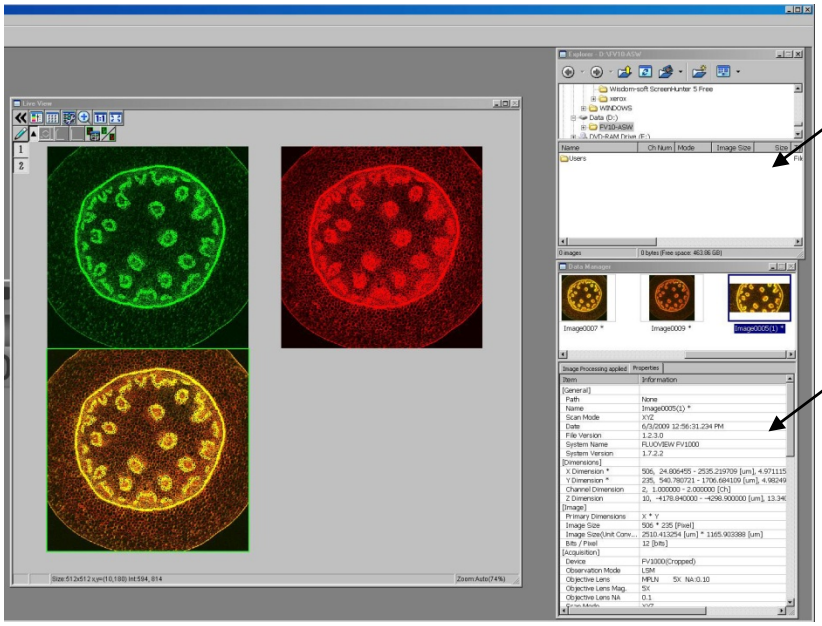




B. Outline of Acquisition Setting / Image Acquisition/ Image Viewer Windows

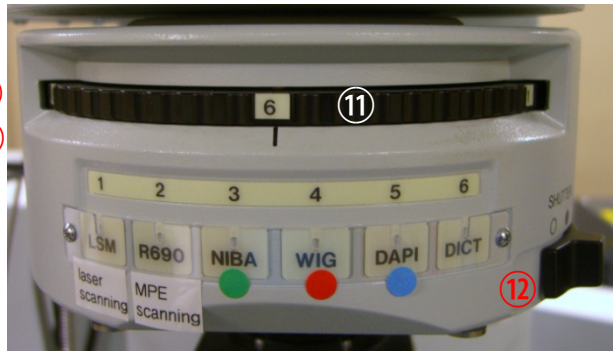






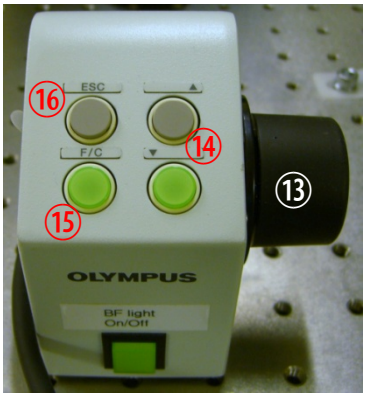
C. Viewing with Transmitted or Epifluorescence Light

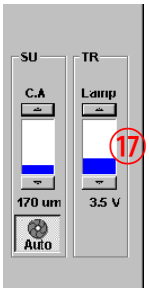
Before scanning the sample with laser light, look at the sample and find the region of interest first with either transmitted or fluorescent light.



Click on transmitted light button ⑨ for transmitted light viewing or on epifluorescence light button ⑩ for fluorescence viewing (Before click on the light button you will used, click off the pressed button first).

Turn the filter wheel ⑪ to #6 (DICT) for transmitted light viewing, or to #3 (Green NIBA), 4 (Red WIG), or 5 (DAPI) for fluorescent light viewing. Open the shutter ⑫.





