# Olympus FV1200 MPE Microscope User Guide

A. Microscope Start Up

1. Sign-in to the log book with the start time.

2. Turn on mercury lamp power supply ① by pushing the ON/OFF button and releasing it (The light should lights up in **Blue**).

3. Turn on microscope controller ② and XY stage controller ③.

4. Turn on the touch panel controller ④ and touch "Start Operation".

5. Turn on both scanner controllers (MAIN and SIM) ⑤; switch ON and then turn the key ON.
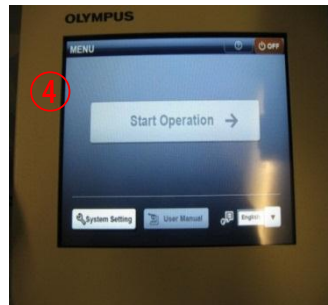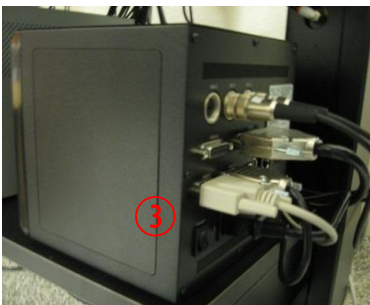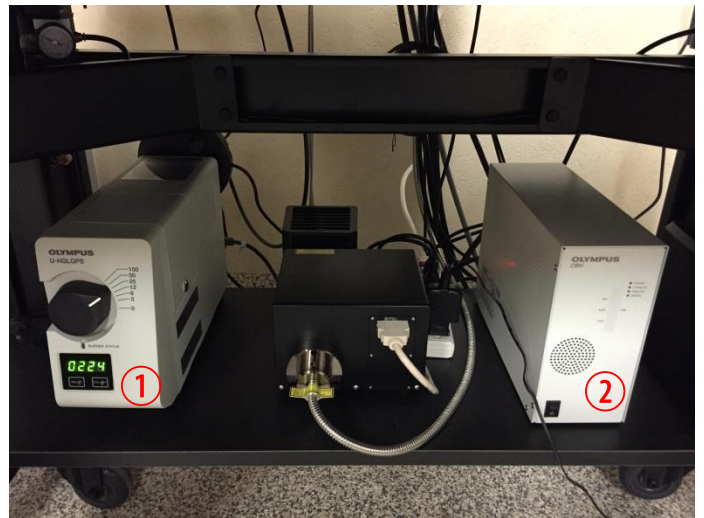
6. Turn on the computer if it is not on.
7. Enter user name/password to log on to Windows7.

B. Laser start up

1. Turn on laser power supplies

   a) Multi Ar laser ⑥: Switch to ON and turn the Key to ON.

   b) MCPSU (405/440/473/559/635nm)⑦ : Switch ON.

   c) LD559 laser ⑧: Switch on and WAIT UNTIL THE TEMPERATURE LIGHT STOPS BLINKING and THEN turn the Key to ON.

