



# Olympus FV1200

## Microscope Users Guide

## Olympus FV1200 User Guide - Contents

This user guide is not intended to replace training, but as an additional aid to help you get the best out of the microscope

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# Live Cell Olympus Turn ON

**A1**

Switch on the controller boxes  
(the black buttons on the left)

**A2**



**B1**

Turn on the 559 laser;

1•turn the power SWITCH on.

2•The TEMP LED (green) will flash.

3•\*WAIT\*

4•After approximately 2 mins the LED will stop flashing.

5•Only then turn the KEY clockwise to the on position.

6•The LASER LEDs (red) will flash

7• \*WAIT\*.

8•When these red LEDs stop flashing the laser is ready to use

**WAIT**

**B2**



**C1**

Turn on the Multiline argon (457/488/515) laser;

1•turn the power SWITCH on.

2•Then turn the key clockwise to the on position  
(there is no need to wait for anything here!).

**C2**



**D**

Turn on the fluorescence bulb



**E**

Turn on the microscope keypad.

The On/Off button is on the BACK.

It's crucial to do this before starting the software



**Scan Mode**

(default is unidirectional)

**Image Size**

(number of pixels:  
default is 1024x1024)

**Zoom / Rotation**

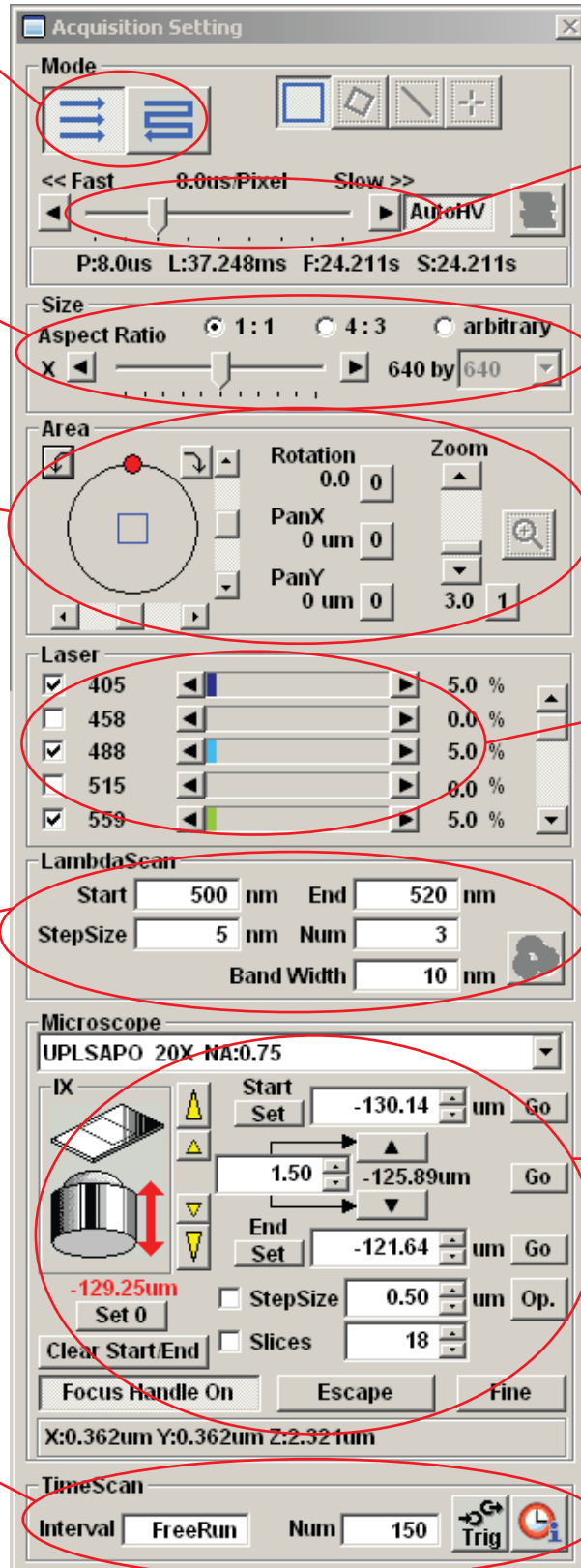
(Image can be optically  
zoomed and rotated)

**Lambda Scanning Setting**

(for spectral unmixing)

**Timelapse Setting**

(for timelapse imaging)

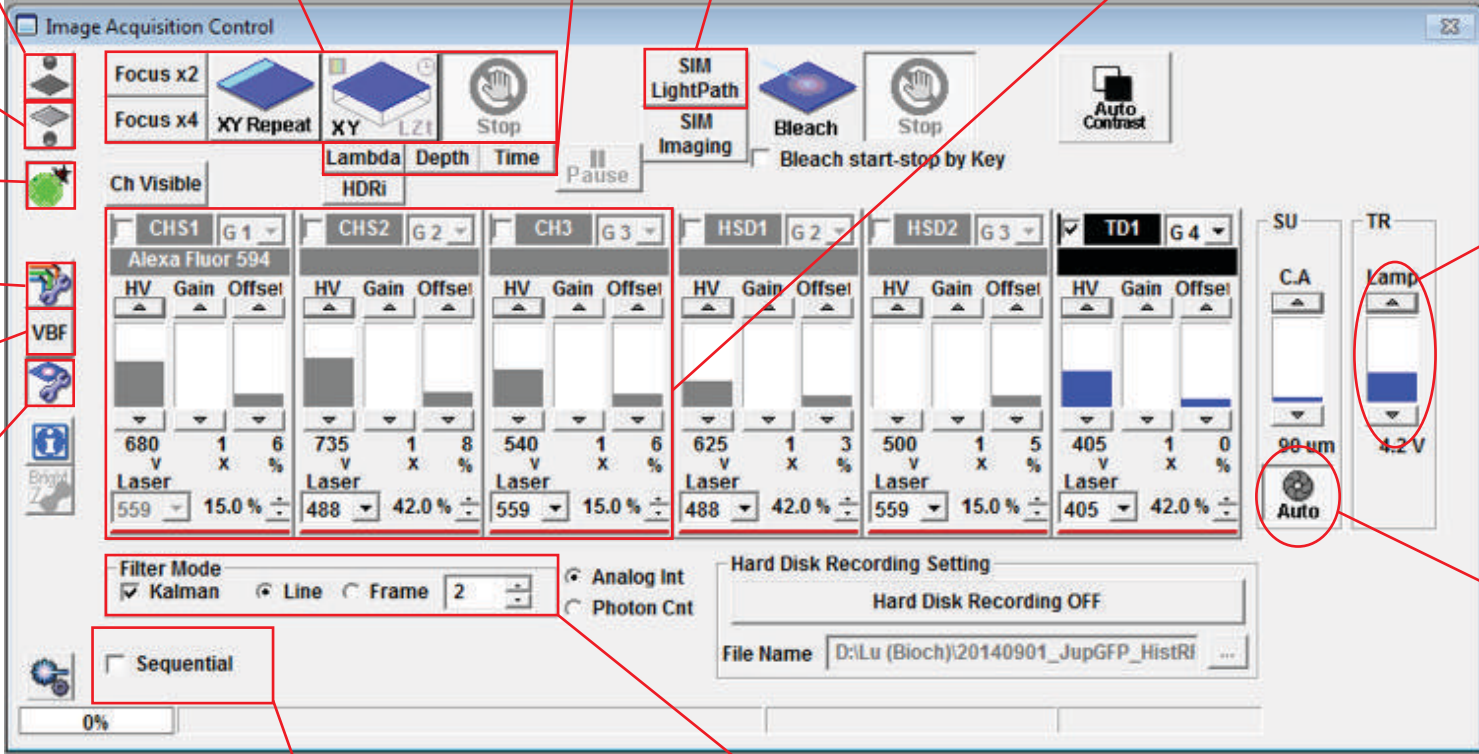


**Scan Speed**

(The slower, the better  
the image, but the more  
bleaching)