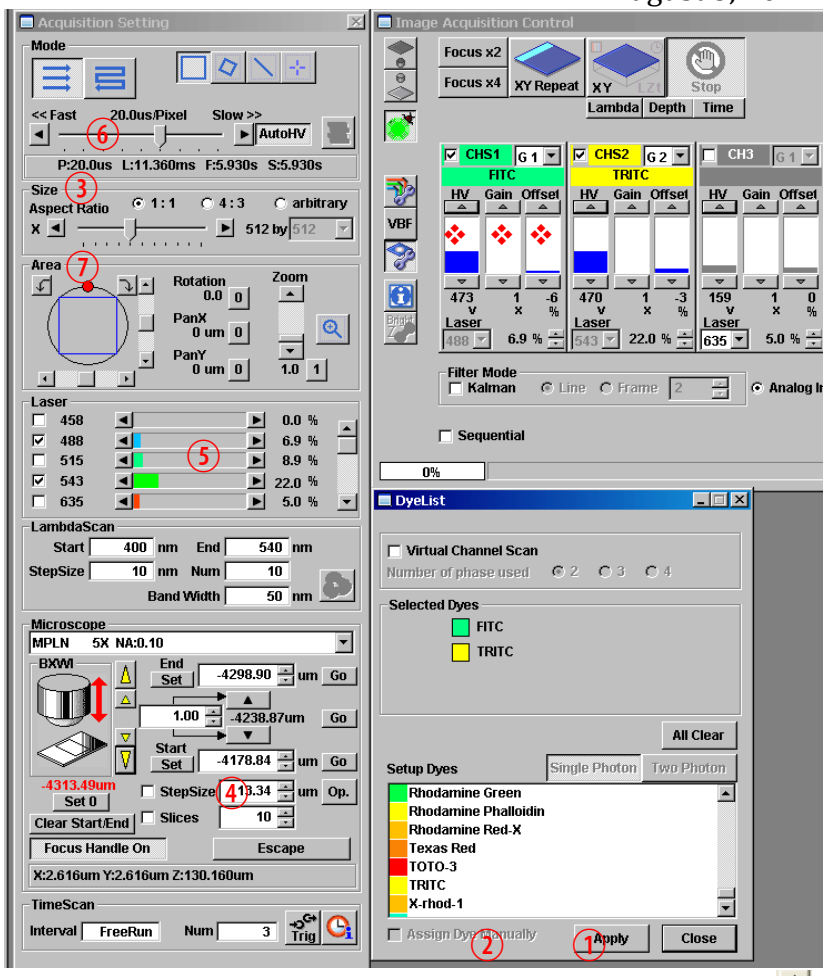
MAKE SURE THE SLIDE AND COVERGLASS ARE CLEAN AND SEALED. Place the slide on the microscope stage. Focus onto the sample with the **focus knob** ⑬ (fine adjustment) or **focus buttons** ⑭ (fast focusing) on the remote controller (Note: the focus knob on the microscope doesn't work if the remote controller is used). The extent (fine or coarse) of movement by focus knob⑬ can be selected alternatively by pressing **F/C button** ⑮.

Press **Esc button**⑯ to move the objective completely away from the sample and press again to bring the objective to the original position.

Adjust the transmitted light level with the lamp controller ⑰ on the **Image Acquisition** control window.

When you are ready for laser scanning, turn the filter wheel ⑪ to #1 position (Laser Scanning label).

D. Image Acquisition



Click off the light source button to turn off the non-laser light.

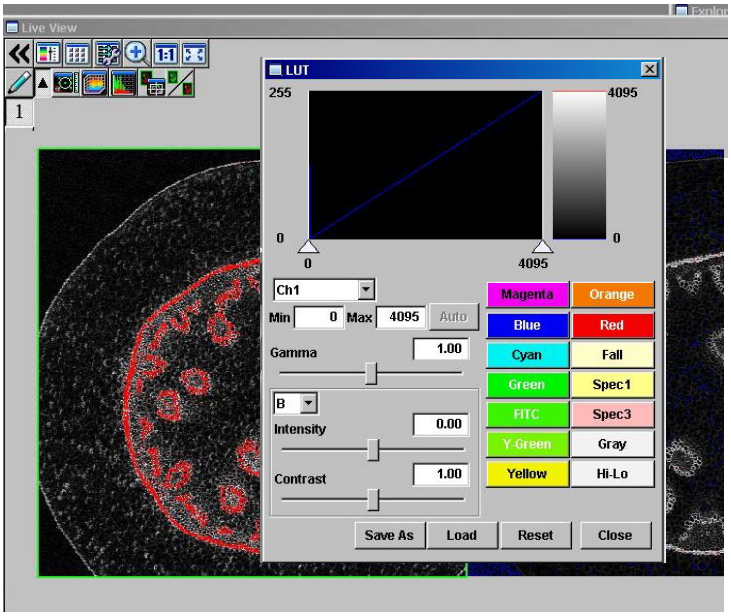
Click on **Dye list** button and double click on the fluorescent dyes you want to use (e.g. FITC and TRITC) from the list. Click **Apply** button① (if **Assign Dye Manually** button② is checked, uncheck before pressing Apply button). It will activate the laser lines and set detection channels up according to the dyes you selected.

