DUNN SCHOOL
BIOIMAGING FACILITY

UNIVERSITY OF
OXFORD

Olympus FV1000

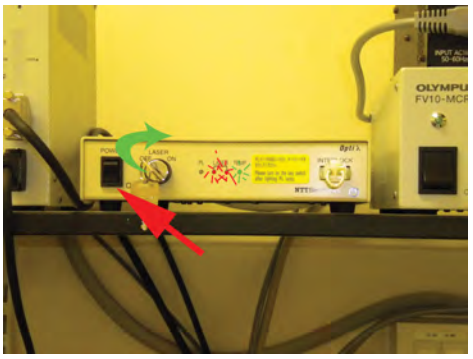
Microscope Users Guide

DUNN SCHOOL
BIOIMAGING FACILITY

Olympus
Turn ON

UNIVERSITY OF OXFORD

A1 Switch on the controller box
(the black button on the left)

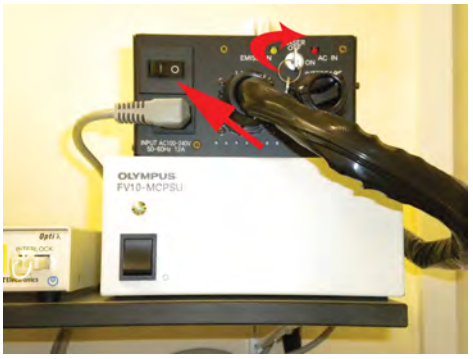


B1 Turn on the 559 laser;

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

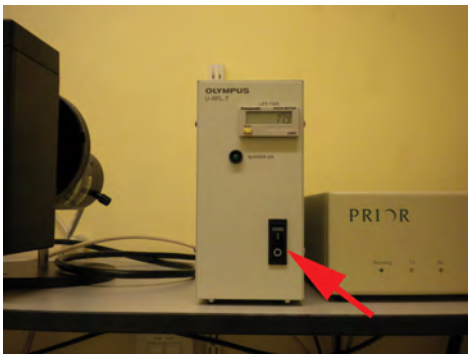
B2



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

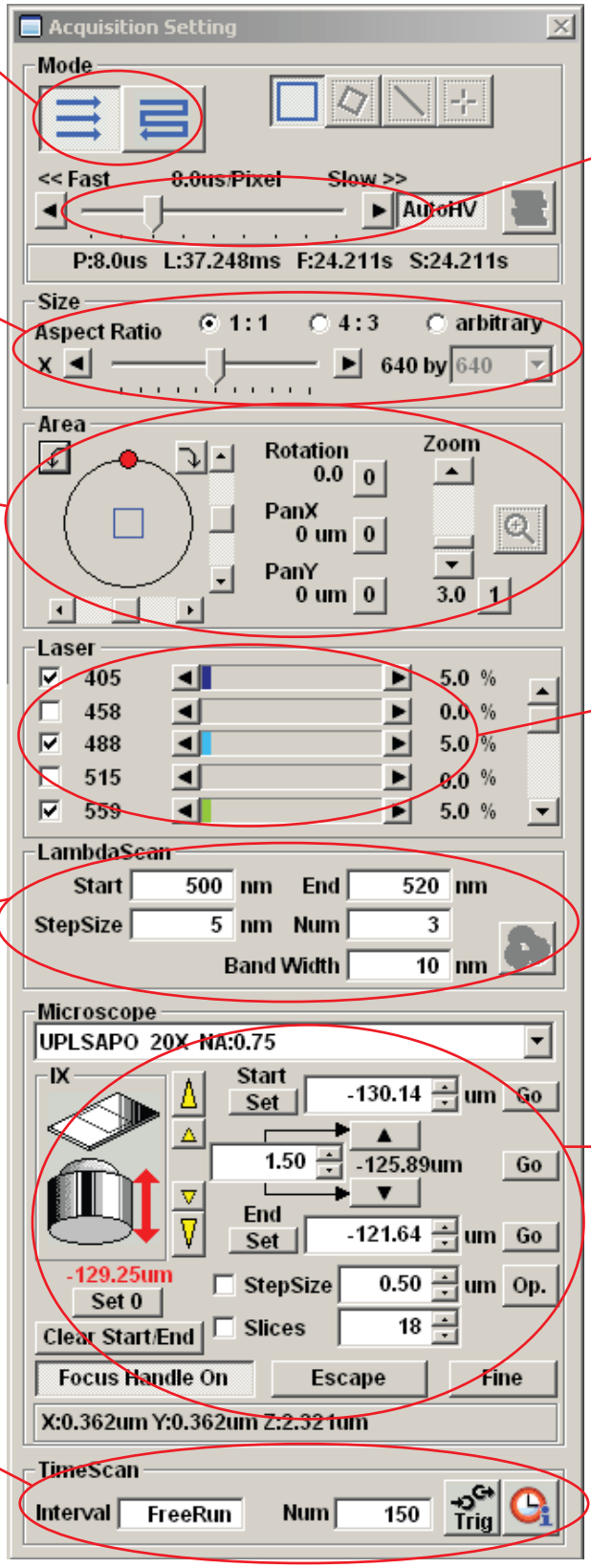
Scan Mode
(default is unidirectional)