Double click FV10-ASW program **and enter User ID and password.**

C. Outline of Acquisition Setting / Image Acquisition/ Image Viewer Windows
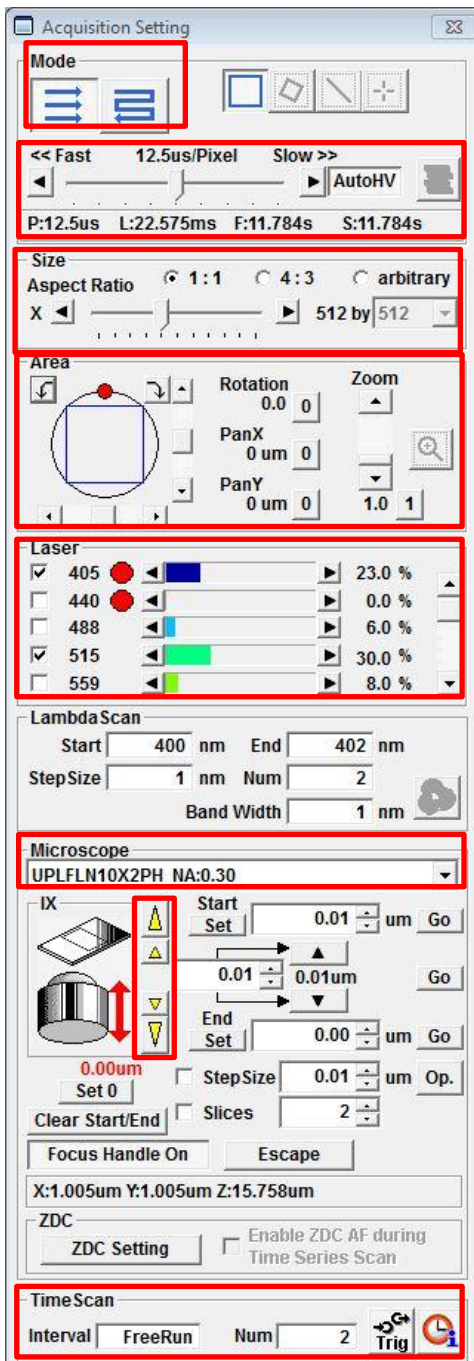
Before taking images, let's get familiar with buttons and sliders on the **Acquisition Setting** Window (such as scan mode, scan speed, image size etc).

Scan mode: the default is unidirectional.

Scan Speed: The higher value, the slower, the better image. Around 12.5~20 us/pixel (microsecond per pixel) is a good speed.

Image size (number of x,y pixel): 512x512 pixel is the default.

Area: the image field can be optically zoomed in and rotated.
To rotate the viewing area, click on the red dot and drag clock- or counter-clockwise (or click). Zoom the imaging area with the slider (click to return to 1x). With zoomed view, you can select the scanning area by moving the blue-lined box around.

Laser power control: Checking the box will activate the laser. The power can be adjusted by pointing the mouse pointer to the slider area and clicking (large change), clicking the arrow heads (small change), or rolling the mouse wheel on the slider area.

**Lambda Scan:** for spectral imaging.

Microscope: Objective selection is displayed. Also selecting the objective on the dropdown list will automatically change the objective.

Z position setting: Clicking the large arrow heads will move the objective up or down to focus onto the sample. The extent of movement is determined by the Step size. Clicking small arrow heads will move objective in half the Step size.
Start Set and End Set determine the z series range.

Time Scan: set up the time lapse series condition (interval and number of image acquisition.

D. Overview of Image Acquisition Control window
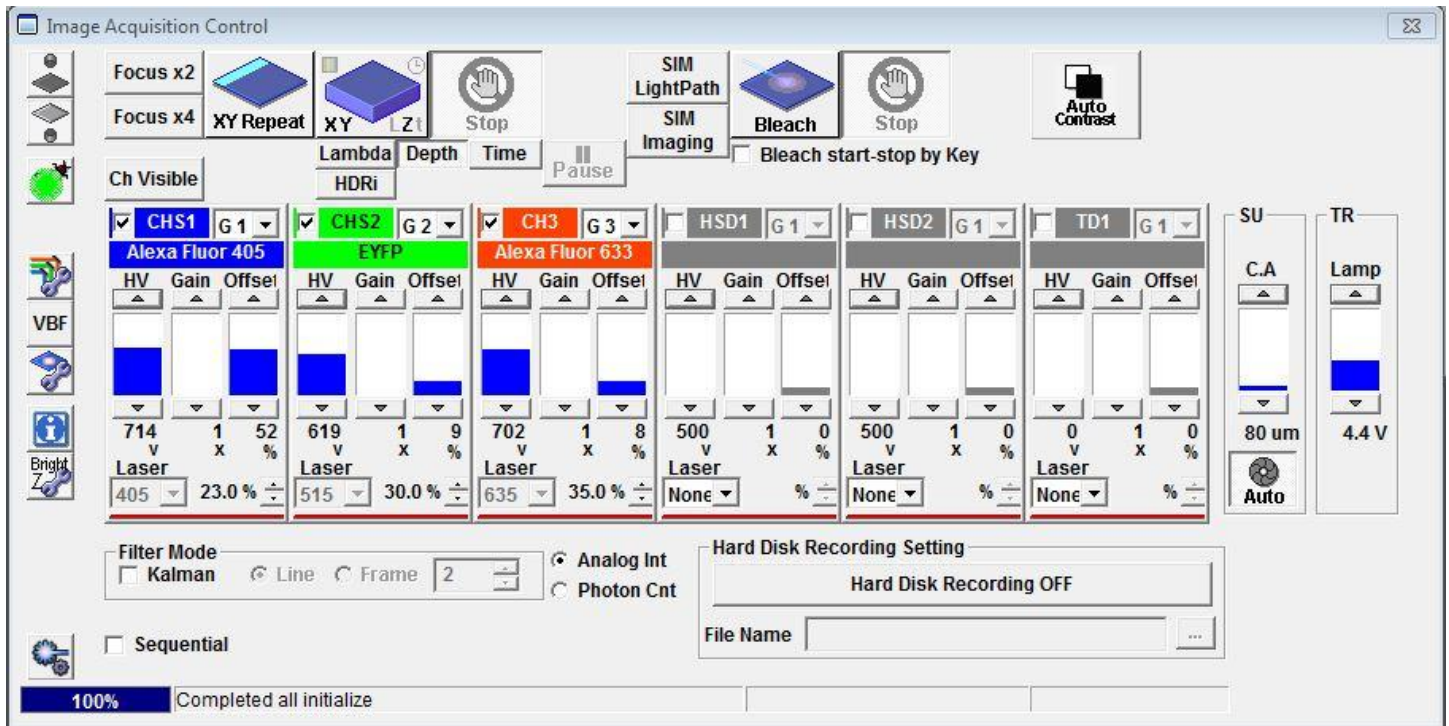
: Click Transmission light or Epifluorescent light to turn it on and off.