**Laser Power Control**

**Microscope Control**  
(Objective selection /  
focus / setting z-series)



The screenshot shows the 'Image Acquisition Control' software interface. It features a top toolbar with buttons for 'Focus x2', 'Focus x4', 'XY Repeat', 'XY', 'LZT', 'Stop', 'SIM LightPath', 'SIM Imaging', 'Bleach', and 'Auto Contrast'. Below this is a 'Ch Visible' section with 'HDMI' and 'Pause' buttons. The main area contains five channel settings (CHS1, CHS2, CH3, HSD1, HSD2, TD1) with sub-sections for 'HV', 'Gain', and 'Offset'. Each channel has a 'Laser' section with a wavelength and percentage. On the right, there are 'SU' and 'TR' sections with 'C.A' and 'Lamp' controls. At the bottom, there are 'Filter Mode' (Kalman, Line, Frame) and 'Hard Disk Recording Setting' (OFF) sections. A 'Sequential' checkbox is also present.

Transmission Light (Bright Field Viewing)

Epifluorescence Light (Fluorescence Viewing)

Dye List Setting

Light Path Setting

Spectral Window Setting

Stimulus Setting (bleaching)

Sequential Scan Mode

Frame Averaging

Transmission light Control

Confocal Aperture (automatic) Control

Imaging Mode Selection

Scan Buttons

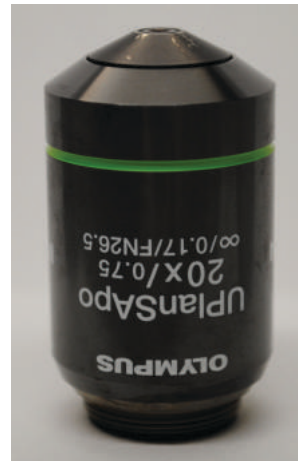
SIM lightpath (for bleaching)

Image Detection Channel Setting



10X  
0.40NA

**NO Oil**



20X  
0.75NA

**NO Oil**



40X  
1.30

Oil



60X  
1.40

Oil



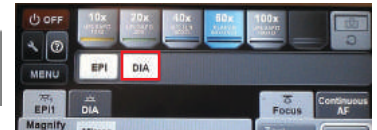
100X  
1.40

Oil

## 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 for transmitted light viewing  
[or click the "DIA" button on the microscope keypad]  
or



Click on epifluorescence light button for fluorescence viewing  
[or click the "EPI" button on the microscope keypad]



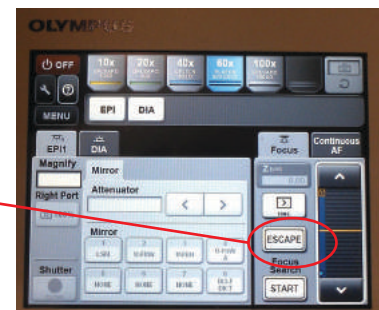
(before clicking on the light button you will use, click off the pressed button first).

Click on the filter wheel keypad buttons for DAPI (U-FWU), green (U-FBN) or red (U-FGW) for fluorescent viewing. Make sure the shutter is open.



Make sure the slide and coverslip are clean and sealed.  
Place the slide on the microscope stage.  
Focus on the sample.

Press the Esc button 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 on the Image Acquisition control window (or alternatively the "DIA" trans-lamp controller on the microscope keypad).



## Image Acquisition

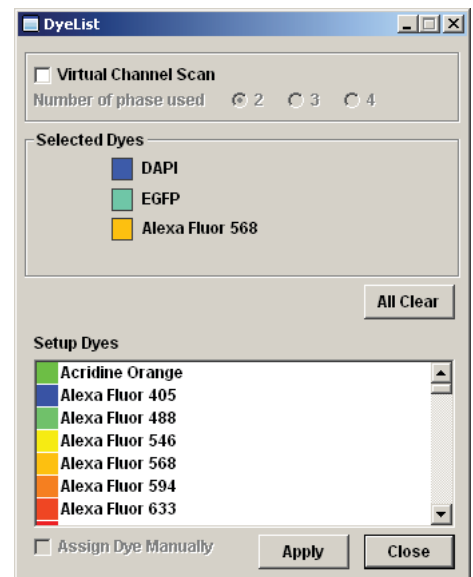
Click off the light source button to turn off the non-laser light.



Click on the Dye list button

Double click on the fluorescence dyes you want to use (eg. FITC and TRITC) from the list.

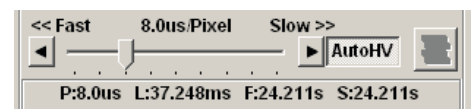
Click Apply button. This will activate the laser lines, and set detection channels up according to the dyes you selected.



Choose Image Size / Aspect Ratio (Generally 1024x1024) fromm Size panel



Press AutoHV button (if not already pressed) to automatically adjust the HV and offset values according to the scan speed change.



Click Focus X2 button to scan the sample. It will scan fast at 2us /pixel and show low-quality (pixelated) image in a Live View Window.



While scanning, focus onto the region of interest with the fine control focus or by clicking arrowhead buttons in the microscope window (large arrowhead moves the objective by the extent set by stepsize, and the small arrowheads move the objective by half of the large arrowhead stepsize).



Click Stop button to stop the scanning.



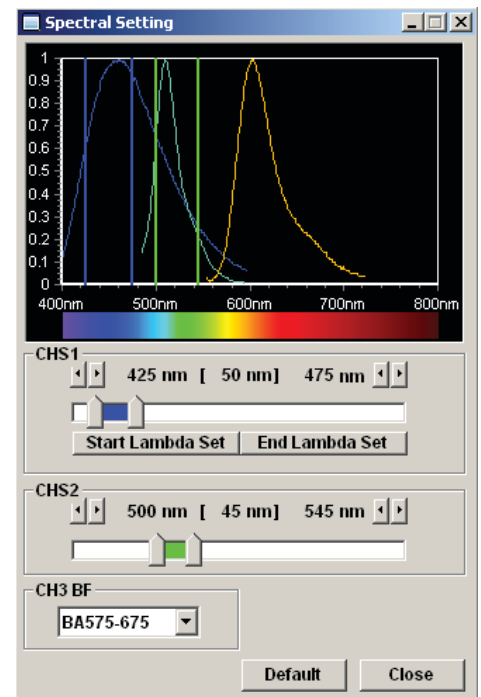


## Image Acquisition

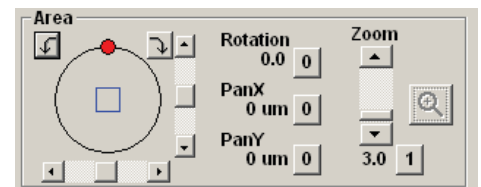
You can optimise the image quality with Hi-Lo LUT (Look-Up Table). Click LUT button to bring the LUT control window and select Channel and click Hi-Lo button (Ctrl-H shortcut key will do the same without opening LUT window). Now the Image is displayed using this specific LUT where red pixels represent intensity beyond scale (saturated) and blue pixels represent zero pixel value. To maximise the signal to noise ratio of the image, adjust the acquisition settings (laser power level, HV and Offset) such that the image shows a few red pixels and a few 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 a neighbouring channel. To minimize this, the detecting range of each channel can be adjusted by changing Spectral Settings. Click on VBF, it will bring up the Spectral Scanning window. Change the range of spectrum for each channel (CHS1 and CHS2) by sliding, widening, or narrowing the tabs or arrowheads.



