WinFluor V3.4.4

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Fluorescence Image & Electrophysiology Acquisition Program

Introduction > Main Features

WinFluor is a combined image and analogue signal acquisition and analysis program, which supports the simultaneous collection of cell fluorescence images (at multiple excitation wavelengths) and patch clamp current and voltage or other signals. Recorded images and electrophysiological signals can be displayed, analysed and plotted together on the same screen.

The main features of the software include:

- High speed image acquisition (500 frames/sec, depending on camera).
- Multi-spectral (excitation) imaging (2-9 wavelengths per file).
- 1 - 8 analogue input channels.
- Continuous image/analogue recording to disk.
- Stimulus pulse generation.
- Time course analysis for up to 50 regions of interest.
- Detection and analysis of event waveforms
- Display of of excitation spectra
- Pixel intensity histograms.
- Line profile plots.
- Averaging of images.
- Creation of ratio and ion concentration images
- AVI movie creation.
- Import/Export of images to PIC, TIF and STK file formats.

Cameras

- Andor Ixon and Luca and others supported by Andor SDK library
- Princeton I-Pentamax, Photometrics Coolsnap and Cascade and others supported by PVCAM library.
- Q-Imaging Rolera XR, Retiga and other supported by QImaging library.
- PCO Pixelfly
- Hamamatsu Orca ER, Orca 285, C4880-81, Image-EM and others supported by Hamamatsu DCAM library
- CCIR video cameras (Data Translation or National Instruments Frame grabbers)
- Cameras supported by National Instruments IMAQdx library.

Light Sources

- Cairn Optoscan monochromator
- Till & PPI monochromators
- Sutter LS-10 filter wheel
- Sutter DG-4 filter changer
- Cairn OptoLED and other LED light sources

Analog/Digital Interface Cards

- National Instruments E or M Series interface card can be used for analogue input/output and timing, The PCI-6229 card or USB-6229-BNC (32 A/D inputs and 4 D/A outputs) is recommended.

Patch Clamps

- Axon Axopatch 1D and 200 and Multiclamp 700A and 700B,
- Biologic VP500 and RK400,
- Cairn Optopatch,
- WPC 100,
- Heka EPC-8,
- NPI Turbo-Tec 03, Turbo-Tec10, SEC05LX,
- Dagan PCOne, 3900A,
- Warner PC501A/B, OC725C.

The Strathclyde Electrophysiology Software package is a suite of programs for the acquisition and analysis of electrophysiological signals, developed by the author at the Strathclyde Institute for Pharmacy & Biomedical Sciences, University of Strathclyde. At the discretion of the author, the software is supplied free of charge to academic users and others working for non-commercial, non-profit making, organisations. Commercial organisations may purchase a license to use the software from the University of Strathclyde (contact the author for details). The author retains copyright and all rights are reserved. The user may use the software freely for their own research, but should not sell or pass the software on to others without the permission of the author. Except where otherwise specified, no warranty is implied, by either the author or the University of Strathclyde, concerning the fitness of the software for any purpose. The software is supplied "as found" and the user is advised to verify that the software functions appropriately for the purposes that they choose to use it. An acknowledgement of the use of the software, in publications to which it has contributed, would be gratefully appreciated by the author.

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Getting Started > Installing WinFluor

To install WinFluor:

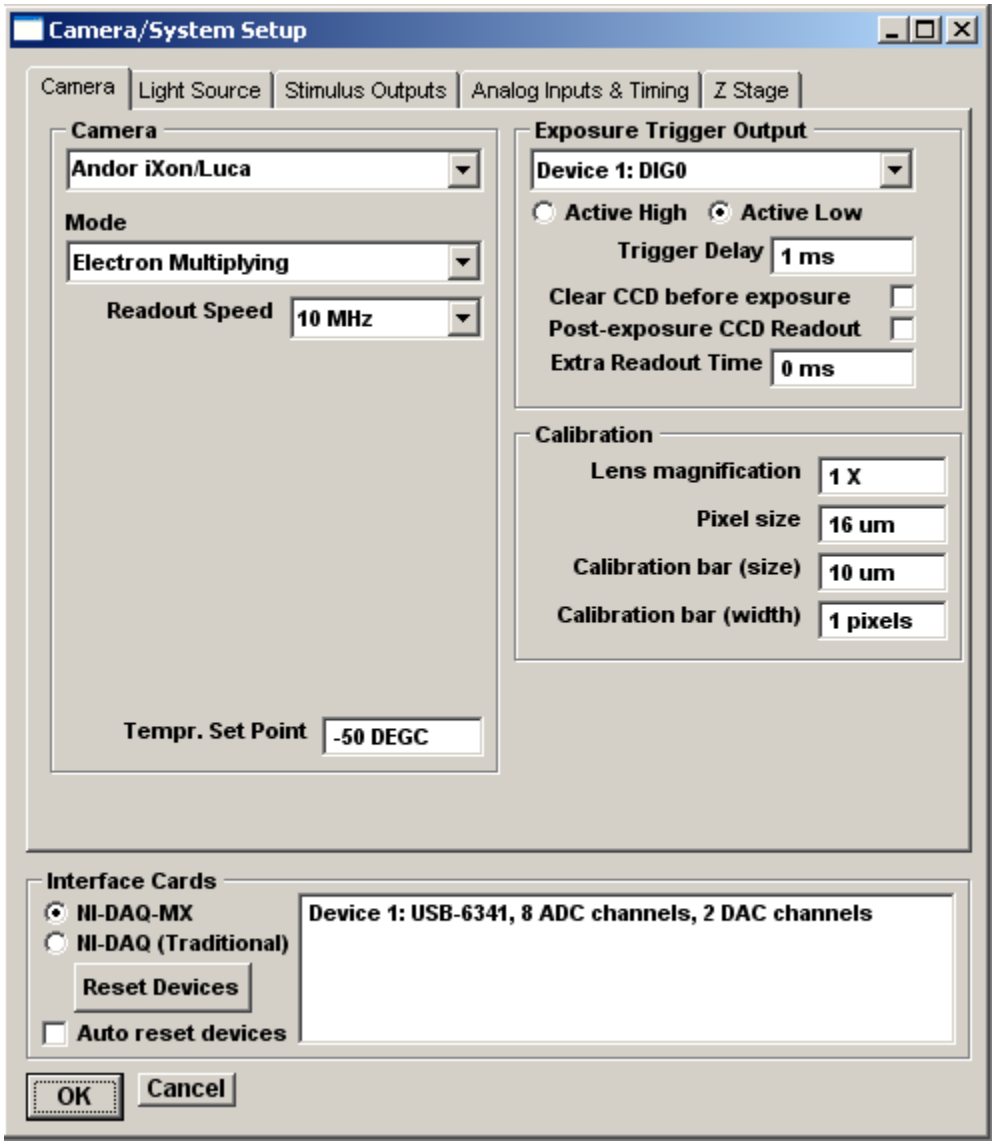
1. Install the National Instruments NI-DAQ software supplied with your NI interface card(s).
2. Install (PCI) or attach (USB) the NI interface card(s) to your computer. If you have more than one PCI card installed connect the cards together using an RTSI bus cable. Check that the card(s) are detected when the computer is re-started.
3. Install the driver software and support library supplied with your camera. (Note. For Andor cameras, the Andor Software Development Kit library must be installed.)
4. If the camera uses a specialised interface board, install it in the computer.
5. Check that the camera is configured and functioning correctly using the test software supplied with the camera.
6. Install the WinFluor program using the setup program downloaded from