 : Click on **Focus x2**, **Focus x4**, or **XY Repeat** begins scanning without image acquisition.

XY Repeat scans at the imaging speed that you set with scan speed on Mode panel.

Focus x2 automatically scans the sample at 2us/pixel – because of its fast display refreshing rate, this will be good for focusing and searching for ROIs without bleaching your sample.

Focus x4 will be faster, but the display quality may be too rough. Click **Stop** scanning.

To acquire image, click **XY (LZt)** .

 : Click **Lambda** to acquire lambda scan imaging (XYL). Click **Depth** for z-series image acquisition (XYZ). Click **Time** for time-lapse image series (XYt).

 : Click on this will open the **Spectral Setting** window, where the range of emission wavelength can be adjusted to prevent overlap between fluorescences.
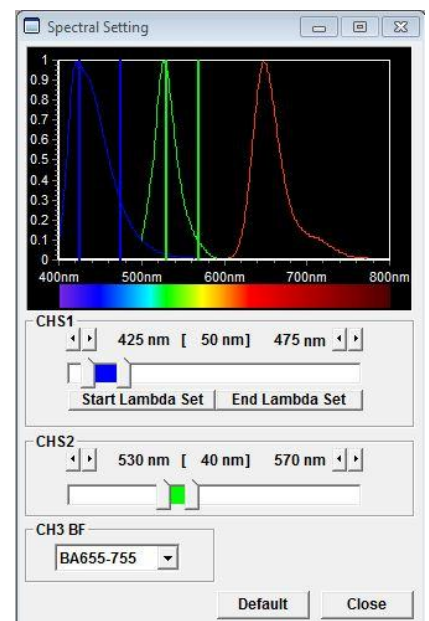
E. Viewing with Transmitted or Epifluorescence Light

Before scanning the sample with laser light, look at the sample and find the region of interest first with either transmitted or fluorescent light. MAKE SURE THE SLIDE AND COVERGLASS ARE CLEAN AND SEALED. Place the slide on the microscope stage.

Move the stage using the joystick on the XY stage controller. Push the speed (Fast/Med/Slow) button① to change how fast the stage moves. (You can also use the side wheels to control x and y separately by pushing the **XY/Z button** to select XY on screen.)