Choose **Image size** (default- 512x512) from **Size** panel ③.

Press AutoHV button (if not pressed) to automatically adjust HV and Offset values according to the scan speed change. Click Focus x2 button to scan the sample. It will scan fast at 2 us/pixel and show low-quality (pixelated) image in a Live View window.

While scanning, focus onto the region of interest with the remote focus controller or by clicking arrowhead buttons in the microscope window (large arrowhead move the objective by the extent set by **StepSize** ④, and the small arrowheads moves the objective by the half of the large arrowhead step size).

Click **Stop** button RepeatStop to stop scanning.

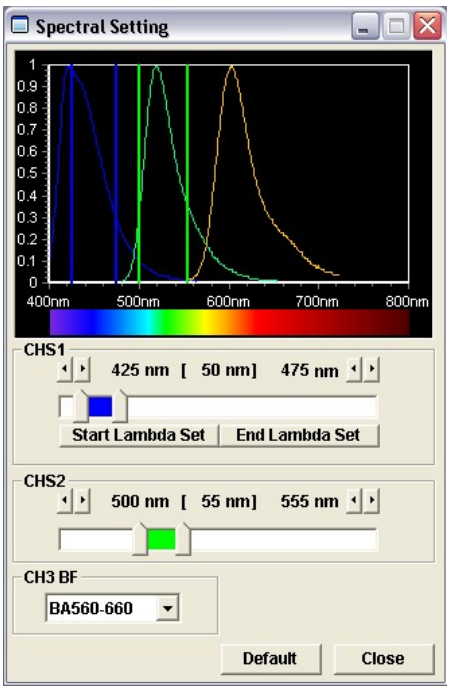


Set the **Laser** output level ⑤ (set the Multi-Ar laser to 1-5% and the HeNe laser to 1-25%, depending on your sample condition, but no lower than 1%).

Set the **scan speed** ⑥ to 10~12.5 us/pixel and click **XY repeat** XY Repeat button to scan (you can scan image faster or slower, depending on your sample).

Detector (CHS) Channel Setting ❖: Adjust the brightness by **HV** (and **Gain**) and background black level (**Offset**) of individual channels. *Keep the HV value below 700 (beyond 700, the background noise will show up).*

You can optimize the image quality with **Hi-Lo LUT** (Look-Up Table). Click **LUT**  button to bring the LUT control window and select Channel and click **Hi-Lo** button (***Ctrl-H*** shortcut key will do the same without opening LUT window). Now the image is displayed using this specific look-up table where red pixels represent intensity beyond scale (saturated) and blue pixels represent 0 pixel value. To maximize the signal-to-noise ratio of the image, adjust the acquisition setting (laser power level, HV, Offset) such that the image shows a few red and blue pixels while you are scanning.



Often excitation/emission profiles of fluorophores you are using may be close, so there could be possible bleed-through of a fluorophore emission to neighboring channel. To minimize this, the detecting range of each channel can be adjusted by changing **Spectral Setting**. Click on 

, it will bring Spectral Setting window. Change the range of spectrum for each channel (CHS1 and CHS2) by sliding, widening, or narrowing the tabs 

or arrowheads.

Use **Area** panel ⑦ to rotate and zoom the imaging area (click on 0 and 1 buttons to return to the original viewing area). To rotate the viewing area, click on the red dot and drag clock- or counter-clockwise. With zoomed view, you can select the scanning area by moving the blue-lined box around. To zoom a specific region in, click  icon and draw a box on the live view area. It will zoom in the area specifically.

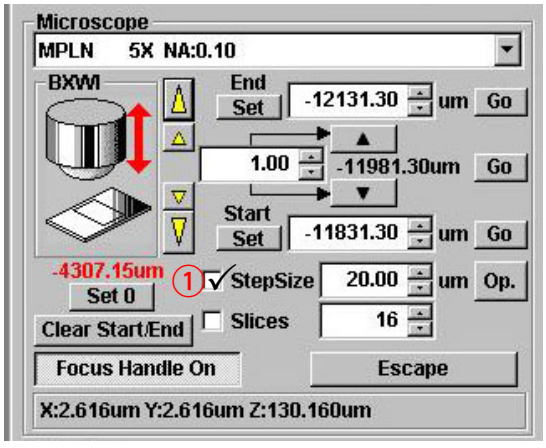
Once you are satisfied with the setting, stop scanning. Set the **scan speed** to slow rates (~10-12.5us/pixel) (the slower the speed set, the better the signal-to-noise ratio, but the more bleaching). Click **XY button** 

to acquire an image. When acquisition is done, a 2D view window will appear.