   http://spider.science.strath.ac.uk/sipbs/page.php?show=software_imaging

Getting Started > Hardware Configuration

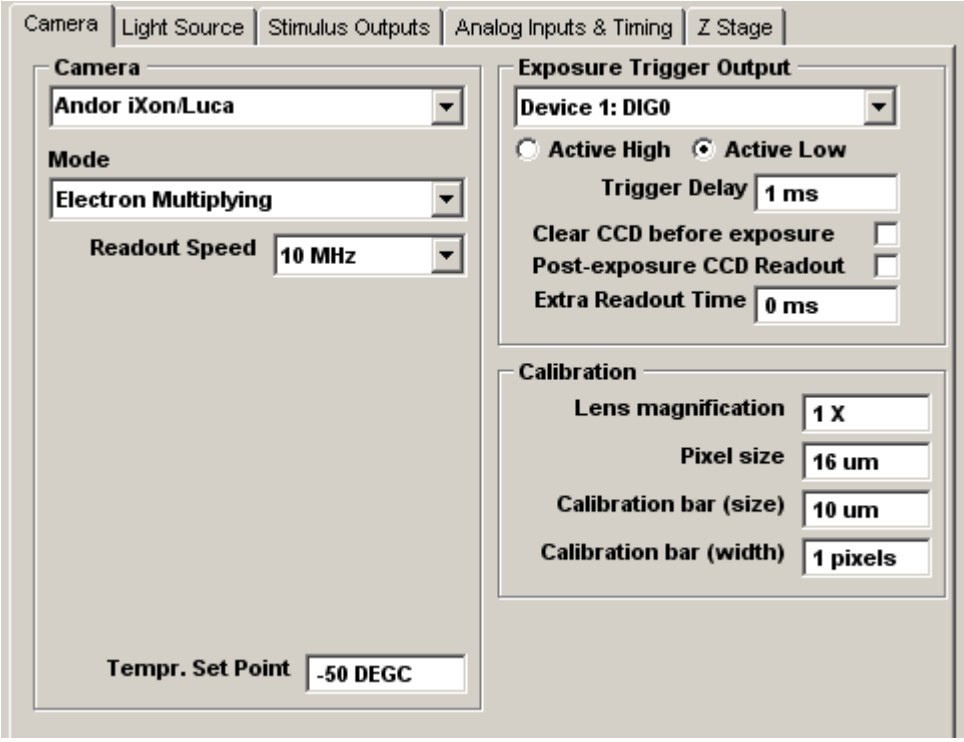
To configure WinFluor to work with your system's camera, light source and timing hardware open the **Camera/System Setup** dialog box by selecting

Setup
Camera/System Setup



Getting Started > Cameras

The camera type in use and camera settings are configured on the **Camera** page.



Camera

Select the required camera support library for your camera from the **Camera** list. ***Note. Before selecting a camera, ensure that the camera software drivers and support library are installed in your computer and the camera is connected and switched on.***

Readout Speed

If your camera supports, multiple readout speeds, select the desired rate (usually the highest) from the **Readout Speed** list.

COM Port

If your camera is controlled via a serial communications line, select the computer COM port to which is it is connected from the **COM Port** list.

Exposure Trigger

Select the D/A or digital output line to be used to trigger camera exposures from the **Exposure Trigger** list and set the polarity of the trigger pulse required by the camera (**Active High** – for a 0V-to-5V pulse or **Active-Low** – for a 5V-to-0V pulse.).

Note. Ensure that the selected output line is connected to the trigger input of the camera. If you do not require the camera to be synchronised to external devices, select **Internal** as the **Exposure Trigger** option

Trigger Delay

the **Trigger Delay** setting determines the delay between the change of excitation wavelength (in multi-wavelength imaging) and the triggering of camera exposure.

Clear CCD before exposure

The **Clear CCD before exposure** option sets the camera to clear the CCD at the beginning of a exposure. (Note. Not all cameras support this option. Enabling this option may reduce maximum frame rate of the camera.)

Post-exposure CCD readout.

The **Post-exposure CCD readout** option forces CCD readout before the next exposure can take place. (Note. This option reduces camera performance.)

Extra Readout Time

In triggered mode exposure mode, camera exposure time is normally set to 90% of the inter-frame interval. The **Extra Readout Time** value (default 0) allocates extra time for CCD readout by reducing the exposure time of the camera.

Tempr. Set Point

The **Tempr. Set Point** setting determines the target temperature for camera equipped with Peltier cooled CCDs.

Image Calibration

Lens magnification

Enter the magnification factor of your microscope objective lens in the **Lens magnification** box.

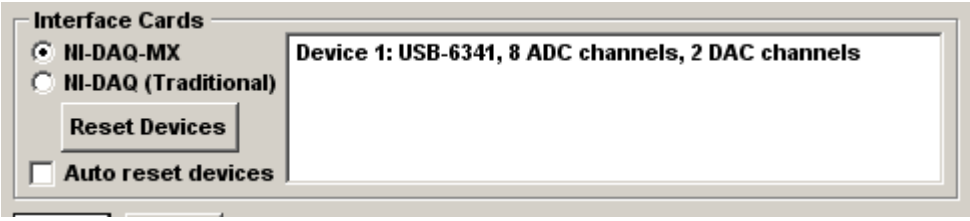
Calibration bar

Enter the desired size (in microns) of the display calibration bar in the **Calibration bar (size)** box. Enter the thickness of the displayed calibration bar (in pixels) in the **Calibration bar (width)** box.

Getting Started > Analogue/Digital Interface Unit

A National Instruments E or M Series multifunction interface unit acts as master clock, providing timing and control pulses to coordinate image capture, light source wavelength changes analogue signal capture and waveform generation. Multiple interface boards, synchronised using the National Instruments RTSI bus, can be used to increase the number of timing and stimulus waveform generation channels.

