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Olympus FV1000

Microscope Users Guide

Olympus FV1000 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

DUNN SCHOOL
BIOIMAGING FACILITY

Olympus
Turn ON

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

B1 Turn on the 559 laser;

WAIT

B2

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

C1 Turn on the Multiline argon (457/488/515) laser;

C2

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!).

D Turn on the fluorescence bulb

Scan Mode
(default is unidirectional)

Image Size
(number of pixels:
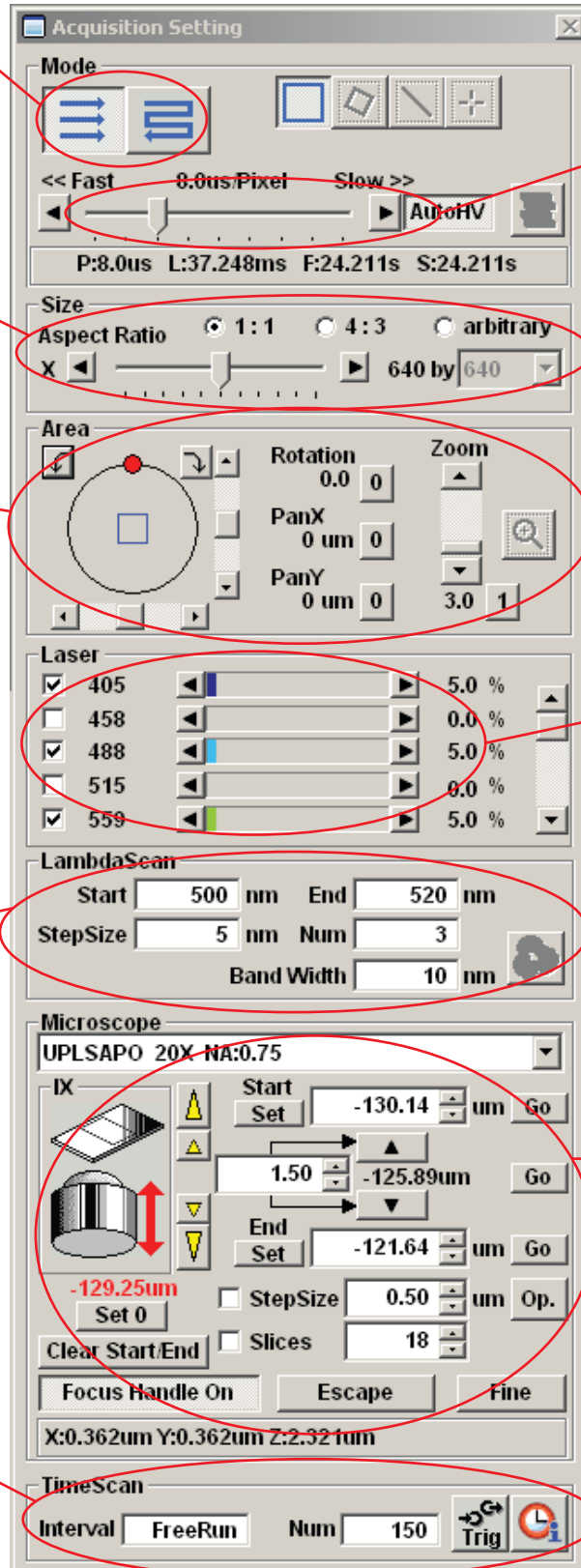default is 1024x1024)

Zoom / Rotation
(Image can be optically
zoomed and rotated)

Lambda Scanning Setting
(for spectral unmixing)

Timelapse Setting
(for timelpase imaging)

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

Laser Power Control

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

Acquisition Setting

Mode
<< Fast   8.0us/Pixel   Slow >>   AutoHV
P:8.0us L:37.248ms F:24.211s S:24.211s

Size
Aspect Ratio   ● 1:1   ○ 4:3   ○ arbitrary
X   640 by 640

Area
Rotation 0.0 0   Zoom
PanX 0 um 0
PanY 0 um 0   3.0 1

Laser
☑ 405   5.0 %
☐ 458   0.0 %
☑ 488   5.0 %
☐ 515   0.0 %
☑ 559   5.0 %

LambdaScan
Start 500 nm   End 520 nm
StepSize 5 nm   Num 3
Band Width 10 nm

Microscope
UPLSAPO 20X NA:0.75
IX   Start
            Set -130.14 um Go
            1.50 -125.89um Go
            End
            Set -121.64 um Go
-129.25um   ☐ StepSize 0.50 um Op.
Set 0   ☐ Slices   18
Clear Start/End
Focus Handle On   Escape   Fine
X:0.362um Y:0.362um Z:2.321um

TimeScan
Interval FreeRun   Num 150   Trig

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Transmission Light (Bright Field Viewing)