E. Z-Series Image Acquisition

Use this mode to obtain optical section through the depth (z dimension) of your sample that can be used for 3D visualization. Click 

button to scan. Use the arrowheads  buttons or the **fine focus knob on the remote controller** to focus into different Z-axial planes (large arrowhead buttons shift a full step size and small ones a half step size). When you find upper limit of your sample, click **End Set** button. Bring the objective down until you find lower limit and click **Start Set** button. Determine the **Step Size** and the number of **Slices**, which correlate with each other. It is recommended to set the **step size** similar to the optical section thickness of the objective you are using, so that there is no gap between the optical sections upon projection into 3D. The step size can be fixed by checking the box ①.



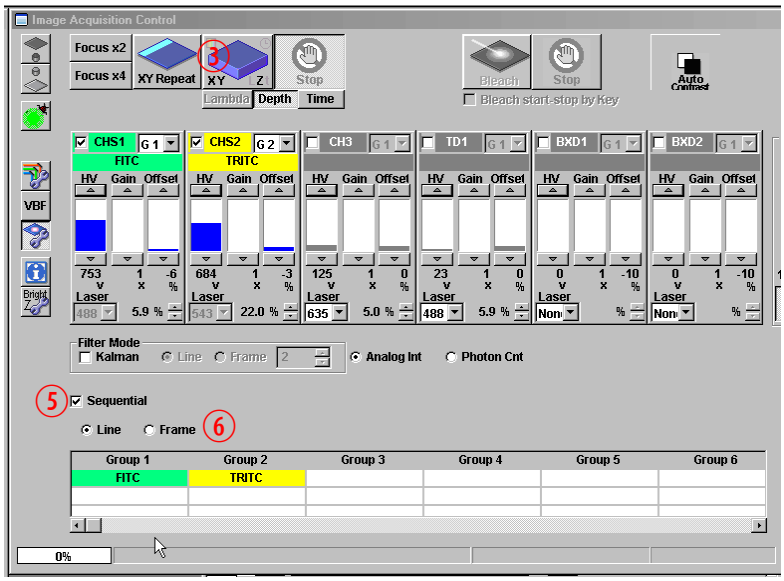
Click Stop 

button. Adjust the Scan Speed if needed, click **Depth** ②button ("**Z**" will be appear on the **XY button** to become XYZ), and then click **XYZ** ③button.

When acquisition is done, **Append Next/SeriesDone** ④ button will appear over the Stop button. Click **Append Next** button to add additional sections at next step (enter the number of series you want to add) or click **SeriesDone** to finish the acquisition. Save the image.

F. Sequential Scanning Mode

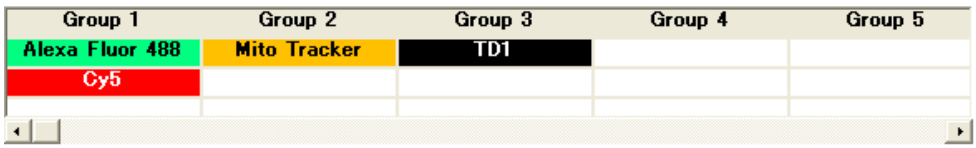
Use sequential scanning to prevent possible cross-talk and/or bleed-through between the emissions of the fluorophores you used.



Check the **Sequential** box ⑤ and it will bring up the sequential scan information window. Choose between **Line** or **Frame** mode ⑥.

Line: by line-by-line, it scans one channel with only a specific laser line and detector on and then sequentially scans the next channel with only a another laser line and detector on.
Frame: it scans sequentially frame-by-frame; it finishes scanning one channel first and then scans the next channel.

If there is no bleed-through between the emissions of some fluorophores you are using, you can group them together to scan them simultaneously as a group. Simply click on the fluorophore name on the list and drag to the other group.