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)

Transmission Light
(Bright Field Viewing)

Epifluorescence Light
(Fluorescence Viewing)

Dye List Setting

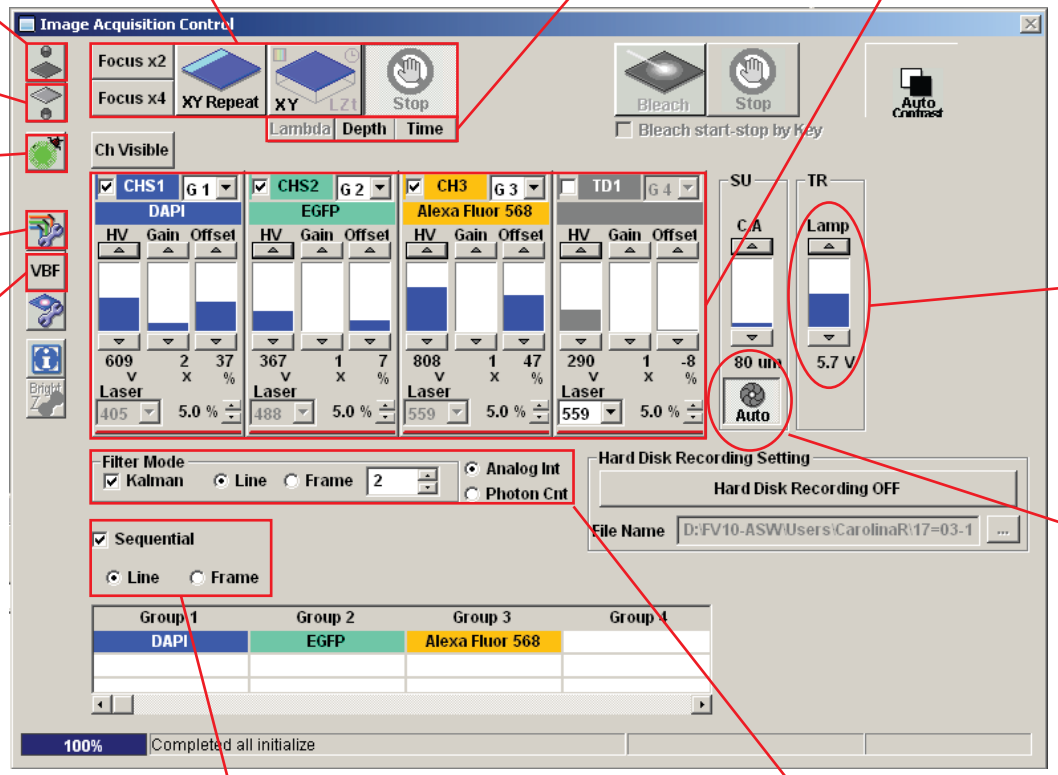
Light Path Setting

Spectral Window Setting

Scan Buttons

Imaging Mode Selection

Imagine Detection Channel Setting



Transmission light Control

Confocal Aperature
(automatic) Control

Sequential Scan Mode

Frame Averaging

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 

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

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

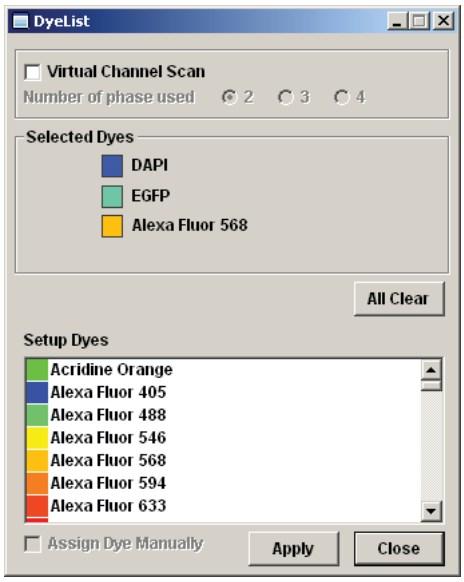
Click off the light source button to turn off the non-laser light.

or

Click on the Dye list button

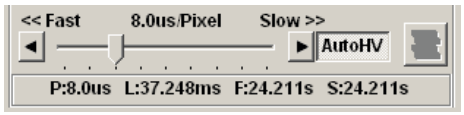
Double click on the fluorescence dyes you want to use (eg. FITC and TRITC) from the list.

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

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