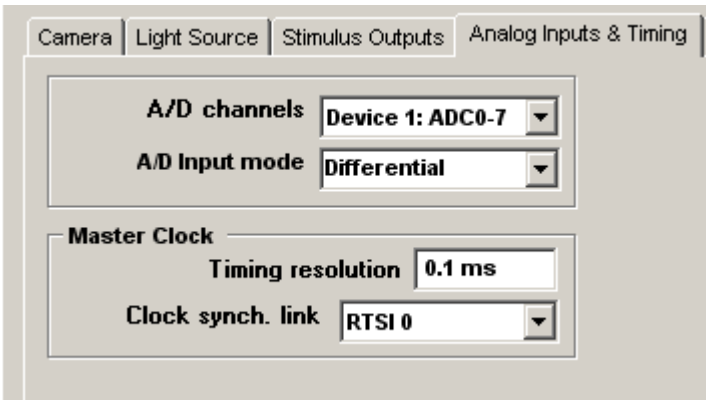
Select the National Instruments software library to be used to communicate with the interface board. If an M or X Series interface card is being used (.e.g. PCI 6221, PCI 6229, USB 6229 BNC), or a combination of M and E Series boards, select the **NIDAQ-MX** option. A list of installed interface cards available to WinFluor with number of available analogue inputs and outputs channels is displayed. Select the **NIDAQ (Traditional)** option when using E series boards when the older 'traditional' NIDAQ software library is installed instead of the NIDAQ-mx library.



Getting Started > Analogue Inputs & Timing

Analogue input channels and the timing of analog input and output is configured on the **Analog Inputs & Timing** page.

WinFluor can acquire analogue signals from up to a maximum of 8 input channels, simultaneously with the capture of images.



A/D Channels

Select the range of interface device analogue input channels to be used for analogue input from the **A/D Channels** list.

A/D Input mode

The A/D input mode for the interface device is selected from the **A/D Input mode** list. Analogue input channels can be configured to operate in either differential or single-ended input mode. In differential mode, the input signals are derived from the differences between pairs of inputs (ADC0 – ADC8, ADC1 – ADC9, etc.) In single-ended mode, the signals are derived from ADC0 .. ADC7 alone, measured relative the AISENSE input.

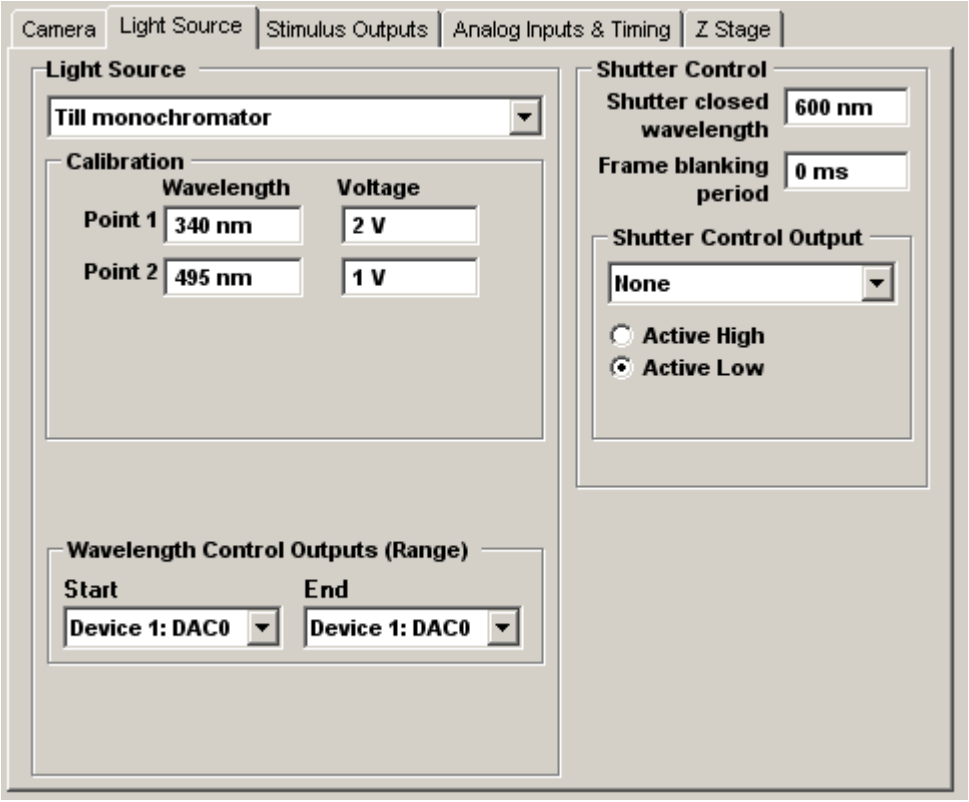
The A/D Input mode setting must match the type of National Instruments input/output box used to connect signals to the interface board. The default setting is **Differential** and this is the only setting that can be used with the BNC-2110 input/output panel or USB-6229-BNC device. Note. If a BNC 2090 19" rack mountable I/O panel is in use and **Differential** mode is selected, ensure that all SE/DI switches are set to DI. (If Single-Ended mode is selected, with a BNC-2090 panel, ensure that the **SE/DI** switches are set to **DI** and the **NRSE/RSE** switch to **RSE**.)

Master Clock Timing

To set the timing resolution of the master clock used for cameras, light source and analogue input and output synchronisation, enter a time interval into the **Timing resolution** box. (The default value is 0.1 ms).

If two or more interface cards are installed in the system and connected together internally using National Instruments RTSI bus cables, select **RSTI 0** as the **Clock synch. link** option. If cards are linked externally using the PFI5 line, select the **PFI 5** option. (Note. The PFI 5 option currently only works NIDAQ (Traditional) library option. Synchronisation using the RTSI bus is to be preferred.)

The type of light source in use and its settings are configured on the **Light Source** page.



If you have a computer-controllable light source attached to your system, select the type of source from the **Light Source** list.

Wavelength Control

Select the group of interface outputs used to control light source wavelength from the **Wavelength Control Outputs (Range)** lists. Select the first output line in the group from the **Start** list and the last output line from the **End** list.

Shutter Control

If the light source requires a shutter control line, select the output line to be used to open/close the light source shutter from the **Shutter Control** list and set the polarity of the trigger pulse required by the shutter (**Active High** for a 0V-to-5V pulse or **Active-Low** for a 5V-to-0V pulse.).

Shutter closed wavelength

The **Shutter closed wavelength** entry sets the wavelength monochromators are set to when the excitation **Off** setting is selected in the WinFluor recording windows.This is typically chosen to be a wavelength blocked by the dichroic or barrier filters in the microscope epi-cube.

Frame blanking period

The **Frame blanking period** entry (default 0 ms) defines the period of time for which illumination is turned off at the end of each camera acquisition frame.

Getting Started > Light Sources > Cairn Optoscan Monochromator

The connections and settings for Cairn Optoscan monochromator attached to a PCI-6229 interface board.

Note Cairn Reseach can supply a custom input/output panel configured to connect the Optoscan to a National Instruments PCI-6229 card.