Press **Esc button** (on LED touch panel ② or on FV10 program) to move the objective completely away from the sample and press again to bring the objective to the original position.
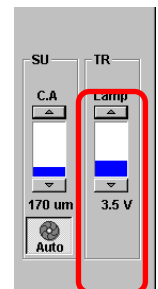

Fluorescent image observation

1. Select an objective lens using the touch panel controller (TPC).

2. Click of the FV10-ASW program or [EPI] button of TPC.

3. Select the appropriate **fluorescence filter** by touching a mirror button on TPC. Adjust focus using the focusing knob.

4. After finding area of interest, click or [EPI] button on the TPC to close the fluorescence light shutter and change the light path to the laser

scanner.

Transmitted light image observation

1. Click or [DIA] button of the TPC to turn on bright field light.
2. Click on [BF], [DIC], or [Phase] button on the TPC. Adjust the light intensity with the lamp controller or by touching Lamp arrows on [DIA] tab.
3. For DIC viewing, insert the DIC slider and adjust the DIC contrast using the prism controller.

4. After observation, click ![shutter icon] or [DIA] button on the TPC to close the shutter.

F. Image Acquisition (Multicolor imaging)

1. Click on **Dye list** ![dye list icon] button on **the Image Acquisition Control Window** and double click on the fluorescent dyes you want to observe from the list. Click **Apply** button (if the **Assign Dye Manually** check box is checked, uncheck it before pressing Apply button). It will activate the laser lines and the detection channels according to the dyes you selected.
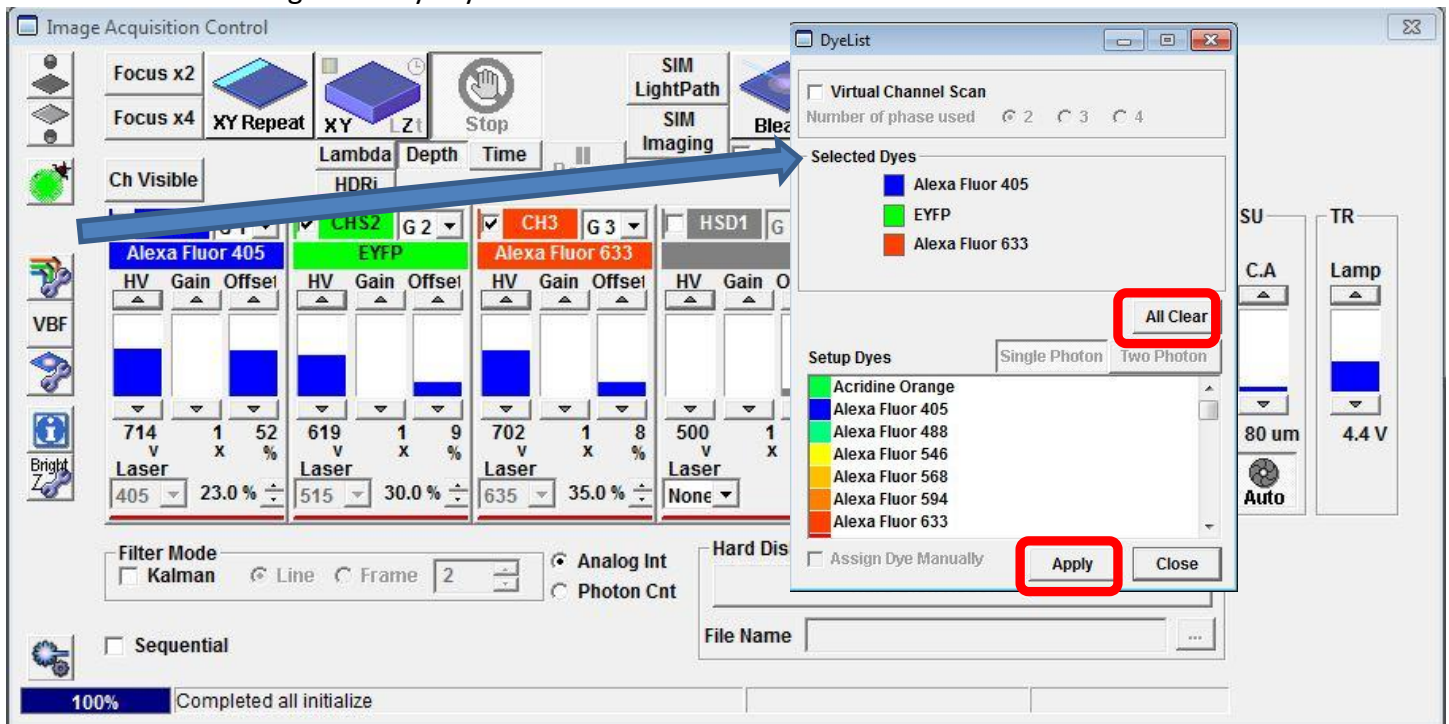
If the Selected dyes upon opening the program are not ones that you want to use, click the **All Clear button** first before selecting your dyes.