Epifluorescence Light (Fluorescence Viewing)
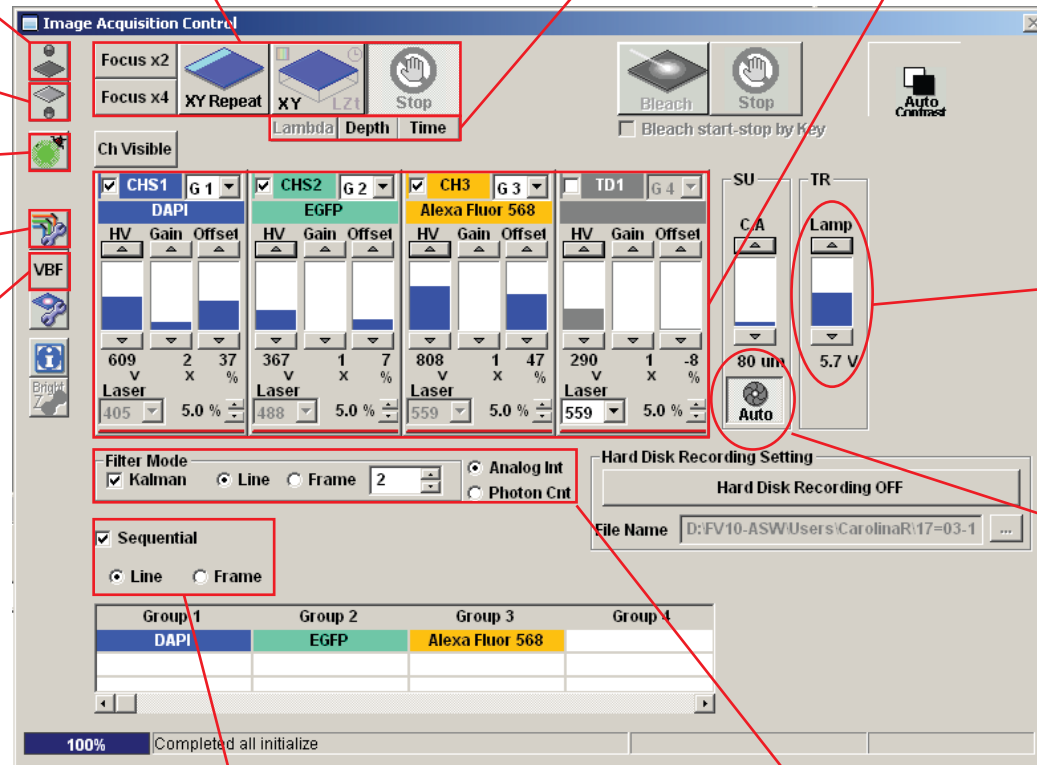
Dye List Setting

Light Path Setting

Spectral Window Setting

Scan Buttons

Imaging Mode Selection

Imagine Detection Channel Setting

Transmission light Control
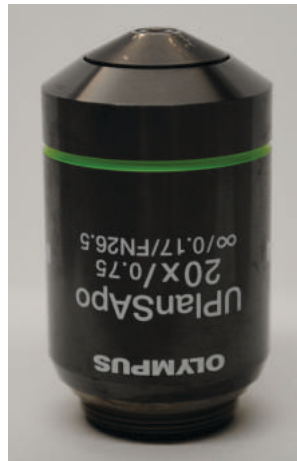
Confocal Aperature (automatic) Control

Sequential Scan Mode

Frame Averaging

10X	20X	40X	60X	100X
0.40NA	0.75NA	1.30	1.35	1.40
NO Oil	**NO Oil**	Oil	Oil	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 on epifluorescence light button for fluorescence viewing

Click on the filter wheel keypad buttons for DAPI (WU), green (NB) or red (WG) 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.
Switch between course and fine adjustment using the F/C button.
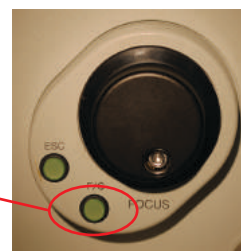
Press the Esc button to move the objective completely away from the sample and press again to bring the objective to the original position.
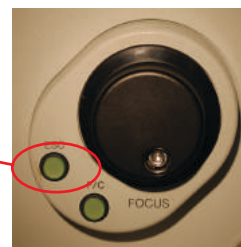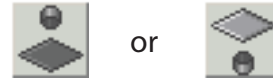
Adjust the transmitted light level with the lamp controller on the Image Acquisition control window (or alternatively the lmap controller on the front of the microscope).
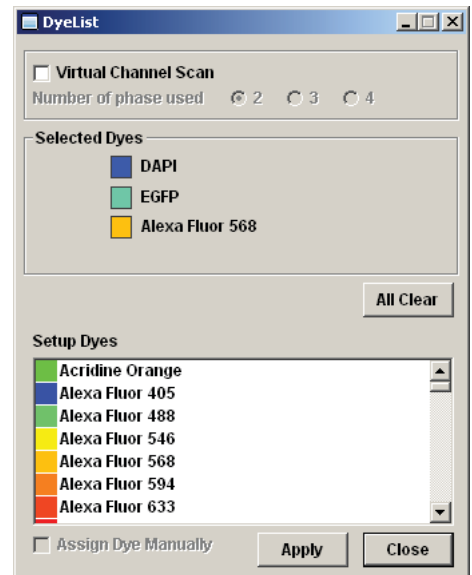
Image Acquisition

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

or

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 accroding to the dyes
you selected.