Signal connections				
PCI-6229 Name		A PCI 6229 68p connector (left)	B PCI 6229 68pin connector (right)	
AO0	Sig	22,		Voltage stimulus #1 (sig)
	Gnd	55		
AO1	Sig	21,		Optoscan Input Slit (V+)
	Gnd	54		Optoscan Input Slit (V-)
AO2	Sig		22,	Optoscan Grating (V+)
	Gnd		55	Optoscan Grating (V-)
AO3	Sig		21,	Optoscan Output Slit (V+)
	Gnd		54	Optoscan Output Slit (V-)
P0.0	Sig	52,		Camera Ext Trigger (sig)
	Gnd	53		Camera Ext Trigger (gnd)

WinFluor Camera/System Setup settings		
Camera exposure trigger		Device 1: DIG0
Shutter		None
Wavelength Control	Start	Device 1: DAC1
	End	Device 1: DAC3
Voltage Stimulus #1		Device 1: DAC0
Voltage Stimulus #2		None
Digital Stimulus	Start	Device 1: DIG1
	End	Device 1: DIG7
Analogue Input		Device 1: ADC0-7

Getting Started > Light Sources > Sutter DG-4 Filter Changer

The connections and settings for Sutter DG-4 filter changer attached to a PCI-6229 interface board.

Camera/System Setup

Camera exposure trigger		Device 1: DIG0
Wavelength Control	Start	Device 1: DIG1
	End	Device 1: DIG4
Voltage Stimulus #1		Device 1: DAC0
Voltage Stimulus #2		Device 1: DAC1
Digital Stimulus	Start	Device 1: DIG5
	End	Device 1: DIG7

Signal Connections

PCI 6229 68 way connector (left)				
Name	Pin			
P0.0	52 53 gnd	------ ------	Camera Ext Trigger	
			Sutter DG-4 25 pin parallel socket	
			Pin	Name
P0.1	17	------	2	Bit 0 Command (FILTER)
P0.2	49	------	3	Bit 1 Command (FILTER)
P0.3	47	------	4	Bit 2 Command (FILTER)
P0.4	19	------	5	Bit 3 Command (FILTER)
DGND	18	------	25	GROUND
DGND	18	------	6	Bit 4 Command
DGND	18	------	7	Bit 5 Command
DGND	18	------	8	Bit 6 Command
DGND	18	------	9	Bit 7 Command

Getting Started > Light Sources > Till or PTI Monochromator

Connections and settings for WinFluor system with a PCI-6229 interface board, camera and Till monochromator.

Signal connections				
PCI-6229 Name		A PCI 6229 68p connector (left)	B PCI 6229 68pin connector (right)	
AO0	Sig	22,		Voltage stimulus #1 (sig)
	Gnd	55		
AO1	Sig	21,		Voltage stimulus #2 (sig)
	Gnd	54		
AO2	Sig		22,	Till grating control input (sig)
	Gnd		55	Till grating control input (gnd)
P0.0	Sig	52,		Camera Ext Trigger (sig)
	Gnd	53		Camera Ext Trigger (gnd)

Camera/System Setup Settings		
Camera exposure trigger		Device 1: DIG0
Shutter		None
Wavelength Control	Start	Device 1: DAC2
	End	Device 1: DAC2
Voltage Stimulus #1		Device 1: DAC0
Voltage Stimulus #2		Device 1: DAC1
Digital Stimulus	Start	Device 1: DIG1
	End	Device 1: DIG7
Analogue Input		Device 1: ADC0-7

Getting Started > Light Sources > Sutter Lambda 10-2 Filter Wheel

The connections and settings for a Sutter Lambda 10-2 filter wheel attached to a PCI-6229 interface board.

Camera/System Setup settings

Camera exposure trigger		Device 1: DIG0
Shutter		Device 1: DIG1 (Active High)
Wavelength Control	Start	Device 1: DIG2
	End	Device 1: DIG5
Voltage Stimulus #1		Device 1: DAC0
Voltage Stimulus #2		Device 1: DAC1
Digital Stimulus	Start	Device 1: DIG6
	End	Device 1: DIG7

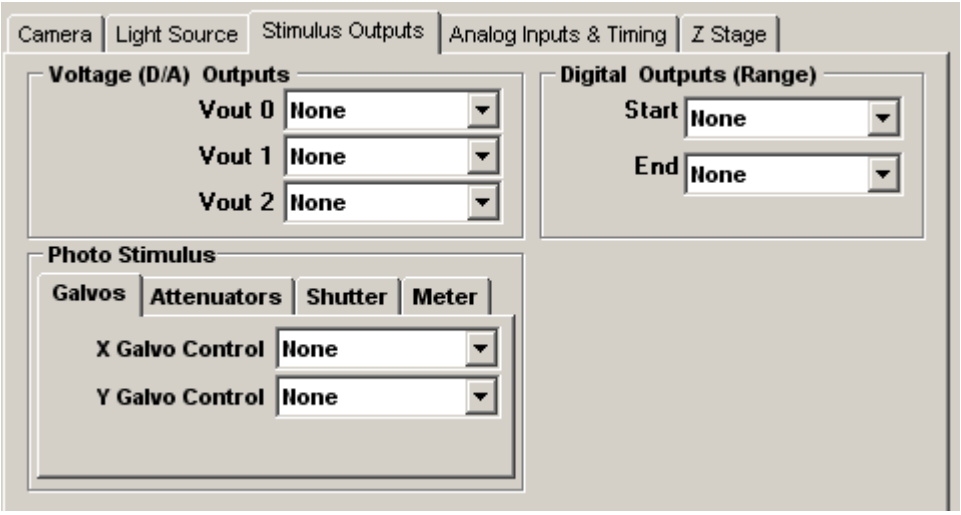
Signal Connections

PCI 6229 68 way connector (left)				
Name	Pin			
P0.0	52 53 gnd	------ ------	Camera Ext Trigger	
			Sutter LS-2 25 pin parallel socket	
			Pin	Name
P0.1	17	------	14	SHUTTER
P0.2	49	------	2	FILTER 0 bit
P0.3	47	------	3	FILTER 1 bit
P0.4	19	------	4	FILTER 2 bit
P0.5	51	------	5	FILTER 3 bit
DGND	18	------	25	Ground
DGND	18	------	6	SPEED 0 bit
DGND	18	------	7	SPEED 1 bit
DGND	18	------	8	SPEED 2 bit
DGND	18	------	9	Wheel Select (Wheel A)

Getting Started > Stimulus Outputs

WinFluor can generate analog or digital stimulus waveforms simultaneously with image and analogue signal recording. If sufficient output channels are available, up to 3 voltage stimulus waveform output channels and TTL digital pulse output channels can be supported.

Stimulus output channels are configured on the **Stimulus Outputs** setup page..



Voltage (D/A) Outputs

To select an analogue output channel for voltage stimulus waveform #0, select a DAC channel from the **Vout 0** list, or select **None** to disable it. (Note. When a patch clamp is in use, **Vout 0** is typically used to provide the command voltage signal.)

To select an analogue output channel for Voltage stimulus waveforms #1 or #2, select a DAC channel from the **Vout 1 or Vout 2** list, or select **None** to disable it.

Digital Outputs

To select a range of digital pulse stimulus channels, select the first digital output line from the Digital Outputs (Range) **Start** list and the last digital output from the **End** list, or select **None** to disable digital outputs.

Photo Stimulus

The **Photo Stimulus** settings configure WinFluor's laser stimulated point photolysis feature (currently only available when used with Prairie Technology Ultima microscope).