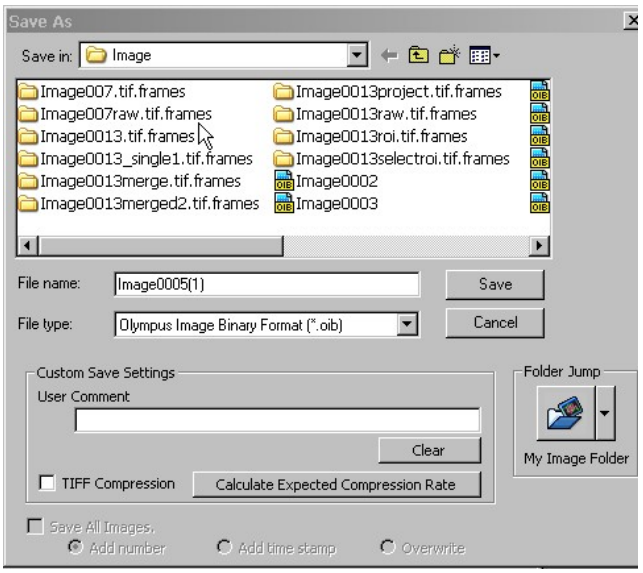


Note: it will be good to set up the acquisition condition for each channel before using the **Sequential** mode. To do so, first in simultaneous mode (unchecked Sequential box), set the acquisition condition by turning on only the specific laser for the specific channel.

Scan with **Focus x2** or **XY Repeat** button to see to check if there is any bleed-through. If not, stop scanning, and set the Scan speed at a slower rate and click **XY(Z)** button to acquire the image.

G. Saving and Exporting Images

1. Click on the image window to be saved.

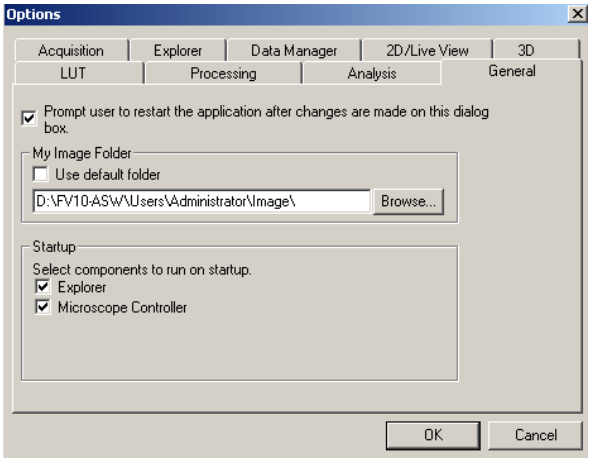


2. Click on  icon or select ***File/Save or Save as*** from menu.

3. A **Save as** window will appear. In case the image should be saved in the Image folder of a Log-in user, click on 

button and it will direct to your image folder. Select **Olympus Image Binary Format *.oib** file type, type file name, and click on **Save** button. (Log-in user and its associate folder will be created as you become self-user).

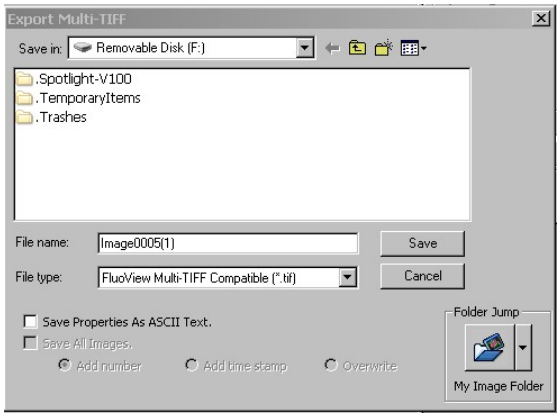
4. To set up your image folder where you can jump directly by clicking **My Image Folder** button, select ***Tools/Option*** menu. Click **General** tab and specify the path of your image folder with **Browse..** button. [*Upon creating a user account, each user will have its own folder in **D** drive (Do not create any folder in **C** drive.).* Click OK button.



5. Oib file type contains all the metadata including all the acquisiotn parameters and it can be opened in FV10-ASW program. The light version of this program is available for installing in user's own computer. Otherwise, the image file can be exported as other file format so that it can be opened and processed in other imaging software.

To export images:

1. Select ***File/Export*** or ***File/Export Multi-Tiff*** menu. Click **My Image Folder** button if the current folder is not yours.



2. With ***Export Multi-Tiff*** option, it saves the image as a single tiff file that can contain multiple frames, like z- or timelapse- series. This file type can be opened in Image J software.

   Check the **Save Properties As ASCII Text** box to save the metadata that contain information about the image acquisition setting. This data will be useful to find out the imaging condition later with other programs.

3. ***File/Export*** command brings a window with different options.
   This command will export images into a folder that contain individual images of each channel, z-steps, or time-points.

   Select **File type**① for export.