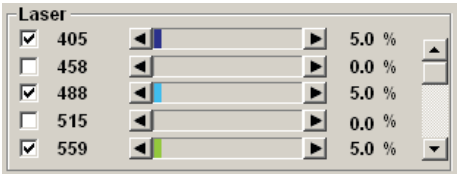
Click Focus X2 button to scan the sample.
It will scan fast at 2us /pixel and show low-quality (pixelated) image in a Live View Window.

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).

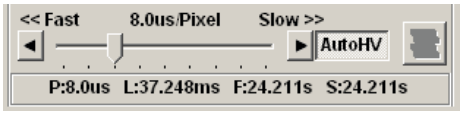
Click Stop button to stop the scanning.

Image Acquisition

Set the Laser output level



Set the scan speed as required.



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).

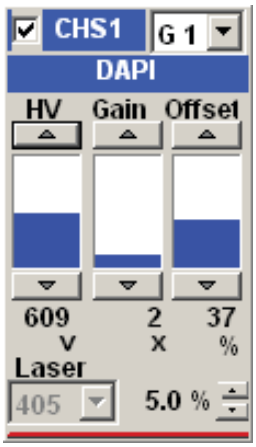
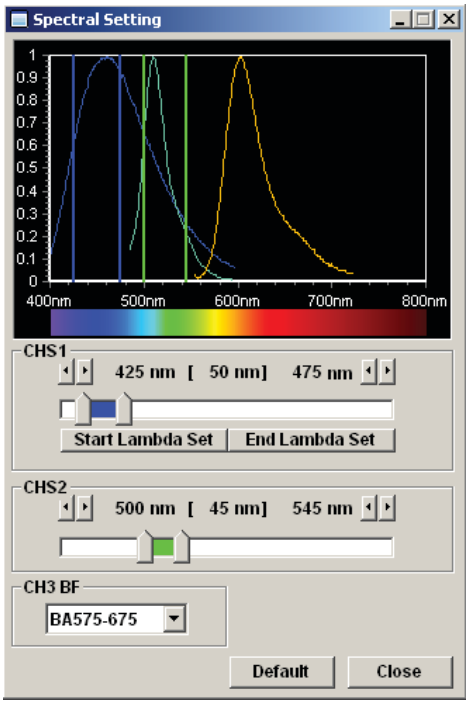


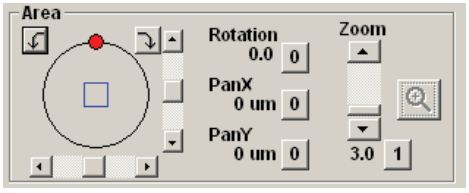
Image Acquisition

You can optimise the image quality with Hi-Lo LUT (Look-Up Table).
Click LUT button to bring the LUT control window
and select Channel and click Hi-Lo button
(Ctrl-H ahortcut 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 bos around.
To zoom on a specfic region, click the iconnand 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.