2. Choose **Image size** (default- 512x512) from **Size** panel ![size panel image].

3. Press ![AutoHV button] button (if not pressed) to automatically adjust HV and Offset values according to the scan

    speed. ![scan speed panel]. Slide the scan speed knob to select the image scan speed (around 20 us/pixel).

4. Click ![Focus x2 button] 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 (![arrow buttons]) in the Microscope window.

5. Click **Stop** button ![RepeatStop button] to stop scanning.

6. Set up proper acquisition condition each fluorescence colors by adjusting laser power, PMT detector sensitivity, and offset. The following procedure is for Alexa Fluor 405, EYFP, and Alexa 633 as an example.
   a) Check the Laser 405 only (check off other lasers, i.e. 515 and 635) to set up the imaging condition for Alexa 405.

   b) Click [Focus x2] and focus onto the brightest focal plane of your sample.

   c) Press **Ctrl-H** keys to change the color LUT to Hi-Lo; the live display will change to blue/white/red color scheme, where red pixels represent saturated intensity (beyond scale), blue ones complete darkness (0 intensity), and white in between.

   d) Adjust the laser power (at least 5%) and HV and Offset of CHS1 to obtain some brightest pixels in red and the background in blue.
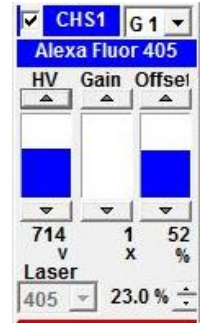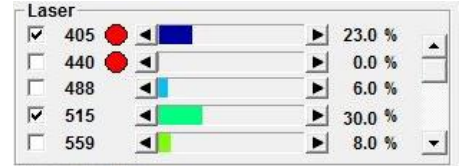
   e) Click [RepeatStop] to stop the fast scanning and click [XY Repeat] to scan at the imaging speed and to check the image quality. Adjust more if needed.
7. Repeat the above procedure for 515 nm (EYFP) and for 635 nm (Alexa fluor 633).
8. Check on all the selected lasers.
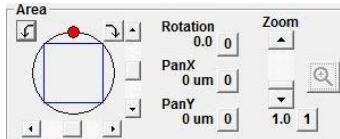
9. Set the Zoom and Rotation as required.

10. Click [XY LZt] button to capture images. When acquisition scanning is done, a 2D view window will appear.
11. Save the image in .oib or .oif format. (.oib format is more convenient.)

(Optional Spectral Setting for Multicolor Imaging).
Often excitation/emission profiles of fluorophores in multicolor imaging may be close, so there could be possible bleed-through of a fluorophore emission to neighboring channels. To minimize this artifact, the emission detection range of each channel can be adjusted by changing **Spectral Setting**.

Click on [VBF], it will bring **Spectral Setting** window. Change the range of spectrum for each channel (CHS1 and CHS2) by sliding, widening, or