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



Once you are satisfied with the settings, stop scanning. Set the scan speed to a slower rate (the slower the speed set, the better the signal to noise ratio, but the more bleaching).

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



## z-Series Image Acquisition

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

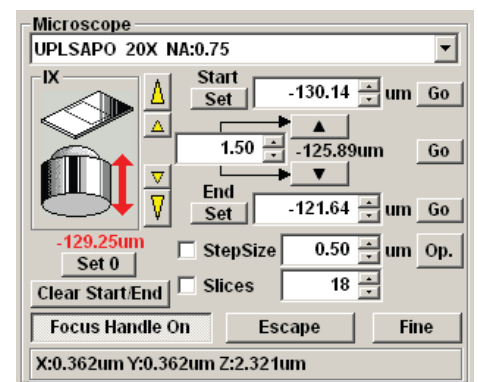
Click Focus x2 or x4 button to scan.



Use the arrowhead buttons or the fine focus on the microscope to focus into different z-axial planes (large arrowhead buttons shift a full step size and the small arrowheads shift half a step size).



When you find the upper limit of your sample, click the **Set End** button. Bring the objective down until you find the lower limit and click **Set Start** button. Determine the step size and the number of slices, which correlate with each other. It is recommended to set the step size to half the optical section (Nyquist sampling) by pressing the Optimal (op.) button.



Click the stop button. Click the **Depth** button ("Z" will appear in the **XY button** to become XYZ), and then click on the XYZ button.



When acquisition is done, **Append Next / Series Done** button will appear over the stop button. Click **Append Next** button to add additional sections at next step (enter the number of additional slices you want to add), or click **Series Done** to finish the acquisition.



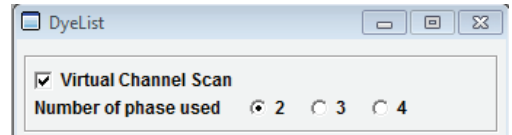
Save the image.

## 4 Colour Image Acquisition

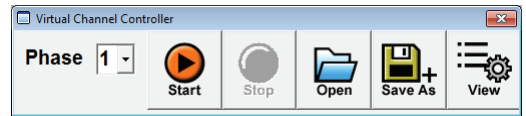
Click on the Dye list button



Tick "Virtual Channel Scan"

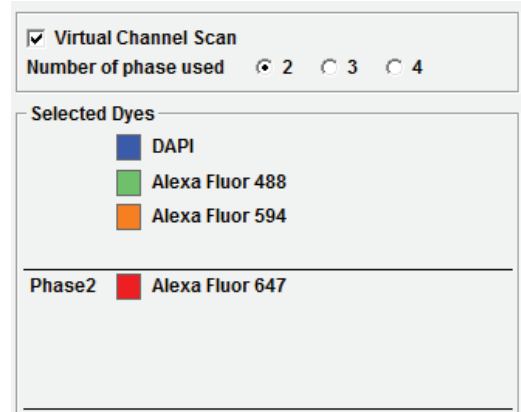


This opens up the 'Virtual Channel Scan' window

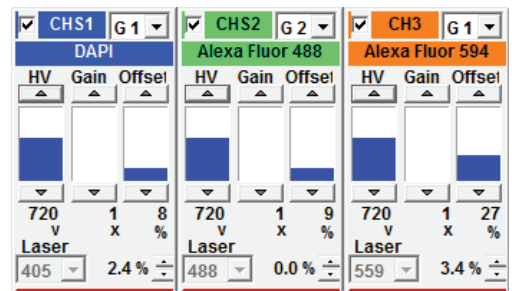


From the Dye List drag and drop the first three dyes into Phase 1

and the 4th dye into Phase 2



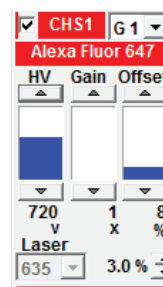
Set the HV, Gain, Offset and Laser Power for the first 3 dyes (which are in Phase 1)



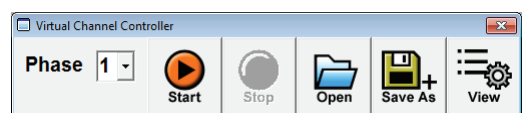
Using the 'Virtual Channel Scan' window select Phase 2



Set the HV, Gain, Offset and Laser Power for the 4th dye (which is in Phase 2)



Click on the 'Start' button to acquire the image



## Saving and Exporting Images

Click on the image window to be saved.

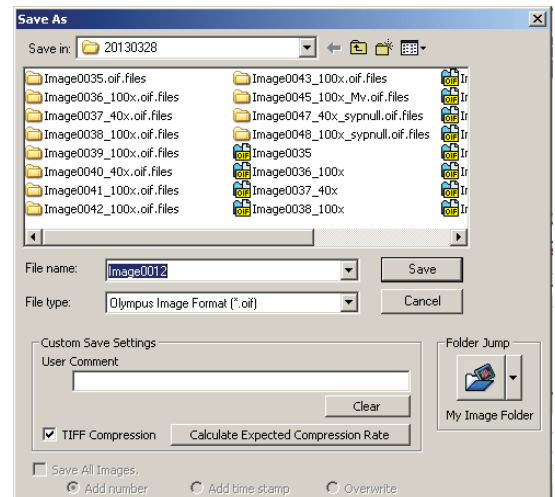
click on the save icon or select **File>Save or Save as** from menu.

A **Save As** window will appear. Save the image in your own folder on the D: Data drive.

Select **Olympus Image Binary Format \*.oib** file type, type file name, and click on **Save** button.

oib file type contains all the metadata including all acquisition parameters and it can be opened in the FV10-ASW software. The light version of this program is available for installing on the user's own computer (PCs only).

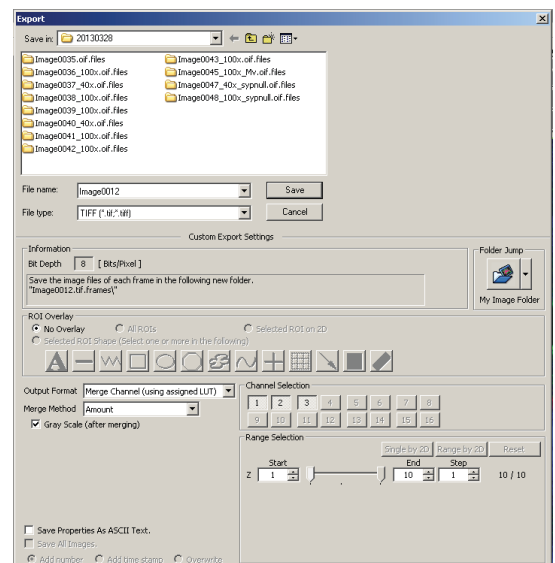
The installation file can be copied from the desktop.