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

Click XY Repeat button to 'live' scan the sample.

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
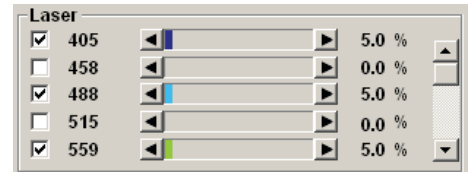arrowhead stepsize).

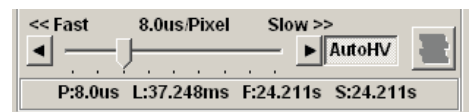Click Stop button to stop the 'live' scanning.

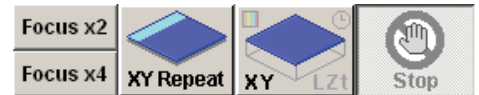# Image Acquisition

Set the Laser output level

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

Click XY Repeat button to scan

Detector (CHS) Channel Setting:
Adjust the brightness of HV (and Gain) and
background black level (offset) of individual
channels.

Keep the HV value below 720 (beyond 720
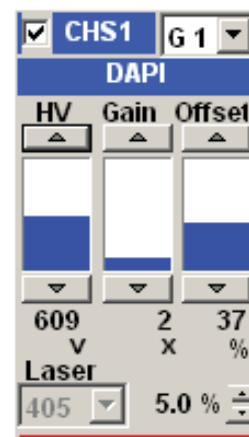the backgorund noise will show up).

Image Acquisition

You can optimise the image quality with Hi-Lo LUT (Look-Up Table).
(This is the display with red dots and blue dots)
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 represents 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 scaninng.

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 an CHS2) by sliding, widening,
or narrowing the tabs or arrowheads.
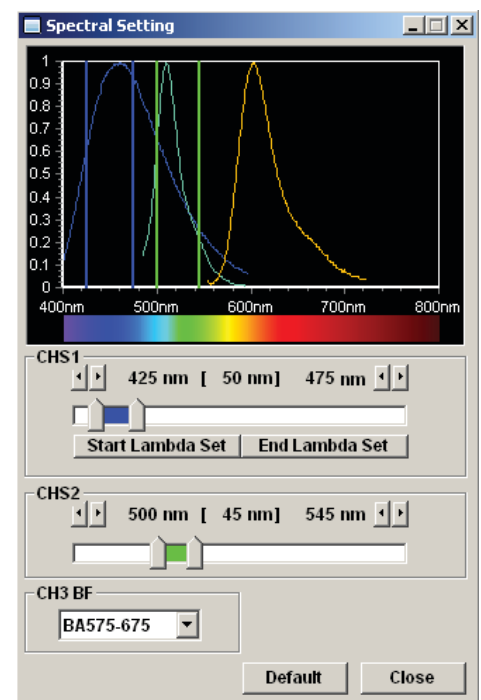
Use Area panel to rotate and zoom the imaging area.
Click on '1' button to retrun to the original viewing zoom.
To rotate the viewing area, click on the red dot and drag
clockwise or counter clockwise.
With zoomed view you can select the scanning area by moving
the blue lined box around.

To zoom on a specfic region, click the "magnifying-glass" icon
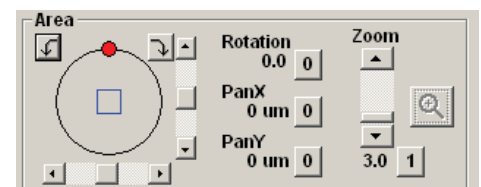and draw a box on the live view area.
This zoom will scan only the area specified.

Once you are satisfied with the settings, stop scanning.
Set the scan speed to a slower rate (the slower the scan speed,
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.

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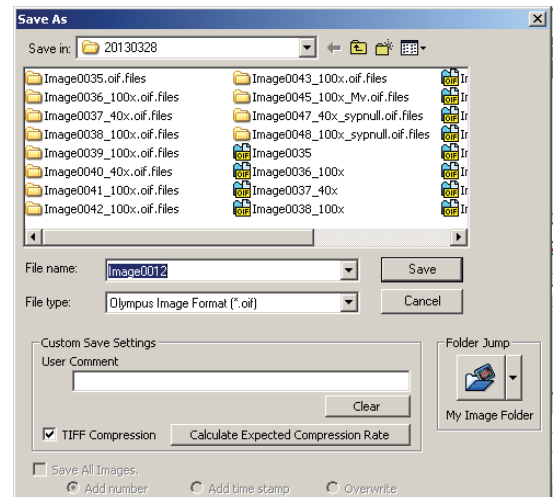
Alternatively the .oib files can be opened using fiji / image J free software (http://fiji.sc/Fiji).