narrowing the tabs [425 nm [50 nm] 475 nm] or arrowheads.
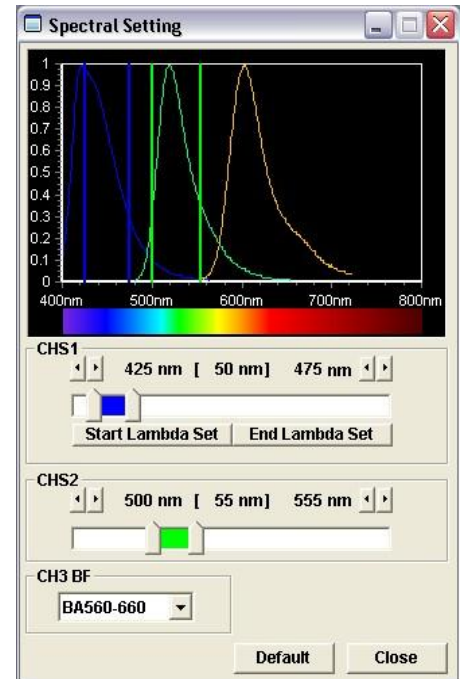
Sequential Scanning Mode

In many cases, the bleed-through between multicolor can't be eliminated by a spectral setting. It can be avoided by sequentially scanning the sample with one laser and one detector at a time.

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

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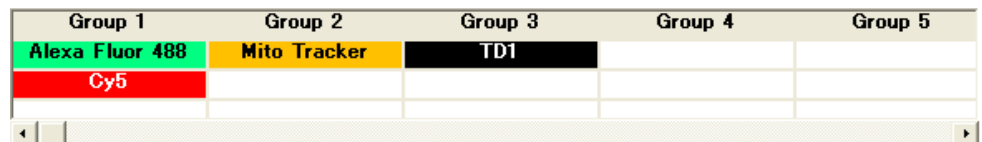
*(Note: it will be good to set up the acquisition condition for each channel before using the **Sequential** mode. To do so, first in simultaneous mode (unchecked Sequential box), set the acquisition condition by turning on only the specific laser for the specific channel. Once you set the imaging condition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

2. 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   button to acquire the image.

3. Save the image.

G. Z-Series (xyz) Image Acquisition

Use this mode to obtain optical section through the depth (z dimension) of your sample that can be used for 3D visualization. Before starting the following procedure, make adjustment for xy multicolor imaging condition as described above.

1. Click Focus x2 button to scan.

2. Use the arrowheads buttons or the **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 that you define).

3. When you find an upper limit of your sample by moving the objective up, click **Start Set** button. Bring the objective down until you find lower limit and click **End Set** button.
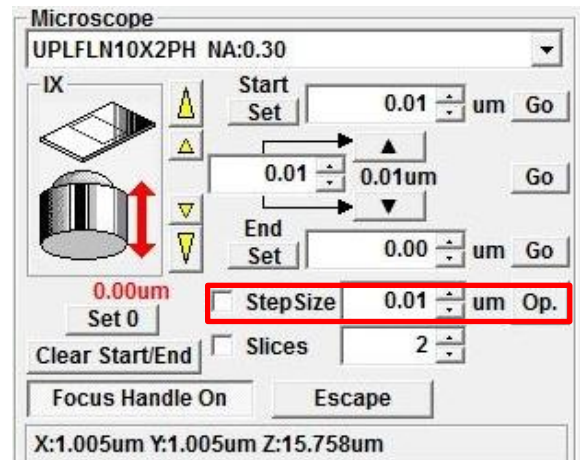
4. Determine the **Step Size** and the number of **Slices**, which correlate with each other. It is recommended to set the **step size** similar to the optical section thickness of the objective you are using, so that there is no gap between the optical sections upon projection into 3D. The step size can be fixed by checking the box.

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

6. When acquisition is done, **Append Next/SeriesDone** button will appear over the Stop button. If you need add additional sections, click **Append Next** button (enter the number of sections you want to add into the Append box
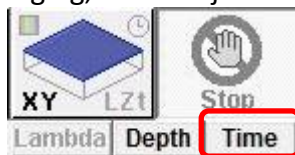
) or click **SeriesDone** to finish the acquisition.

7. Save the image.

H. Time-Series (xyt) Image Acquisition

Use this mode for imaging time-lapse of live specimens. Before starting time series imaging, make adjustment for xy imaging as usual.