Alternatively the oib files can be opened using fiji / image J free software (<http://fiji.sc/Fiji>).

To Export images:

- 1). Select **File / Export**.
- 2). Select **File Type** for export.
- 3). **ROI Overlay** allows saving the file with ROI information.
- 4). **Output Format:**  
**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.
- 5). Check the **Save Properties as ASCII Text** button if you export the metadata.
- 6). Click **Save** button.



## Re-Using Acquisition Settings

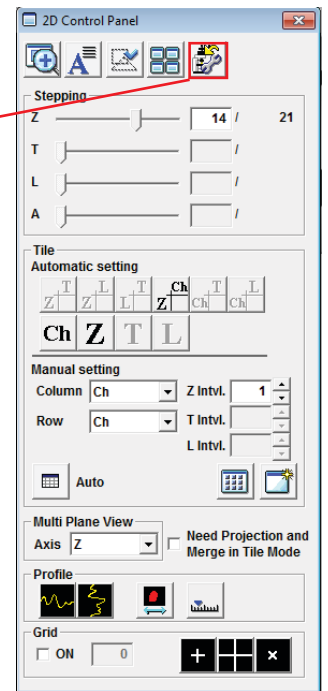
Open a saved image which was acquired with the settings you wish to reuse  
click on the double arrow icon



The '2D Control Panel' window will open

Click the 5th button along the top.  
The 'spanner arrow to microscope button'

This will re-configure the microscope to the same as when this  
image was captured



For 4 colour images where you have used the  
virtual channel scan, the above 're-use' method  
will not work

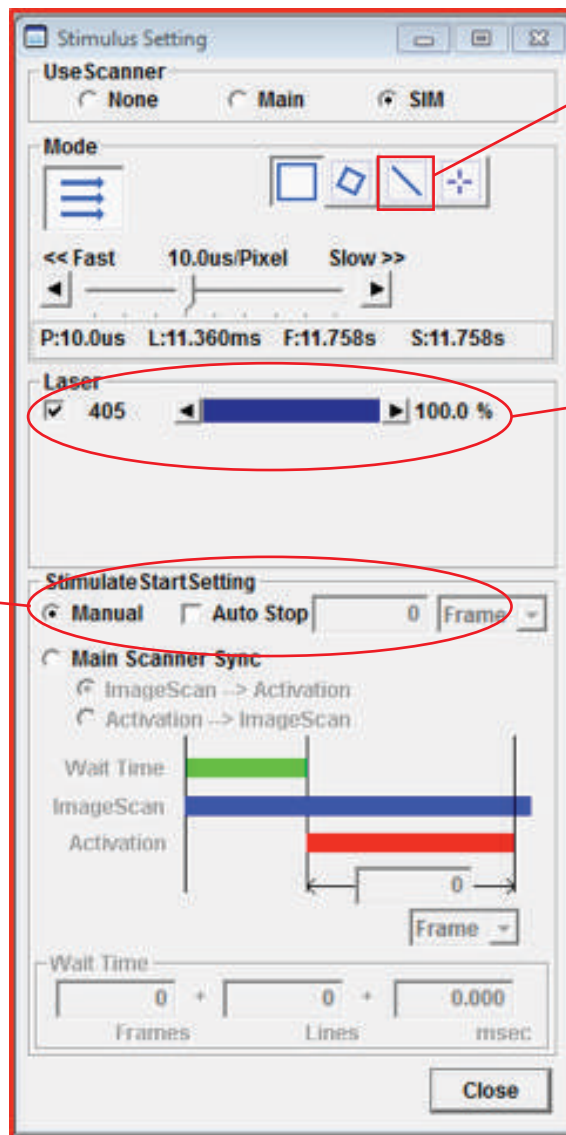
Instead, when you have perfected your 4 colour  
imaging conditions, click the 'Save As' icon.  
This will save the image 'protocol' as a .ovp file.



When you wish to re-use these imaging  
conditions, open the virtual channel scan using  
via the dye list. The open the 'protocol' file using  
the 'open' icon on the 'Virtual Channel Controller'.  
This will re-configure the microscope in both  
phases

## Laser Damage Experiments (I)

Click on the Stimulus Setting button



Select the single line icon and then draw a line in the 'Live View window' across the cell you wish to bleach

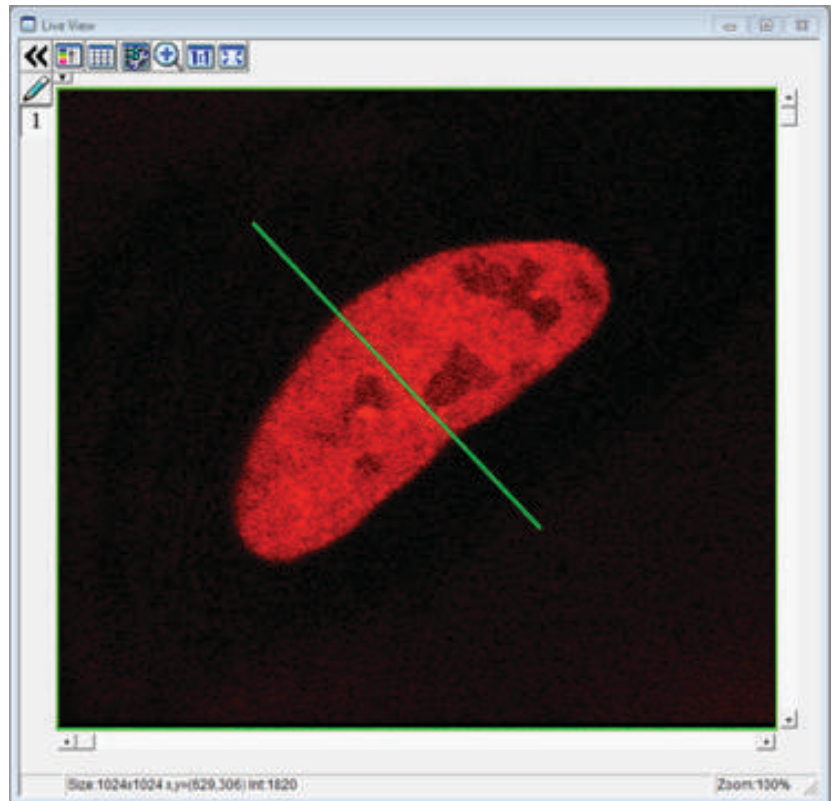
This indicates the 405nm laser will be used for bleaching

You can set the system to manually or Auto Stop the bleaching.