Galvos

Select the analog outputs used to control laser position from the **X** and **Y Galvo Control** list boxes.

Attenuators

Select the analog outputs used to control laser attenuators (Pockel Cells) from the Attenuator **Control Channels 1-3** list boxes.

Shutter

Select the analog outputs used to control laser safety shutter from the Shutter **Control Channel 1** . Enter the shutter opening time into the **Latency** field and select whether the shutter is opened by an 5V signal level (**Active High**) or 0V (**Active Low)**.

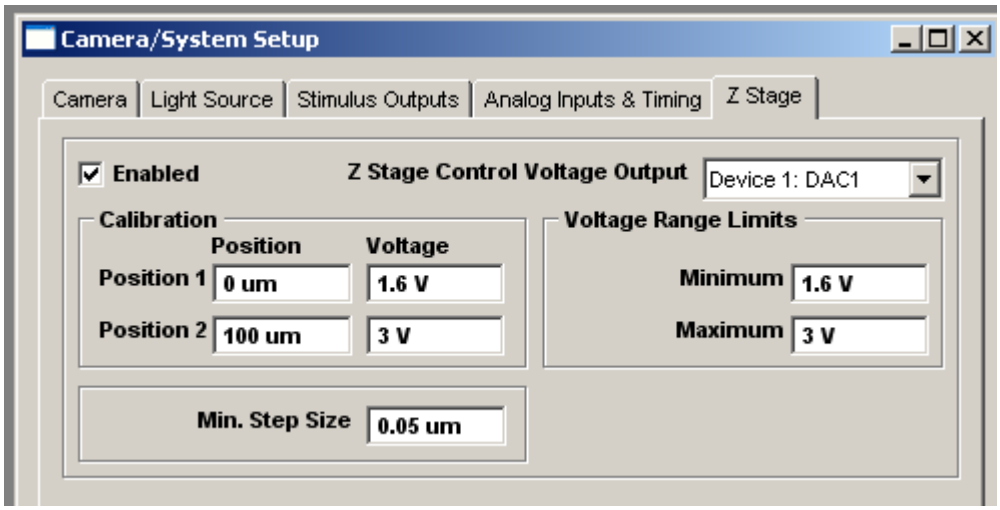
Meter

Select the signal level meter from **Meter Input** list box.

Getting Started > Z Stage Controllers

When a voltage-controlled piezo stage or lens focus controller is available, lens focus can be adjusted from the **Image** and **Image & Signals** recording windows and Z stacks containing sections at a series of Z positions can be recorded.

Support for an voltage-controlled stage or piezo lens focus controllers can be configured using the **Z Stage** setup page.



Enable

Tick the **Enabled** option to enable Z position control in WinFluor's Image and Image & Signals recording windows.

Z Stage Control Voltage Output

Select the analog output channel to be used to control the lens/stage positioner from the **Z Stage Control Voltage Output** list.

Voltage Range Limits

Enter the lower and upper limits of the lens positioner analog input voltage working range into the **Minimum** and **Maximum** fields.

Calibration

Calibrate the lens positioner by entering the analog control voltage and associated lens position (**Position** (microns), **Voltage** (V)) for a pair of calibration points (**Position 1, Position 2**).

Min. Step Size

Enter the smallest change (in microns) in Z position supported by the lens controller into the **Min Step Size** field.

WinFluor V3.4.4

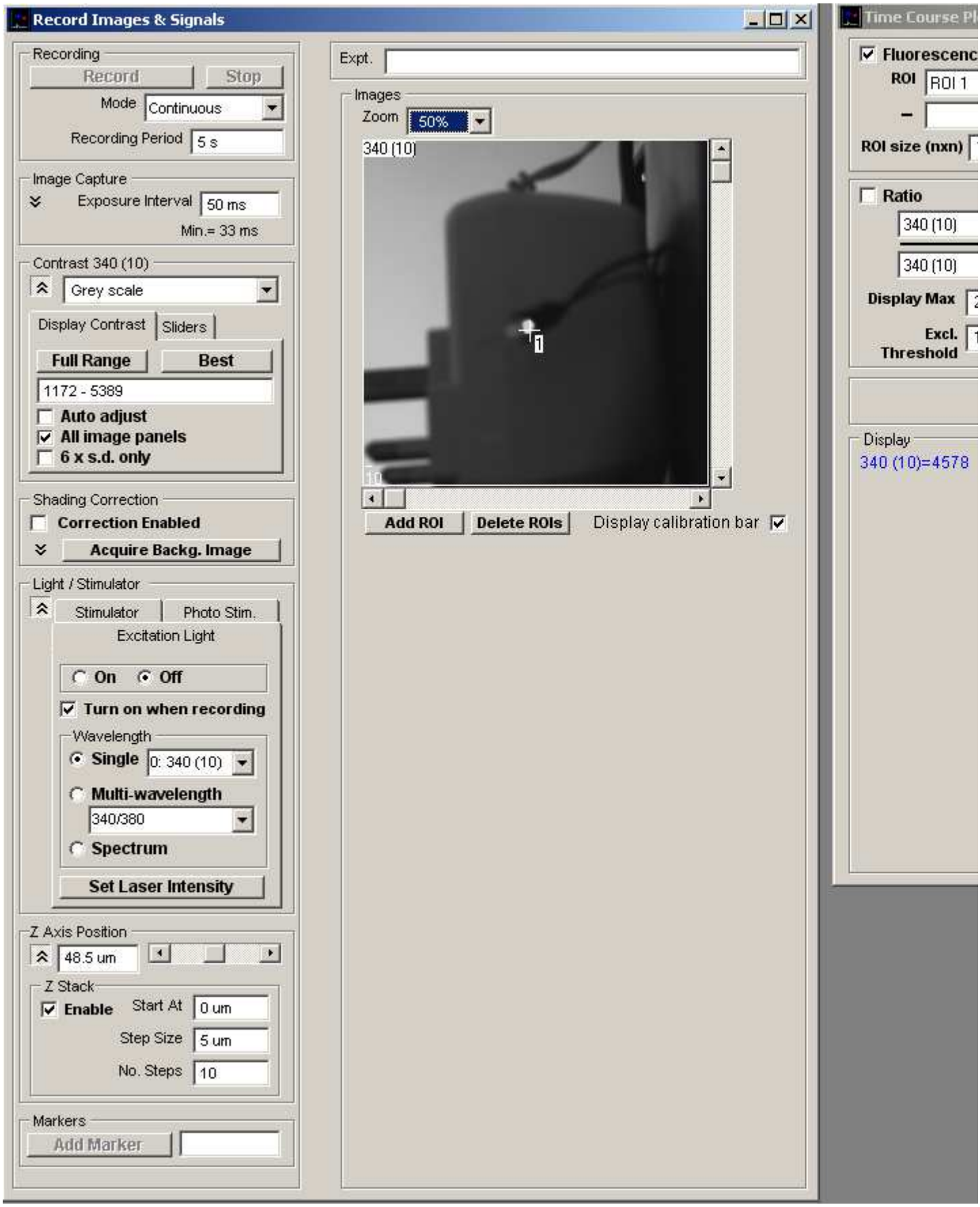
Fluorescence Image & Electrophysiology Acquisition Program

Recording > Images + Signals > Recording Images & Signals

To acquire images from the camera and analog signals and record these to file, select

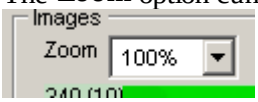
Record
Images & Signals

to display the **Record Images & Signals** window.