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.
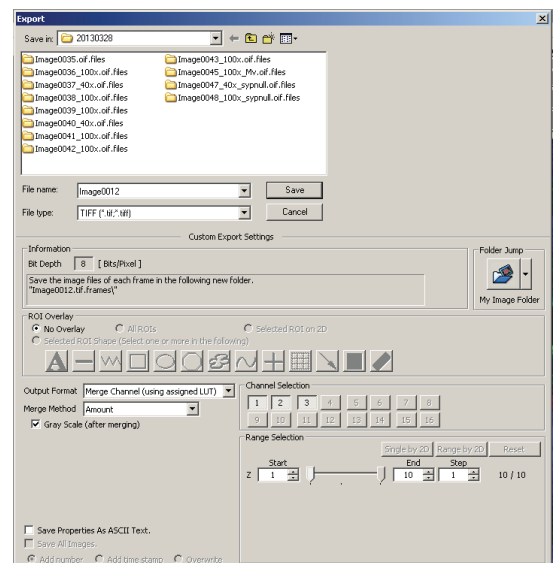
Re-Using Acquisition Settings

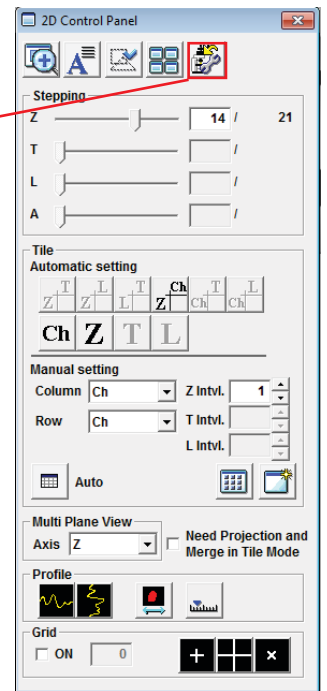Open a saved image which was aqcuired with the settings you wish to reuse

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

z-Series Image Acquisition

Use this mode to obtain an optical section through the depth (z dimension) of your sample.
This 'z-stack' can then be used to visualise your sample in 3D:

Click Focus x2 or x4 button to start 'live' scan.

Use the arrowhead buttons or the fine focus on the miroscope
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 Start** button. Bring the objective down
until you find the lower limit and click **Set End** 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.
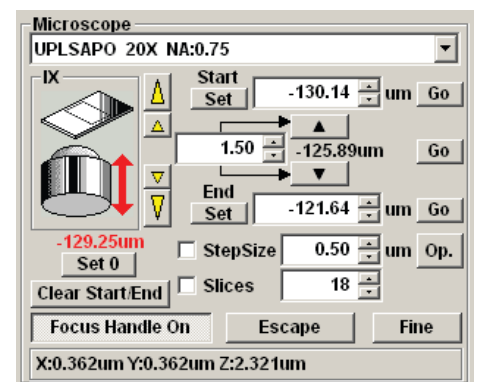
Click the stop button to stop the 'live scan'.
Click the **Depth** button ("Z" will appear in the
XY button to become XYZ), and then click on the XYZ button.

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),
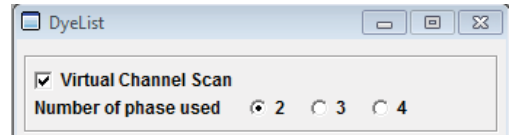or click **Series Done** to finish the acquisition.

Save the image.

4 Colour Image Acquisition

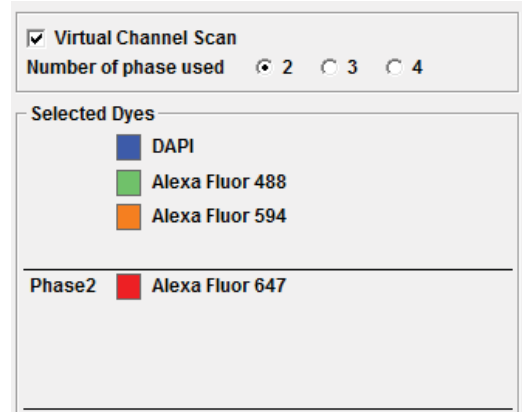Click on the Dye list button

Tick "Virtual Channel Scan"

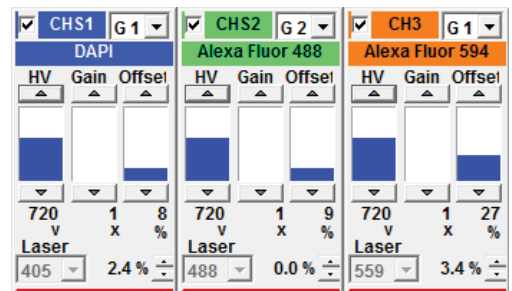This opens up the 'Virtual Channel Scan' window