To set Auto Stop, check the box and input the time you wish to bleach for

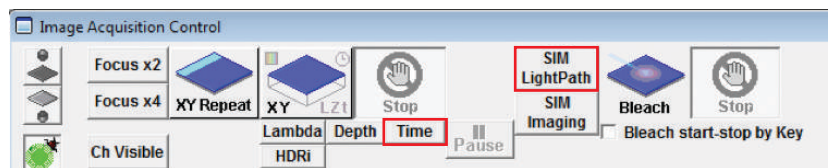
## Laser Damage Experiments (II)

**Draw a line in**  
the 'Live View window' across  
the cell you wish to bleach



**Select SIM Lightpath** icon

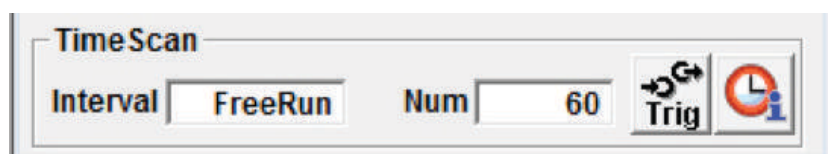
**Activate timelapse** imaging  
by clicking on 'time'



**Adjust capture parameters** (time  
interval (in seconds) and number of  
images required)

FreeRun will capture at the shortest  
possible interval based on scan  
speed and frame size (type 0).

It is possible to set a larger than  
anticipated number of frames to  
capture and then manually stop  
scanning if required.



## Laser Damage Experiments (II)

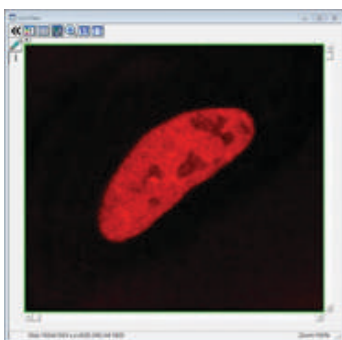
**Pre-bleach:** Begin the image capture by clicking XYt Scan button.  
Allow several frames of pre-bleach scanning.



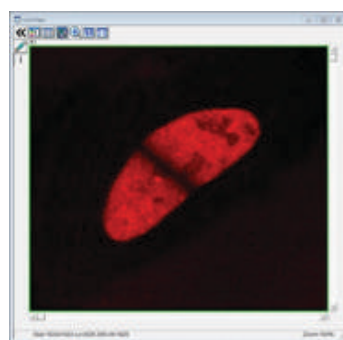
**bleach:** Press the Bleach icon along the top of the Image Acquisition Control window. You will need to press 'Stop' if you have opted to control the bleaching manually.



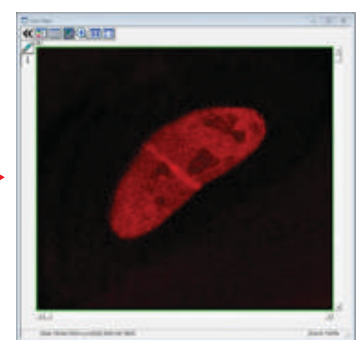
**Timelapse:** If the response is fast, then you can manually stop the scanning. If longer is required, you can add to the image by clicking 'Append New' which will capture additional frames.



pre-bleach



bleach



timelapse



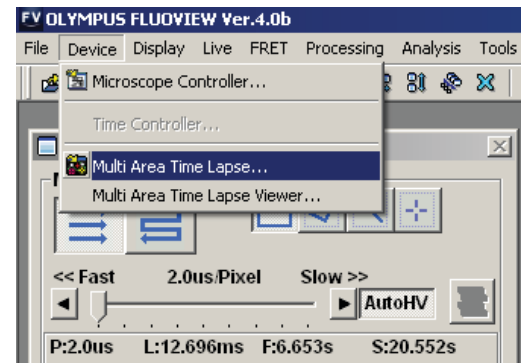
## Stitching (I) - Image Acquisition

### Set up 'Multi-Area Timelapse' mode

BEFORE you place your slide/dish on the microscope, start the FV1000 software, and select "Multi Area Time Lapse" (MATL) from the "Device" tab on the top of the screen.

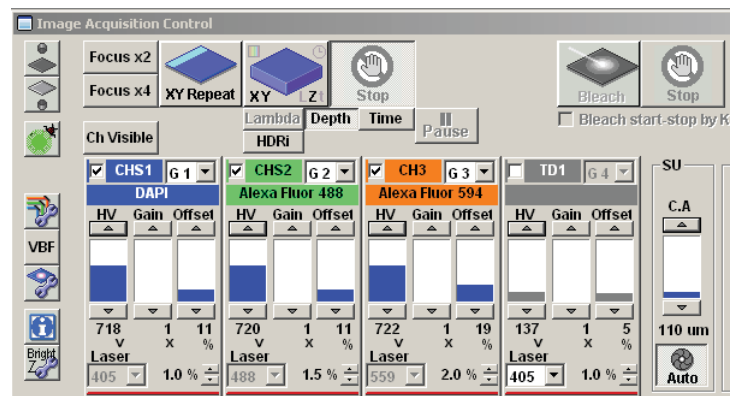
The "XY stage warning" window will pop up with the statement: "This stage will move in a wide range to detect its mechanical origin". Click "OK". This will cause the stage to move around. When it is finished moving, you can place your slide/dish on the stage.

You should see 3 new windows: Stage Control, MATL Control, and Registered Point List (more detail on those later).



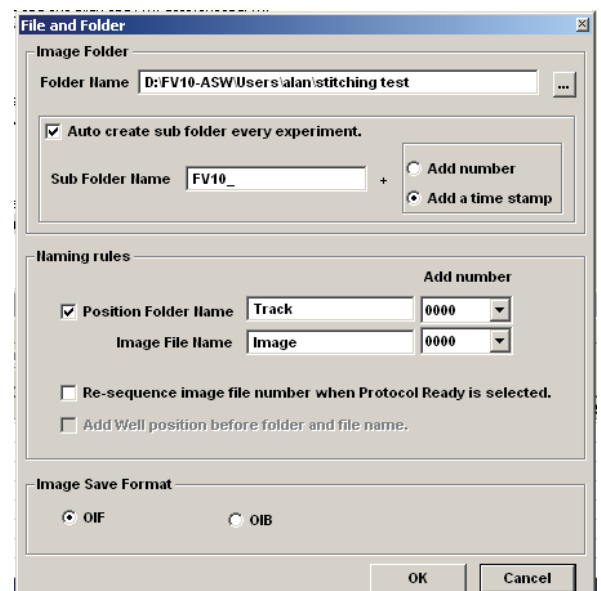
### Set up your scan parameters

Load your dyes (virtual phases cannot be done in combination with MATL), find a region of interest on your slide, set exposure levels, etc. If you wish to merge Z-stacks, set your start and end points, then click the "Depth" button in the "Image Acquisition Control" window. It is essential to click "Depth" BEFORE setting up your matrix, otherwise the program will function only in 2 dimensions.



### Set Destination for saved files

Click the 4th button from the right (disc & wrench) on the Registered Point List window, which will allow you to set the folder destination (Folder Name window). You also have the option to name your image files (this will show up later on the Registered Point List spreadsheet).