1. Click Time button . It becomes **XY_t**.
2. In the **TimeScan** option, enter the **interval time** (in second) between one acquisition start and the next acquisition start (for example; to set up a 1 min interval, type 60 and press Enter key. If you put 0 and enter (FreeRun), the interval will be the acquisition time that is required for each frame).
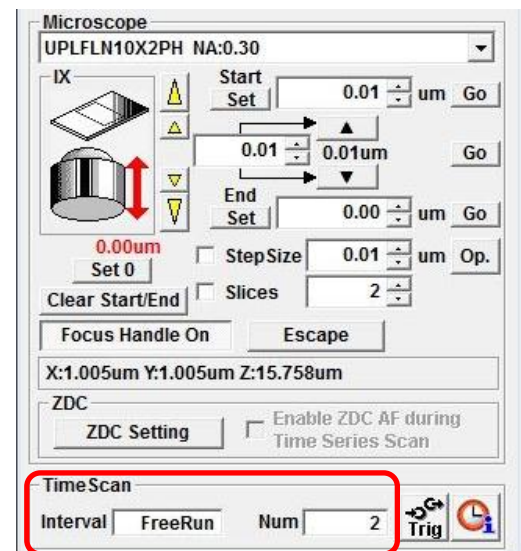
3. Enter the number of acquisition frame in **Num** box.

4. Click [XY LZt] button to start the timelapse.
5. Click **SeriesDone** button to finish the sequence and save the image.

I. 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 [My Image Folder] 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).
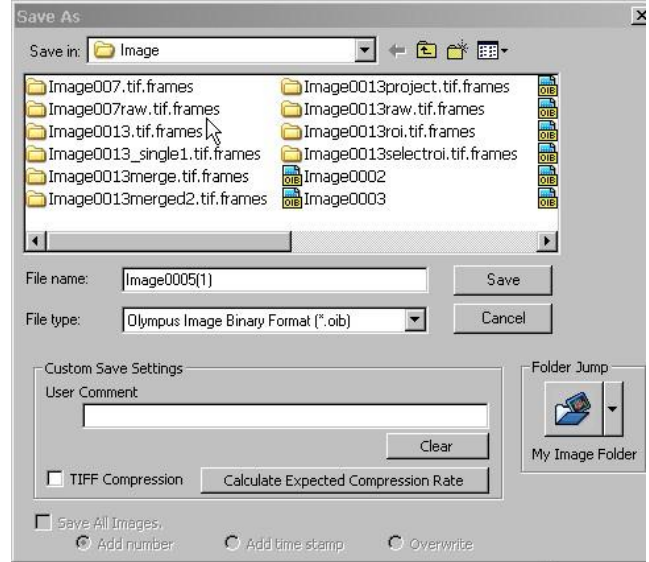
4. To set up your image folder where you can jump directly by clicking **My Image Folder** button, select *Tools/Option* menu. Click **General** tab and specify the path of your image folder with **Browse..** button. [*Upon creating a user account, each user will have its own folder in **D** drive (Do not create any folder in **C** drive.).* Click OK button.
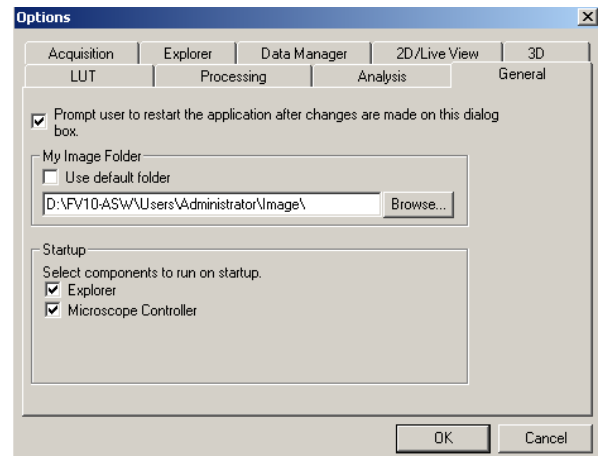
5. Oib file type contains all the metadata including all the acquisiotn parameters and it can be opened in FV10-ASW program. The light version of this program is available for installing in user's own computer. Otherwise, the image file can be exported as other file format so that it can be opened and 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.
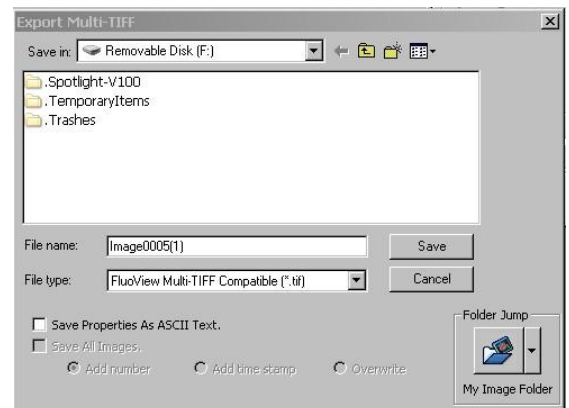
Check the **Save Properties As ASCII Text** box to save the metadata that contain information about the image acquisition setting. This data will be useful to find out the imaging condition later with other programs.