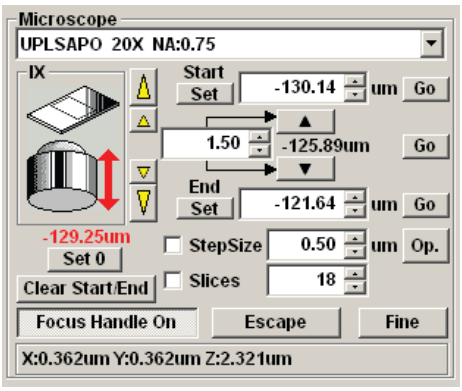
z-Series Image Acquisition

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 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 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.

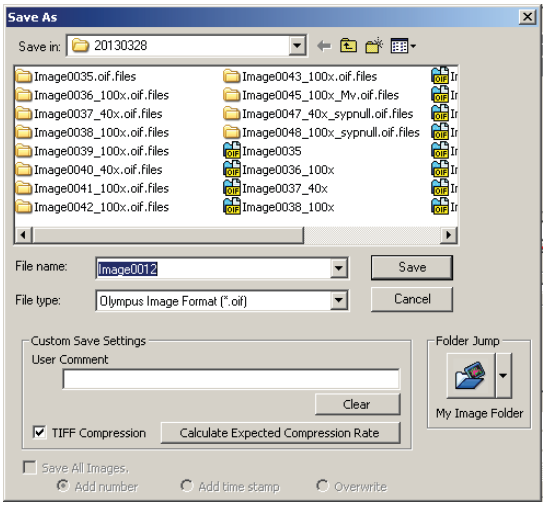
Save the image.

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.

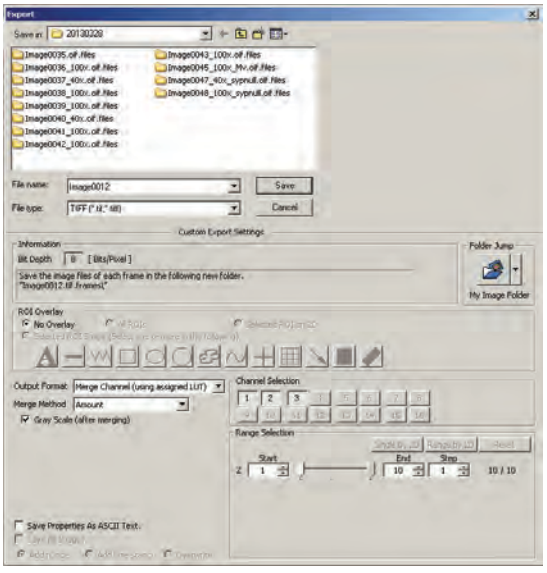


oib file type contains all the metadata including all acquisition parameters and it can be opened in the FV10-ASW software. The light version of this program is available for installing on the user's own computer (PCs only).

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.

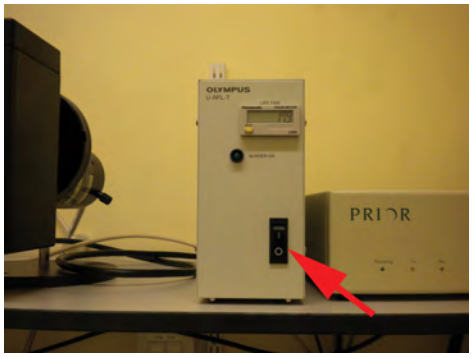
6). Click **Save** button.

DUNN SCHOOL
BIOIMAGING FACILITY

Olympus
Turn OFF

UNIVERSITY OF OXFORD

Check the booking calendar. Are you the last user?

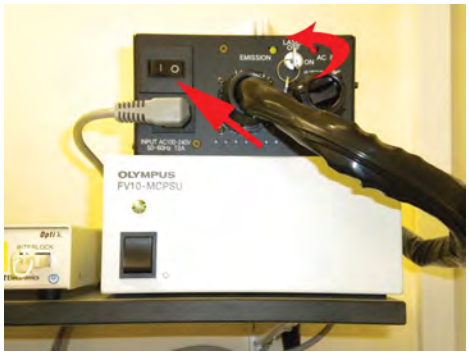


α1

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;



1•Turn the key anti-clockwise into the off position.
WAIT
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.
γ2
2•Turn the power SWITCH off
(there's no need to wait here!)

δ1

Log off the computer.

δ2

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