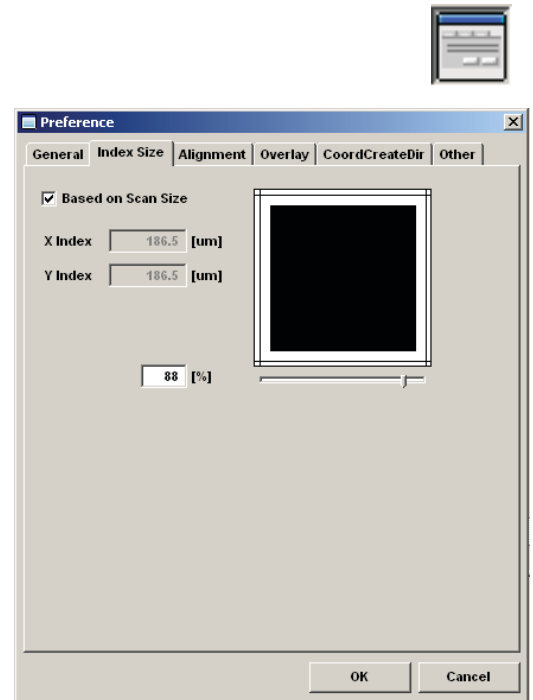
## Stitching (II) - Image Acquisition

### Set overlap

The 3rd button from the right on the MATL Control window opens the "Preferences" window. Click the "Index Size" tab. The overlap setting is in the window next to the % sign. 100% = 0 overlap. Some overlap is required for optimal image stitching to produce the most seamless combined image. The optimal amount of overlap is determined by the total magnification:

$$((\text{Obj. mag.} \times \text{X zoom\#}) / 8) + 3 = \text{ideal overlap\%}$$

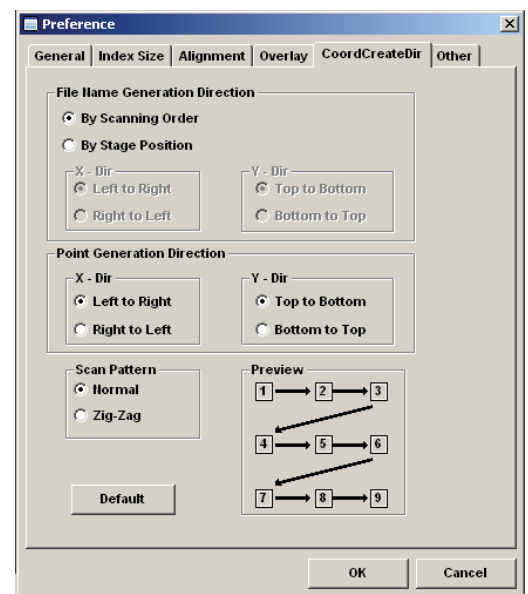
For example, if you are using a 60X objective with a zoom of 1.5, the ideal overlap is around 14%, and you would set the overlap # to 86%.



### Set scan order and pattern

Click the "CoordCreateDir" tab:

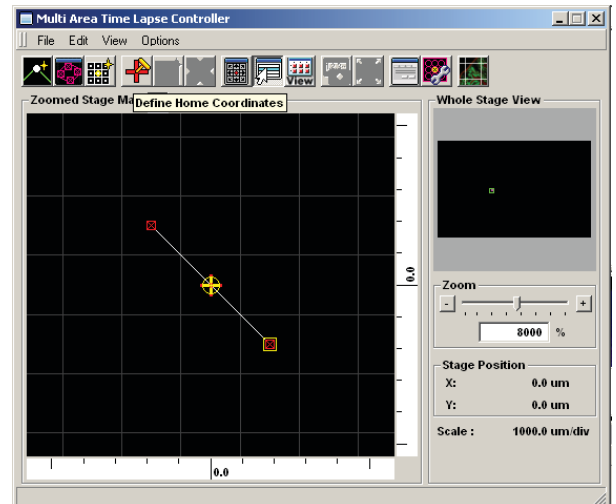
The "Normal" scan pattern is recommended.



## Stitching (III) - Image Acquisition

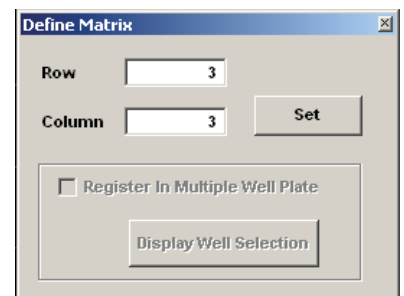
### Define your matrix

The MATL Controller window displays a grid. The yellow crosshair shows the position of the sample in the view field on the grid ("you are here"). The red cross marks the center of the grid. The x y coordinates of the yellow crosshair (relative to the center) are shown to the right of the grid (and in the windows of the stage controller). You can zoom in or out with the slider bar to the right.

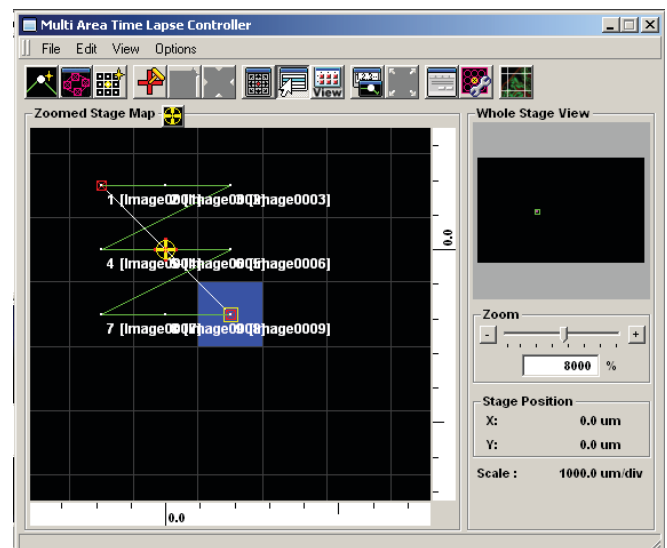


The "Define Matrix" button (3rd from the left in the MATL Controller window) will allow you to specify a set number of squares across and down.

Click "Set" and the yellow crosshairs will be centered in the defined matrix. The coordinates for each square will be loaded into the Registered Point List.



Your matrix should look something like this...



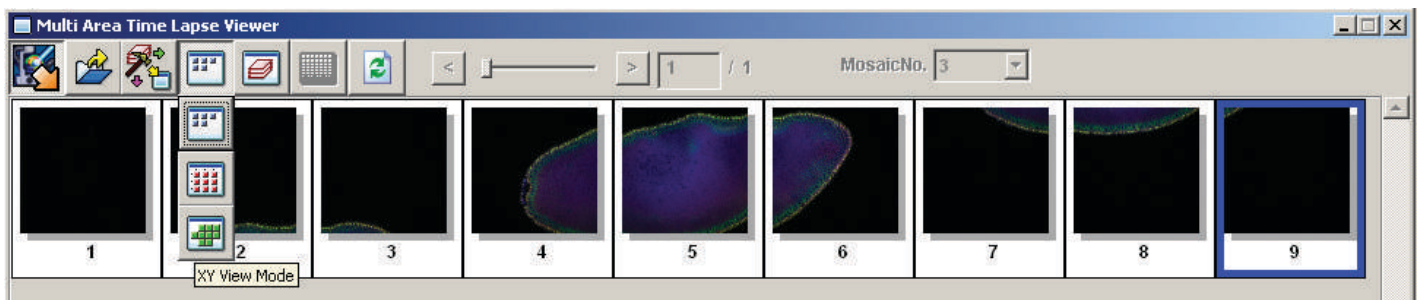
## Stitching (IV) - Image Acquisition

### Take images

You will use the start controls in the “Registered Point List” window instead of the “Image Acquisition Control” window. First click the “Ready” button (bottom left). When the machine is ready to start, the next button, “Play”, will change from a grey arrow to a black arrow. Hit “Play”. You should see windows appear for all your channels in the “Live View” window. If you are Z-stacking you should see a z-stack # indicator below the “Ready” and “Play” buttons in the bottom left corner of the Registered Point List window. “Total Time” (bottom right) gives you an estimate of how long the scan will take. The system will scan the entire Z-stack for square 1 (if applicable), then move to the next square.