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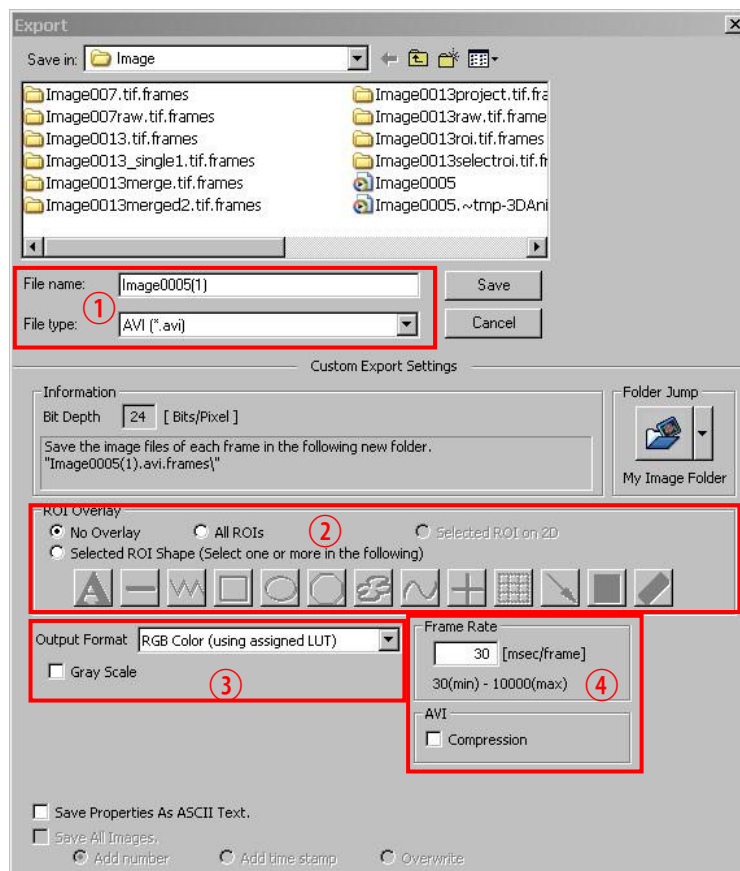
4. Check the **Save Properties As ASCII Text** button if you export the metatdata. Click **Save** button.

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

J. System Shut-Down Procedure

Wipe off the oil or water from any oil objectives you have used during your session with lens paper.

Logging off and transferring data.

1. Exit the FV10-ASW program.
2. **Make sure that you transfer your image file to USB flash memory. All image files more than 6-month old will be removed during monthly clean-up.**
3. Log off Windows. If someone signs up for use within a next 1 hour or so, leave the microscope system ON. If not, shut down the system as described below.

Shutting Off the system.

1. Shut down the computer ①.

2. Turn the mercury lamp off by pushing ON/OFF button② for two seconds and release it.

3. Turn the microscope controller OFF ③and the XY stage controller④.

4. Turn off the touch panel controller with the switch on the back⑤.

5. Turn off the Mulit Ar-laser⑥ by turning the key off and then the I/O switch off.

6. Turn off MCPSU⑦.

7. Turn off LD559 laser by turning the key off first and then the I/O switch off ⑧.

8. Turn off the two laser combiners, Main⑨ and SIM⑩ by turning the key off first and then the I/O switch off.