A graph of the time course of pixel intensity from a selected region of interest (ROI) within the live image (one for each image pane in multi-wavelength sequence) is displayed along with the images. Analogue signals (if they are being acquired) are also displayed in this area.

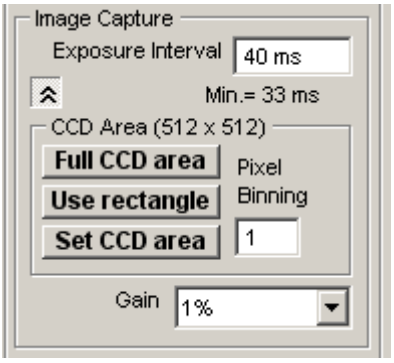
The **Zoom** option can be used to magnify or reduce the region of the image displayed within the image panel.



Zoom factors of 25% - 800% are currently supported. The magnified region of the image displayed within the image panel can be panned horizontally and vertically within the image.

Recording > Images + Signals > Image Capture

The Image Capture group of settings define the size and location of the image to be captured and the time interval between frames



Exposure Interval

Enter the required time interval (in ms) between successive image frames into the **Exposure Interval** box. (The shortest valid inter-frame interval is indicated below the entry box. This will vary depending upon capture region size and CCD binning factor.)

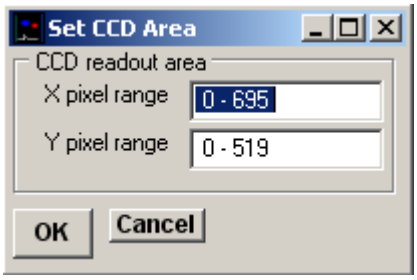
CCD Area

If the camera supports CCD sub-region readout, the CCD area controls allow you to acquire the image from a defined sub-region within the CCD.

To use the full imaging area of the CCD, click the **Full CCD Area** button.

To acquire the image from a CCD sub-area defined on screen, use the mouse to drag in the edges of the capture rectangle superimposed on the live displayed image until it encompasses the region to be acquired, then click the **Use rectangle** button.

To define the CCD sub-area in terms of its X and Y pixel coordinates within the CCD, click the Set **CCD Area** button and enter the required X and Y pixel range into the Set CCD Area dialog box which pops up.



Binning

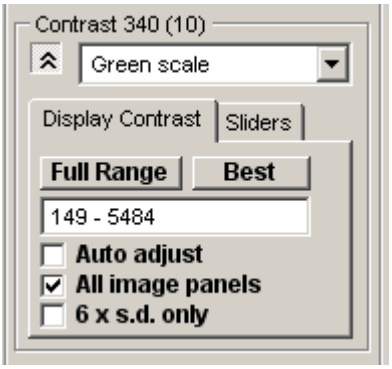
If the camera supports CCD pixel binning, to bin together blocks of pixels to increase image intensity and/or allow faster frame capture rates, enter a value greater than one into the **Pixel Binning** box, then press the <Enter> key. (A value of 2 bins together 2x2 blocks of pixels, increasing image intensity by 4).

Gain

Set the camera CCD readout amplifier gain by selecting a gain factor from the **Gain** list. (With EMCCD cameras the gain is expressed as a percentage of the maximum electron multiplying gain).

Recording > Images + Signals > Display Contrast

The display colour mapping options determines how pixel intensities within images are displayed on screen.



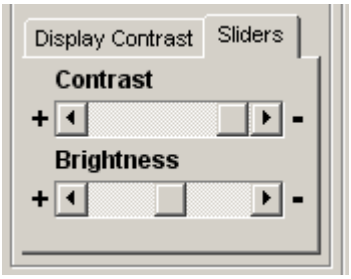
The **Grey Scale** option maps image intensity as shades of grey. **Green, Blue** and **Red Scale** maps intensities into shades of these colours. **False Colour** maps intensities in a rainbow of colours, ranging from minimum to maximum in the sequence - black, blue, green, yellow, red, white.

The range of pixel intensities mapped into the display colour range is displayed in the **Display Contrast** tab. To achieve optimal image contrast, click the **Best** button to adjust the intensity range to the range of pixel intensities within the image. Click the **Full Range** button to set the display to the full range of intensities supported by the camera. The display can be set to a specific intensity range, by entering the required range into the intensity range display box and pressing the **<Enter>** key. When the **Auto adjust** option is ticked, the display contrast is automatically adjusted to the optimal setting as frames as acquired.

The **6 x s.d. only** option is ticked the optimal contrast range is set to 3 standard deviations on either side of the mean image intensity, and to the minimum and maximum intensities within the image when unticked.

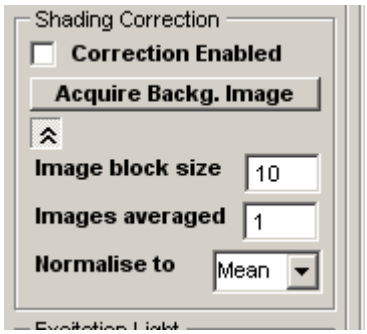
When the **All image panels** option is ticked the contrast is set for all image panels of multi-wavelength files simultaneously. Unticked, the contrast can be adjusted for individual panels, by clicking on a panel to select it, then clicking the **Best** or **Full Range** button.

Display contrast can also be adjusted manually by selected the **Sliders** tab and adjusting the **Brightness** and **Contrast** slider bars to achieve the desired image contrast.



Recording > Images + Signals > Shading Correction

The shading correction function can be used to cancel out uneven illumination intensity across the image by subtracting a smoothed background image.



To apply shading correction:

1. Set the of number pixels (*n x n*) in a smoothing block in the **Image block size** box (default 10).

2. Set the the number of images to be averaged in the **Images Averaged** box (default 1).

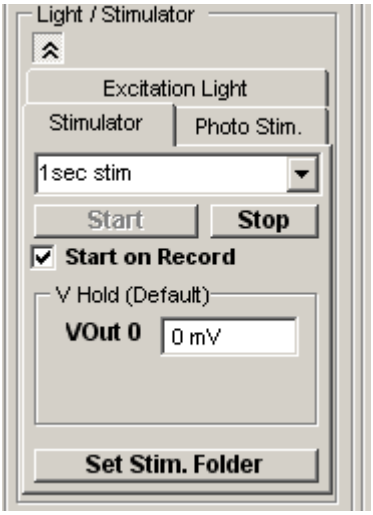
3. Select the grey level to which the corrected image is normalised from in the **Normalise to** list. Select **Mean, Min. or Max.** to normalise to the mean, minimum or maximum grey level within the uncorrected image, or **Zero** to normalise about zero (default **Mean**).