No.	Enable	X	Y	Z	Z Start	Z End	Scan	Laser(405)	Laser(488)	Laser(559)	(CHS1)-HV	(CHS2)-HV	(CH3)-HV	(TD)-HV
1	<input checked="" type="checkbox"/>	-186.3	186.3	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137
2	<input checked="" type="checkbox"/>	0.0	186.3	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137
3	<input checked="" type="checkbox"/>	186.3	186.3	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137
4	<input checked="" type="checkbox"/>	-186.3	0.0	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137
5	<input checked="" type="checkbox"/>	0.0	0.0	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137
6	<input checked="" type="checkbox"/>	186.3	0.0	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137
7	<input checked="" type="checkbox"/>	-186.3	-186.3	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137
8	<input checked="" type="checkbox"/>	0.0	-186.3	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137
9	<input checked="" type="checkbox"/>	186.3	-186.3	1048.83	1048.83	1046.83	XYZ	1.0	1.5	2.0	718	720	722	137

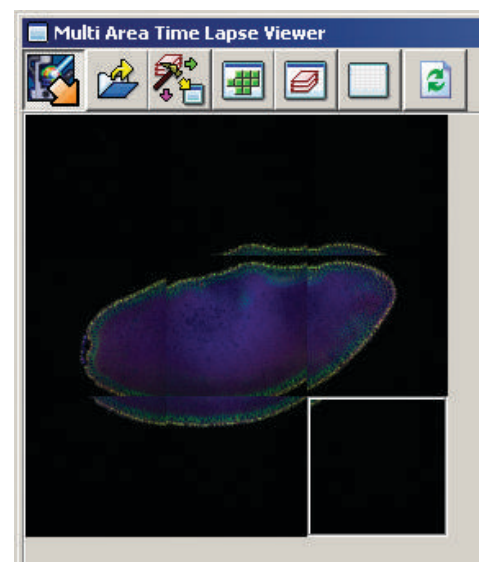
Interval: 00:03:49.5    Repeat: 1    Total Time: 00d 00:00:00.0    Frame Interval Time: 00d 00:00:00.0



### Finding the right stitch

When your scan is finished, go to the “Device” tab and select “MATL Viewer”. This opens a new window with a picture of imaged squares. Click the 4th button (“Select view mode”) and select the bottom button (green tiles) on the drop down menu.

This should arrange all the images in their proper order.

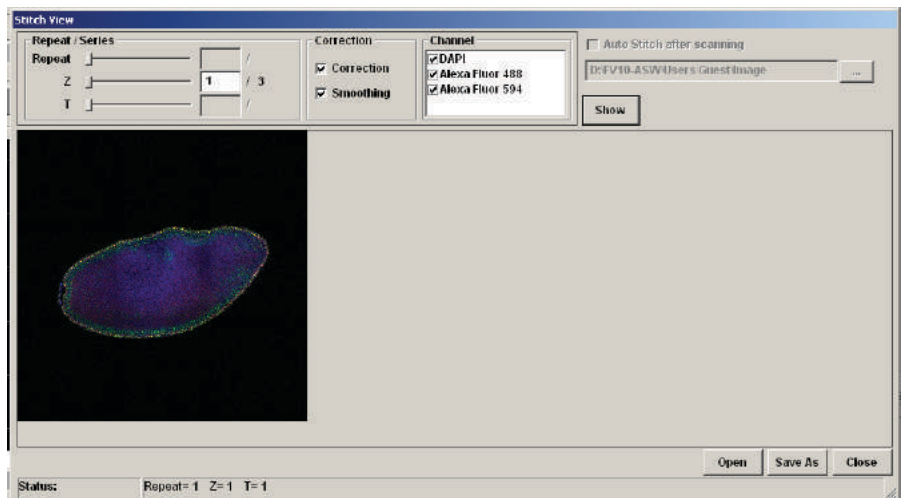
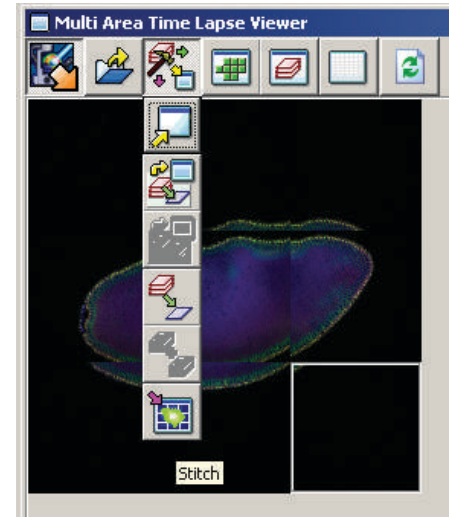


## Stitching (V) - Image Stitching

### Stitching:

To generate a stitched image go to the 3rd button on the MATL Viewer ("select action") and pick the 6th button ("stitch") in the drop down menu.

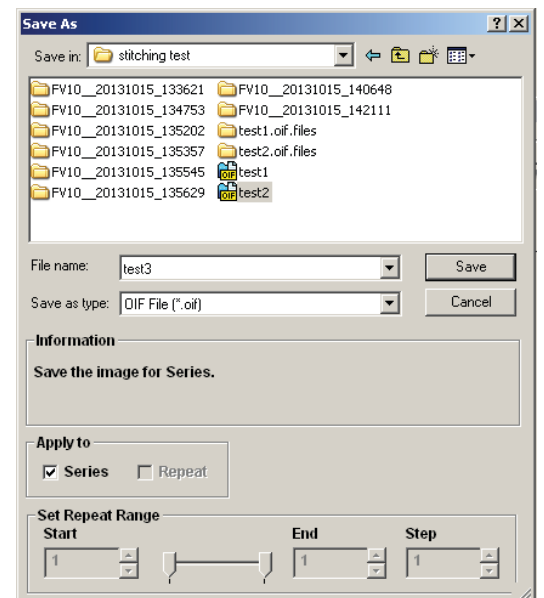
This will open the "Stitch View" window. Click the "Show" button to generate an initial stitched image in the window. Finding the ideal stitch requires a bit of trial and error. If you are stitching Z-stacks, using a plane in the center will usually give you the best results. Use the Z slider to the left to pick a Z plane, and then hit the "Show" button to re-stitch. The channels used are indicated above the stitched image and will be initially all checked. If the image is not optimal you can uncheck some of the channels and re-stitch. The channel with the most contrast works best for guiding stitching. Try different Z-planes and channel combinations to find the best possible image.