From the Dye List drag and drop the first three dyes into Phase 1
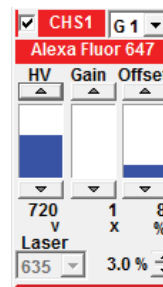
and the 4th dye into Phase 2

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 Phase2

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

Click on the 'Start' button to aquire the image

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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).
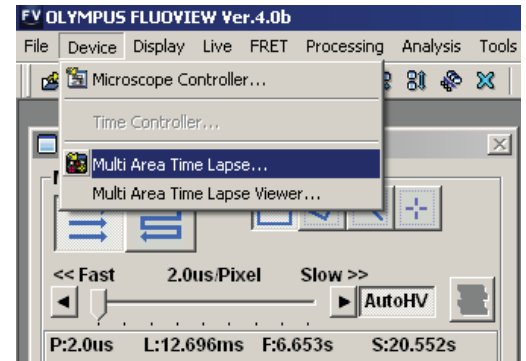
Set up your scan parameters

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.
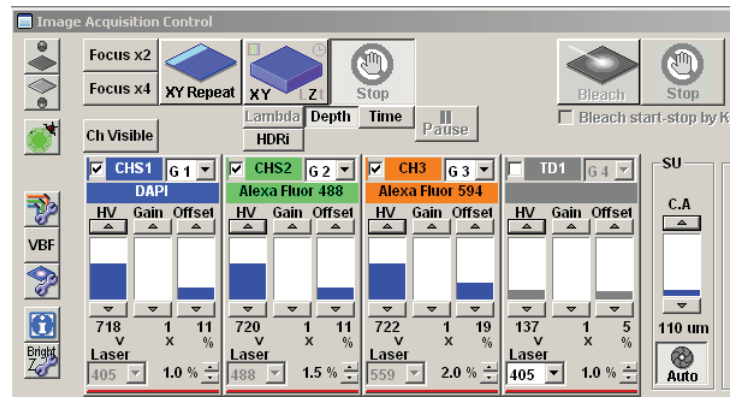
Set Destination for saved files

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).
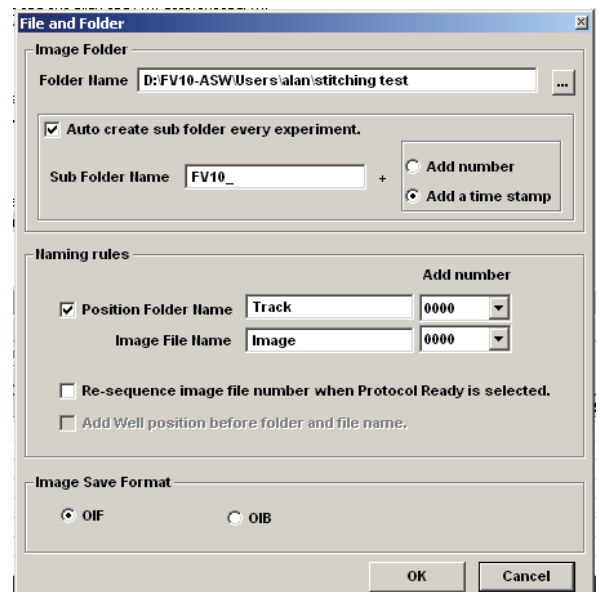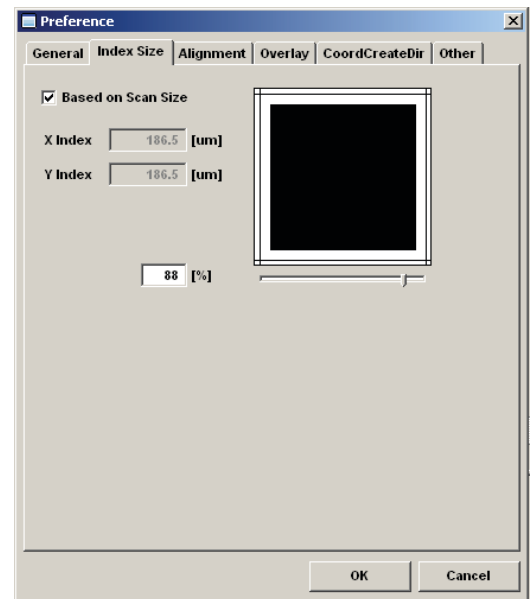
Image Acquisition

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:

((Obj. mag. X zoom#)/ 8) + 3 = 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.
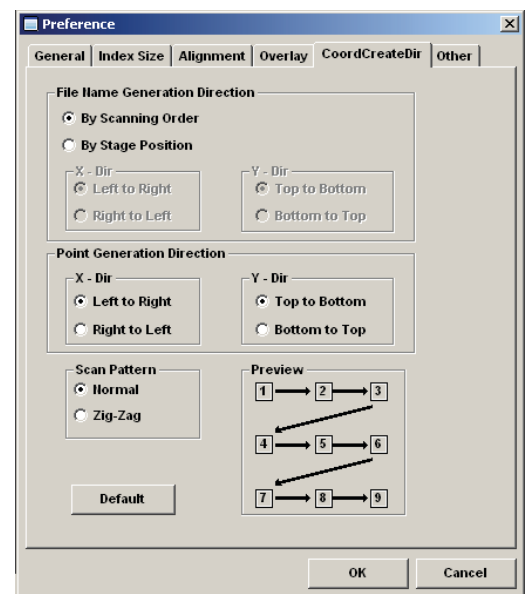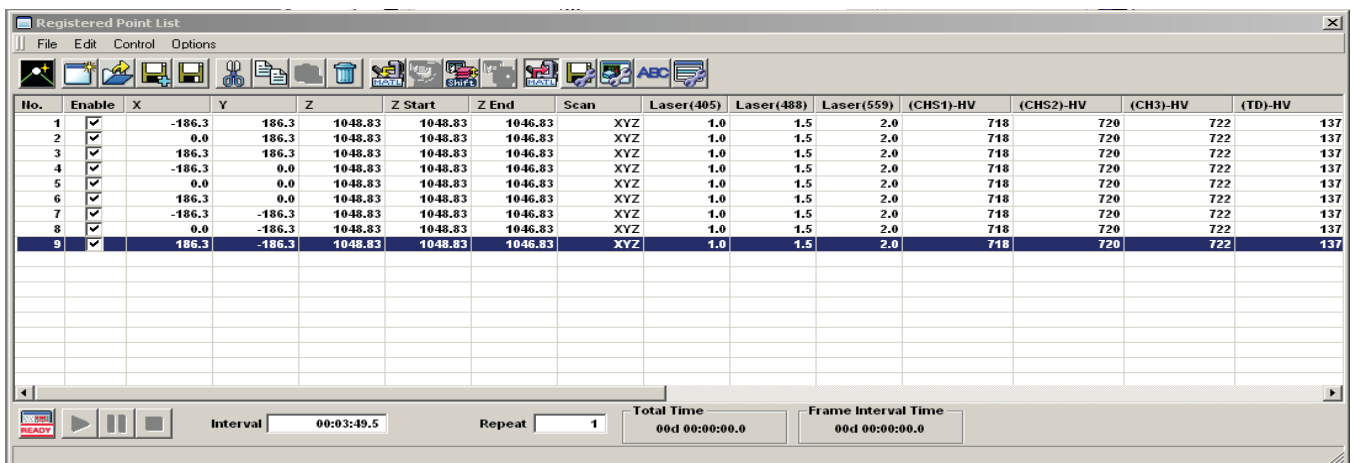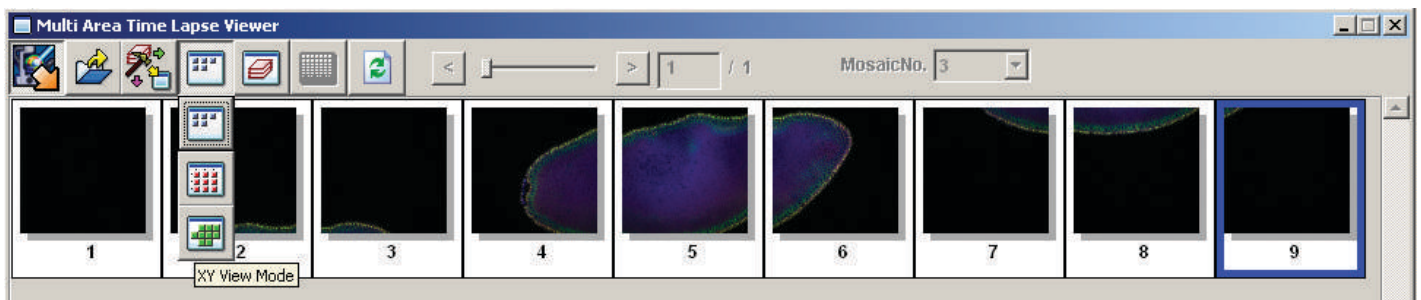
Image Acquisition

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.

Finding the rigth 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.

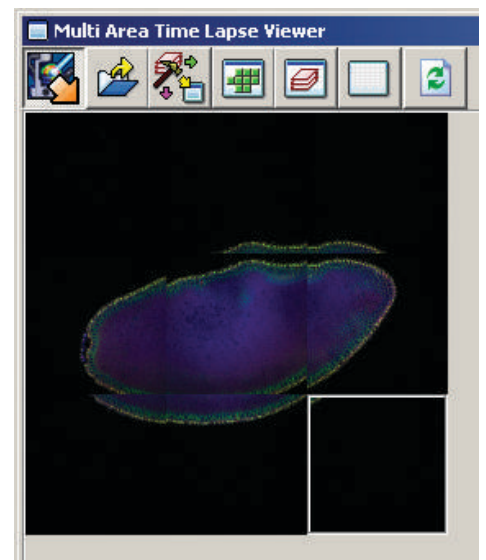This should arrange all the images in their proper order.