4. Click the **Acquire Backg. Image** button to acquire a background image.

5. Select the **Correction Enabled** option to apply the shading correction to live images. Shading correction using the background image can be toggled on and off as required.

Recording > Images + Signals > Stimulator

The application of voltage and/or digital stimulus pulses is handled by the controls on the **Stimulator** page.



Stimulus Protocols

To generate a stimulus waveform, select a stimulus protocol from the **Stimulator** list and click the **Start** button. (Stimulus protocols are also started automatically when the **Record** button is pressed.) Clicking the **Stop** button terminates the stimulus and returns the output voltage to the holding level. Click the **Set Stim. Folder** to change the folder holding the stimulus protocol files.

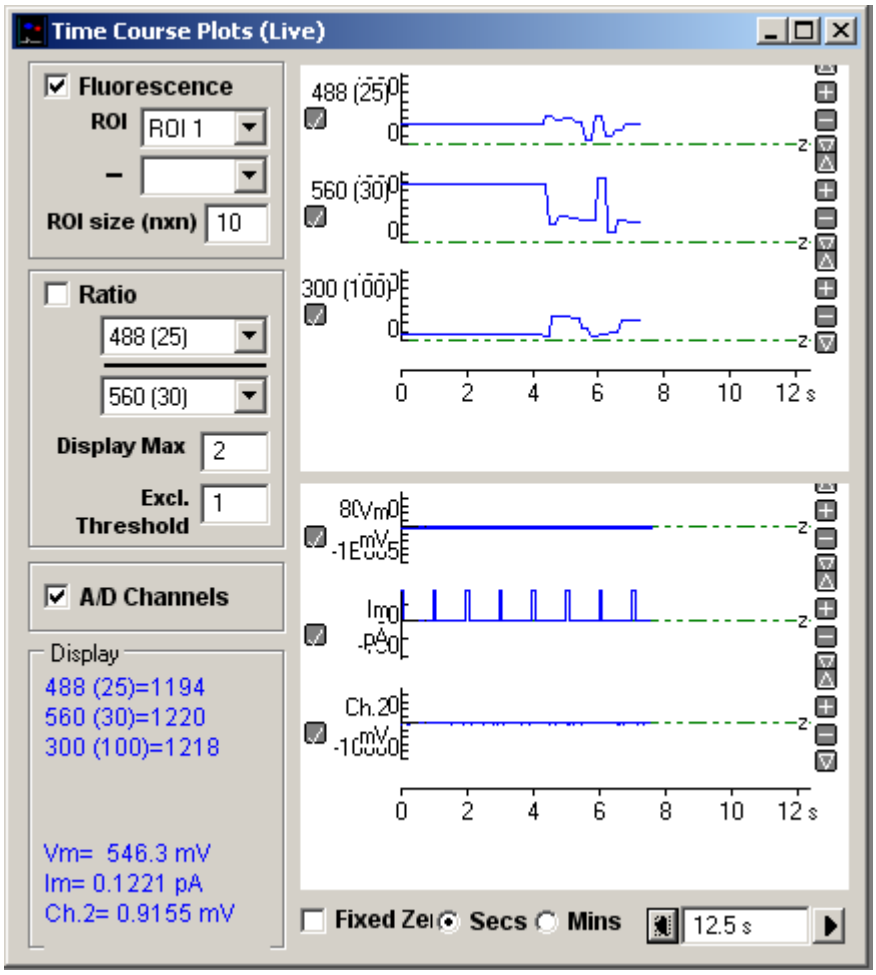
Stimulus protocols can consist of a series of one or more pulses, incremented in amplitude or duration to create a family of pulses. Complex stimulus waveforms can be produced, including series of rectangular steps, ramps, and digitised analogue signals. Individual protocols can also be linked together to automatically apply a series of different protocols during an experiment. Protocols are created using the Stimulus Protocol Editor and stored as protocol files (*.vpr files). (See section ?)

V Hold (Default)

The holding voltage applied to each voltage stimulus channel (**VOut 0-2**) can be set by entering a new voltage into the appropriate box for each channel and pressing the Enter key. (Note. Stimulus voltages waveforms generated during a voltage protocol are added to the holding voltage. To record to file a series of images (with signals if analogue channels are defined):

Recording > Images + Signals > Time Course Window

The Time Course Plots (Live) window displays the time course of the fluorescence intensity at a selected region of interest in the image and analogue signals.



Fluorescence

Select the **Fluorescence** option to display the time course of the fluorescence intensity of a selected the region of interest (**ROI.1** 2 etc.) ROIs consist of *n* x *n* pixel square regions with the centred on the + marker on the image. The size of the ROI square is defined in the **ROI size (nxn)** box.

Initially, **ROI.1** is defined and placed at the centre of the displayed image. It can be relocated within the image by moving the mouse pointer to the new location and double-clicking the mouse.

Up to 10 additional ROIs can be defined by clicking the **Add ROI** button which creates a new ROI at the current ROI.1 position. All ROIs (except ROI.1) can be deleted by clicking the **Delete ROI** button.

Once defined, the fluorescence intensity within the ROI can be selected for display in the time course plot by selecting it in **Fluorescence ROI** list. in the time course window. The difference between 2 ROIs can be plotted by selecting an ROI in the **- ROI** list.

Ratio

Select the **Ratio** option to display the time course of the ratio of intensities from a selected pair of wavelength image at the selected ROIs. The **Display Max** box sets the upper limit of the ratio display range. The **Excl. Threshold** box defines the minimum fluorescence signal level required for computation of a ratio. (If either of the two fluorescence signals fall below this value, the ratio is set to 0).

A/D Channels

Select the **A/D Channel** option to display analogue signal channels (if they are being acquired).

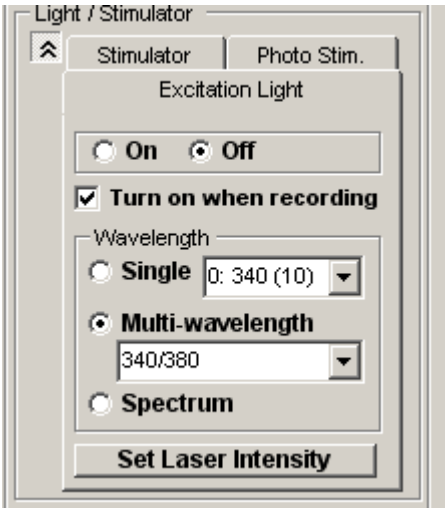
Display

The duration of the time course plot can be set by entering a value (in seconds) into the duration box at the bottom-right of the graph. Clicking the left and right arrow buttons halves and doubles the display duration.

The vertical magnification of each time course plot can be expanded to a selected region by moving the mouse to the upper limit of the region, pressing the left mouse button, drawing a rectangle to indicate the region and releasing the mouse button. The vertical magnification can also be adjusted using the + - buttons at the right edge of each plot.