### Saving the data:

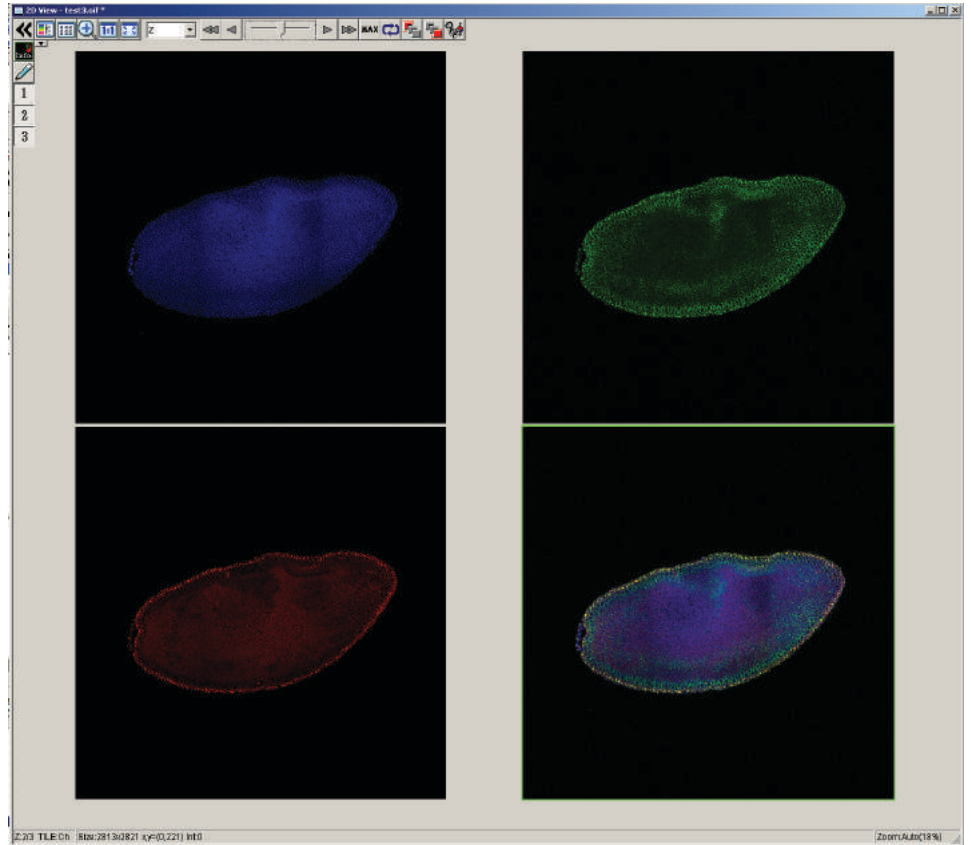
Once you generate a stitch you wish to keep, click the "Save As" button on the bottom right of the Stitch View window.

If you are saving a Z-stack stitch, you must check "Apply to series" to add all of your stacks to the image. Files 1 GB or less can be saved in the "oib" format. Files larger than 1 GB should be saved as an "oif". If you have Z-stacks and/or 20+ tiles you will likely choose "oif".

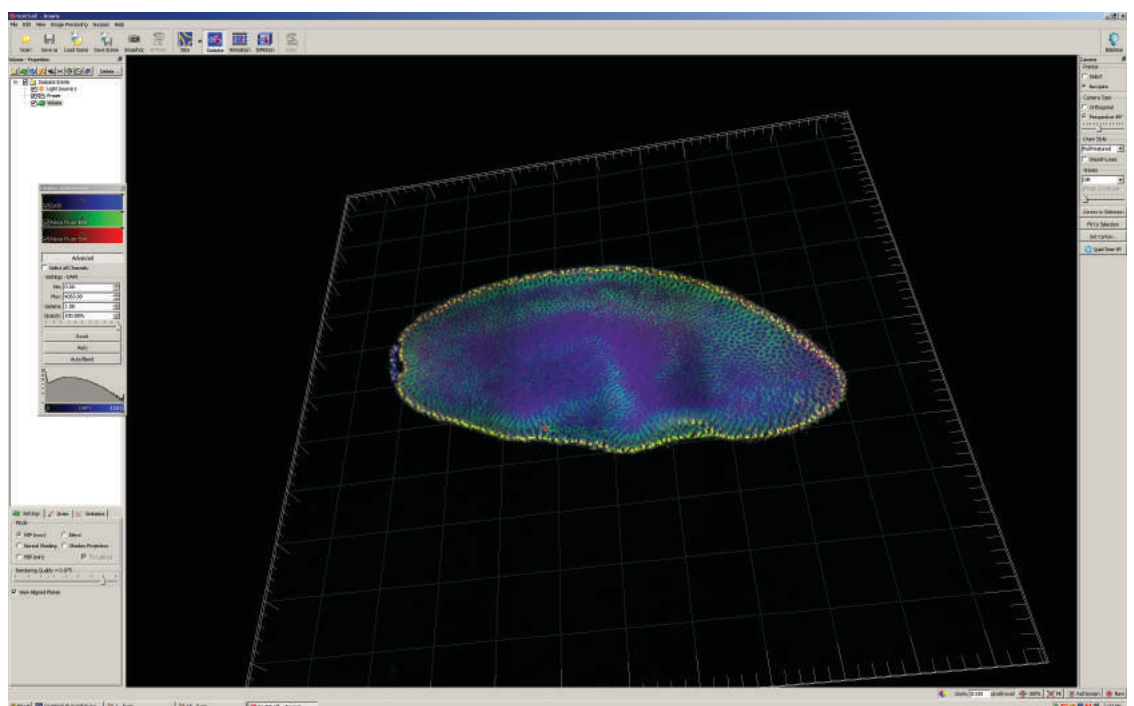


## Stitching (VI) - Image Display

The .Oif file can then be opened in the Olympus FV1000 software, where the z-stack can be scrowled through



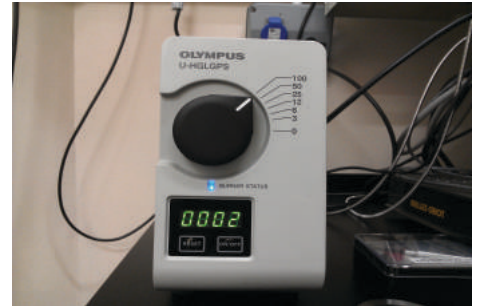
The .oif file can also be opened in Imaris software for further advanced image analysis and generating displays for presentations etc.



Check the booking calendar. Are you the last user?

**$\alpha$ 1**

- 1•Remove your sample from the microscope
- 2•Clean the objective lens using lens cleaning tissue
- 3•Switch off the fluorescence bulb



**$\beta$ 1**

Turn off the Multiline argon (457/488/515) laser;

- 1•Turn the key anti-clockwise into the off position.
- 2•\*WAIT\* for 5 mins.
- 3•When the air blowing from the fan is cool
- 4•Turn the power SWITCH off.

**WAIT**

**$\beta$ 2**



**$\gamma$ 1**

Turn off the 559 laser;

- 1•Turn the key anti-clockwise to the off position.
- 2•Turn the power SWITCH off  
(there's no need to wait here!)

**$\gamma$ 2**



**$\delta$ 1**

Turn off the microscope keypad.  
The On/Off button is on the BACK.



**$\epsilon$ 1**

Log off the computer.

**$\epsilon$ 2**

Switch off the controller box  
(the black button on the left)