   ROI Overlay ② section allows saving the file with ROI information.

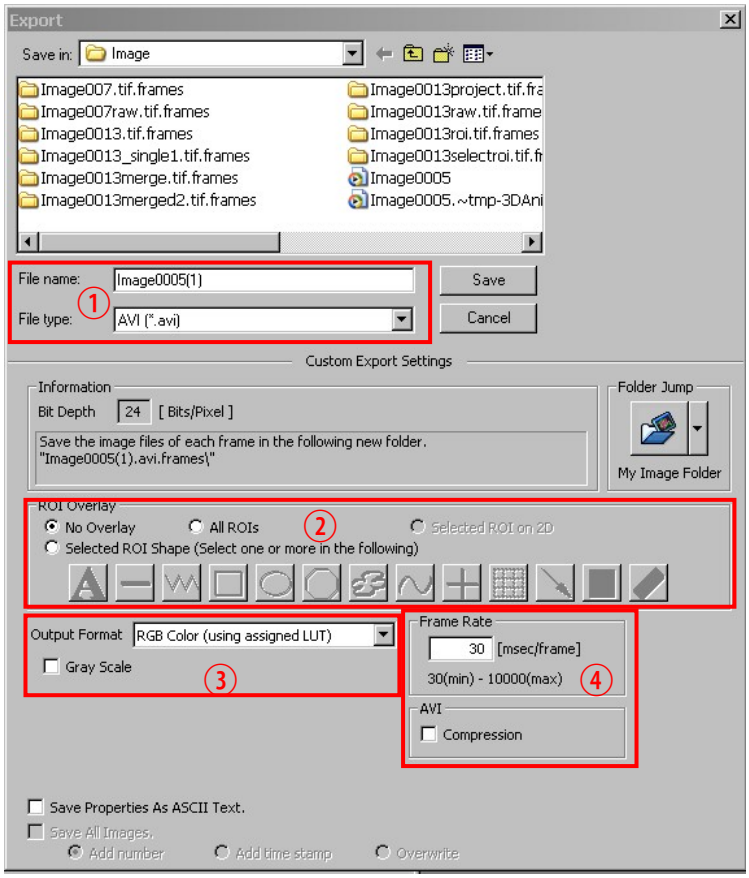
   Output Format ③:

   RGB Color: the red, green, and blue channel will be saved in color as individual *.tif files in a folder.

   RGB Color + Gray Scale box checked: the color channels will be saved in gray scale as *.tif files in a folder.

   Merge Channel**:** a single merged color image will be saved as a *.tif file.

   Frame Rate④ determine the play speed of the movie when the file is exported as AVI.
   Check the AVI box to compress the movie file.

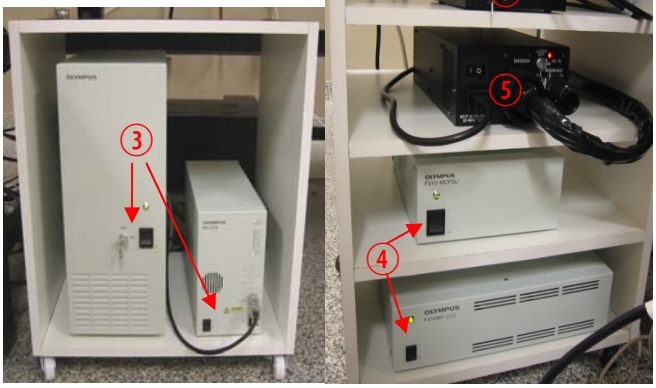


4. Check the **Save Properties As ASCII Text** button if you export the metatdata. Click **Save** button.

5. Transfer your image data to CD or USB flash memory.

G. System Shut-Down Procedure

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



Logging off and transferring data.

1. Exit the FV10-ASW program.
2. **Make sure that you transfer your image file to CD or USB flash memory. All image files more than a month old will be removed during monthly clean-up.**
3. Log off Windows XP. If someone signs up for use within a next 1 hour or so, leave the system ON. If not, shut down the system as follows.

Shutting Off the system.

1. Shut down the computer ①.
2. Turn the mercury lamp off ②.
3. Turn the microscope controller key to OFF and turn off both scanner and controller I/O switches ③.
4. Turn off the laser combiners ④, FV10-MCPSU and FV10-MP-LCU.
5. Turning off the Multi-Ar laser ⑤ by turning the key switch to OFF first and then turn the I/O power switch to OFF.
6. Turn the key of HeNe laser ⑥ to OFF.