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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.
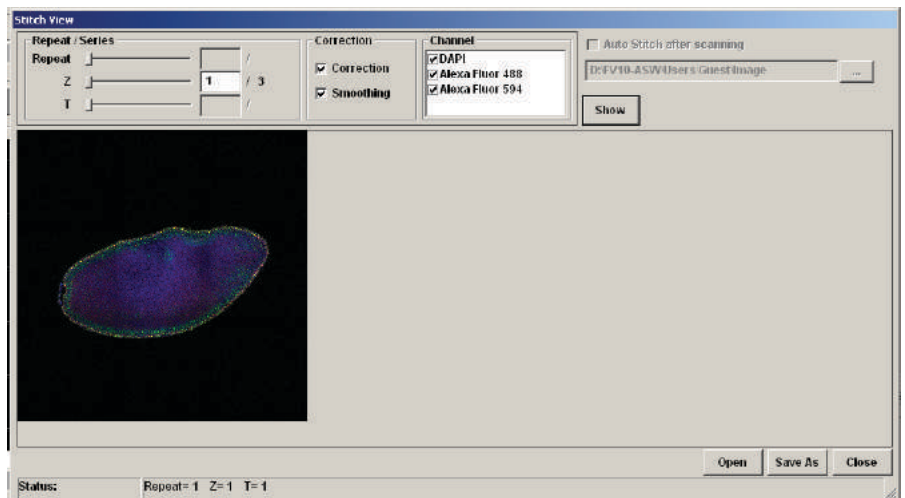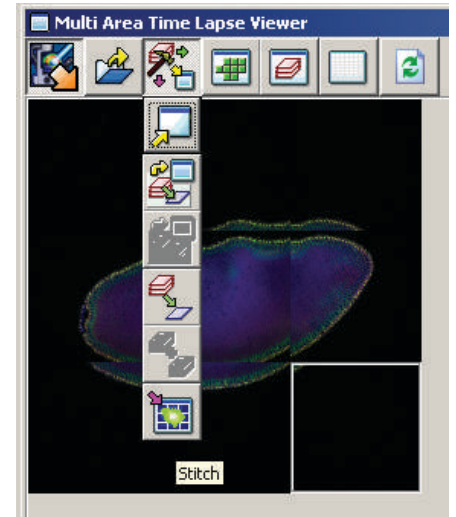
Saving the data:

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".
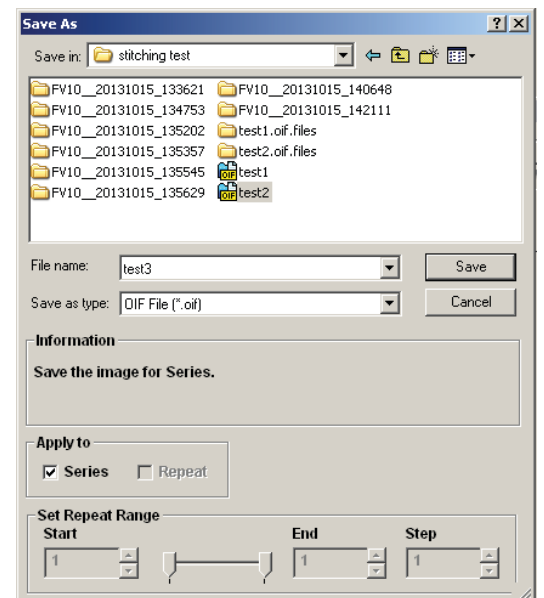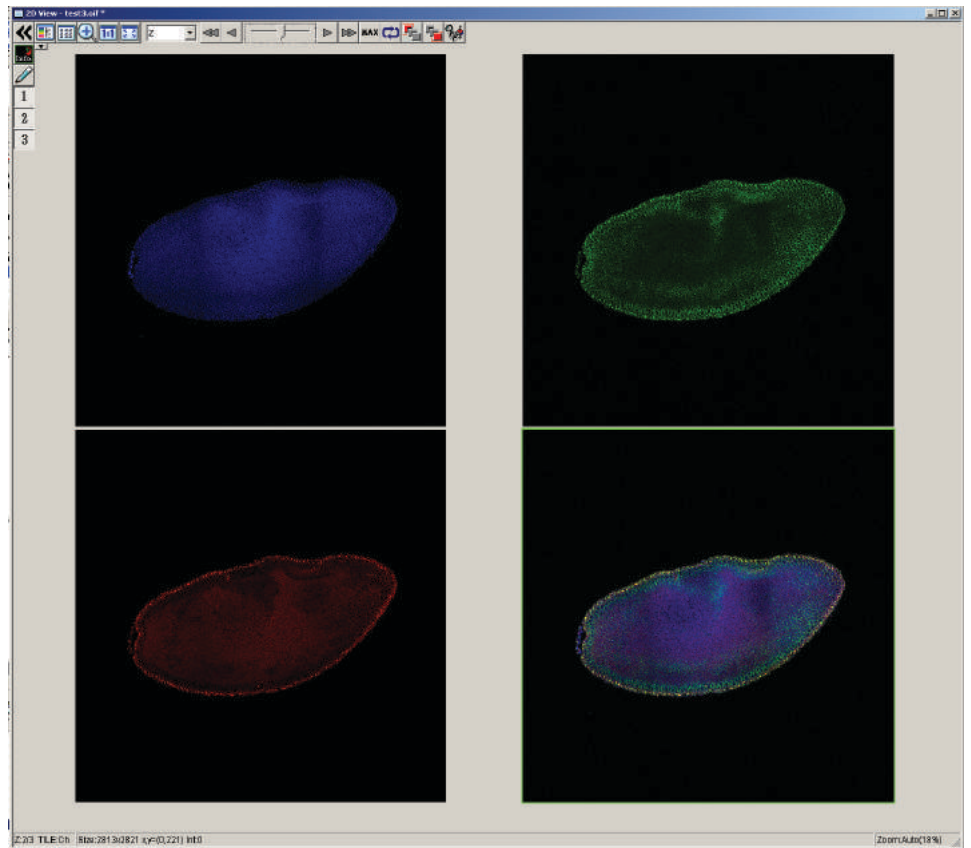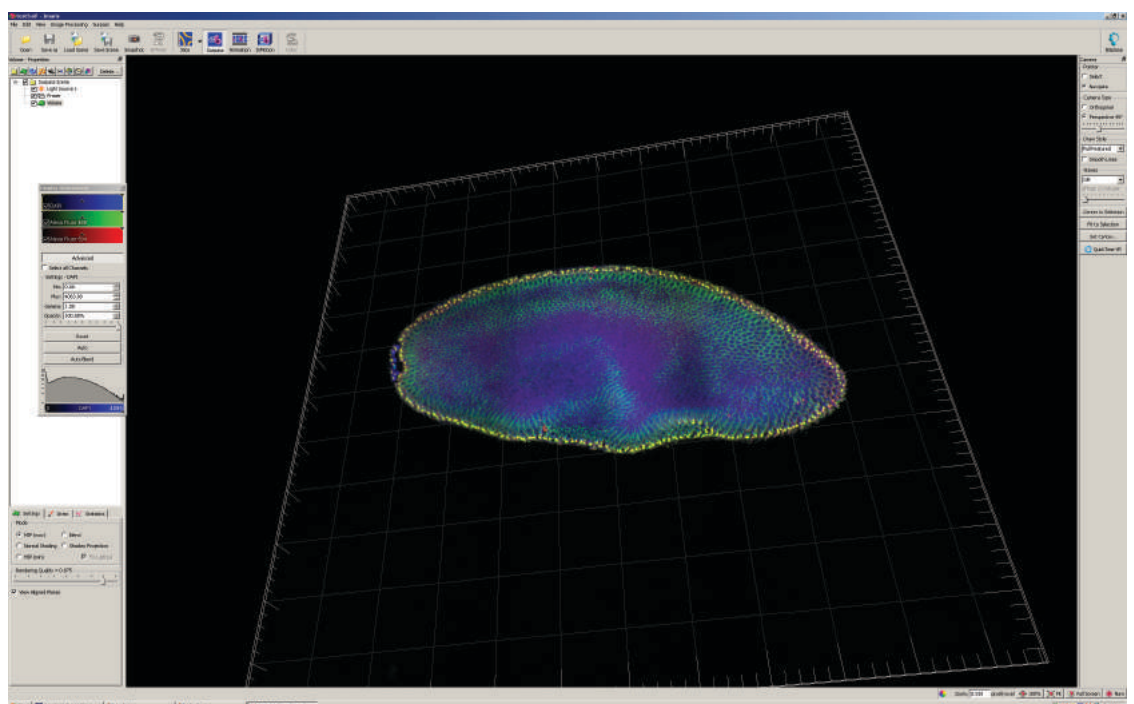
Image Display

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.

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Turn OFF

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Check the booking calendar. Are you the last user?

α1
1•Remove your sample from the microscope
2•Clean the objective lens using lens cleaning tissue
3•Switch off the fluorescence bulb
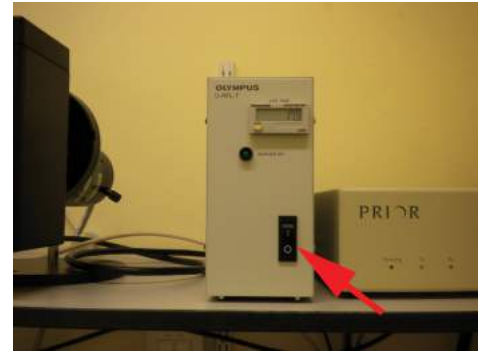
β1

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

WAIT

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

β2

4•Turn the power SWITCH off.

γ1

Turn off the 559 laser;

1•Turn the key anti-clockwise to the off position.
2•Turn the power SWITCH off

γ2

   (there's no need to wait here!)

δ1   Log off the computer.

δ2

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