Recording > Images + Signals > Excitation Light Control

The application of fluorescence excitation light is handled by the group of controls on the **Excitation Light** page.



On/Off

The **On** and **Off** buttons turn the fluorescence excitation light on and off.

Turn on when recording

Select the **Turn on when recording** option if you wish excitation light to be turned on at the beginning and off at the end of recording.

Wavelength

Select the **Single** option to apply a single wavelength, selected from one of the 10 available wavelengths listed below. (The list of available wavelengths is defined in the excitation wavelengths table in the Light Source Setup dialog box.)

Select the **Multi-wavelength** option to acquire multiple (2-9) wavelength image series, using the sequence of 2-9 wavelengths from in the excitation wavelengths table defined. (The wavelength sequence is defined in the excitation wavelengths table in the Light Source Setup dialog box.)

Select **Spectrum** to acquire a spectral series of images with the excitation wavelength being stepped over a defined range of wavelengths. (The spectral range is defined in the excitation wavelengths table in the Light Source Setup dialog box.)

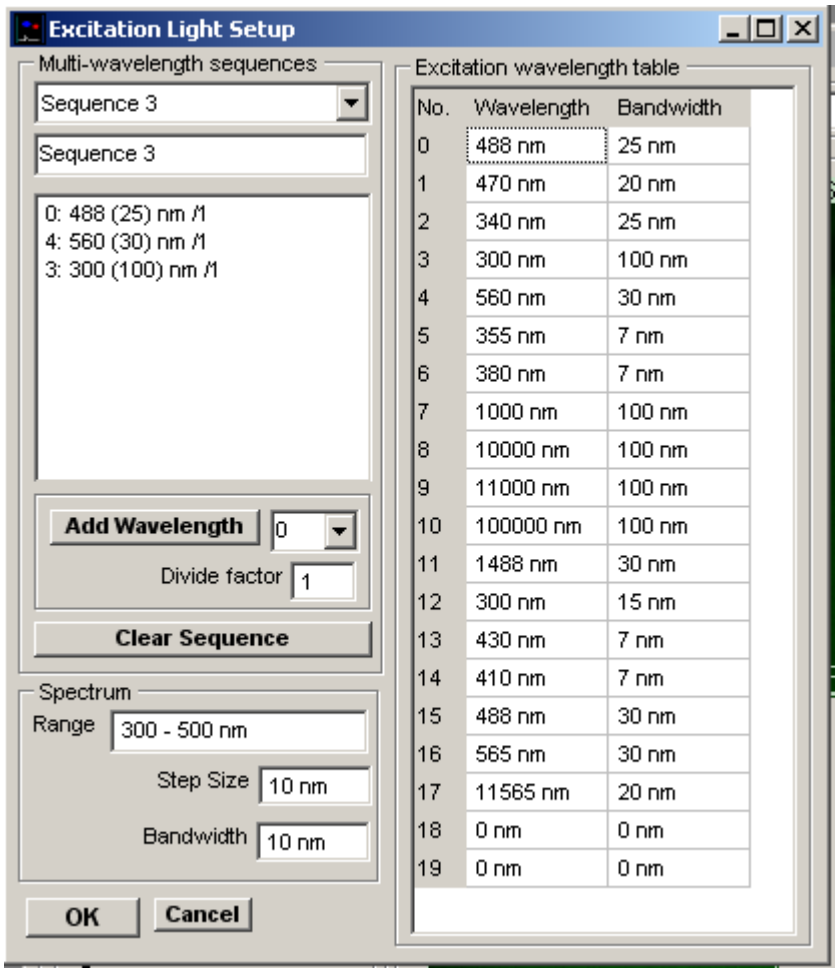
Select **Set Laser Intensity** to set the intensity of a laser source light source (this option currently only applies to the Optoscan + Lasers TIRF light source option.)

Recording > Images + Signals > Excitation Wavelength Sequences

When a wavelength-controllable fluorescence light source is in use, up to 20 separate fluorescence excitation wavelengths can be directly selected by WinFluor and a multi-wavelength sequence of up to 9 of these wavelengths applied sequentially. To configure the light wavelengths and sequences select

Setup
Excitation Light Wavelengths

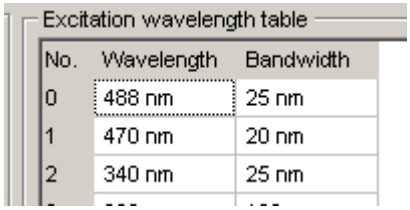
to display the **Excitation Light Setup** dialog box.



Excitation Wavelengths Table

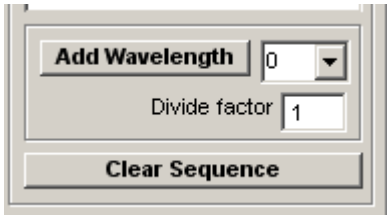
The excitation wavelengths table defines up to 20 excitation light wavelengths available for selection in the excitation light wavelengths list in the Live Image recording window, or available for inclusion in a cyclic multi-wavelength sequence.

Each wavelength option is defined by its centre wavelength in nanometres (in the **Wavelength** column) and width of the passband around that wavelength (in the **Bandwidth** column). (Note. The bandwidth setting of a Till monochromator is ignored). If the light source is a filter wheel, enter the centre wavelengths and passbands of the filters inserted into positions 0-9 (or as many positions exist or are filled) of the wheel.



Multi-wavelength Sequence:

The multi-wavelength sequence list defines a series of up to 9 wavelengths selected from the wavelength table which can be applied in a cyclic sequence at either of two rates when the multi-wavelength excitation option is selected within the Record Images window. Up to 10 sequences can be defined and each given a specific name.



To define a wavelength sequence, click **Clear Sequence** to clear the wavelength sequence list. Select the row number of a wavelength from the Excitation Wavelength table then click the **Add Wavelength** button.

The value in the **Divide factor** box for each wavelength determines the rate at which it is applied within the sequence. When all wavelengths have a divide factor of 1 (or the same divide factor) a simple cycle of the wavelengths within the sequence is produced. When a divide factor of *N* (*N>1*) is applied to one or more of the wavelengths, these wavelengths are applied at *1/N* the rate of the others. For instance, a sequence of 3 wavelengths (0,1,2) where 0 has a divide factor of 5 and the rest 1.

0: 480(10) nm /5
1: 340(10) nm /1
2: 380(10) nm /1

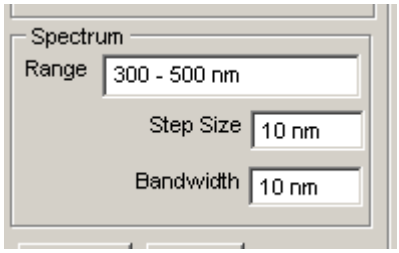
produces the sequence

0 1 2 1 2 1 2 1 2 1 2 0 1 2 1 2 1 2 1 2 1 2 …

Note. Only two divide factor values are allowed with a sequence, one of which must be 1, the other less than 100. Those wavelengths with a divide factor greater than one are always placed at the beginning of the sequence.

Spectrum

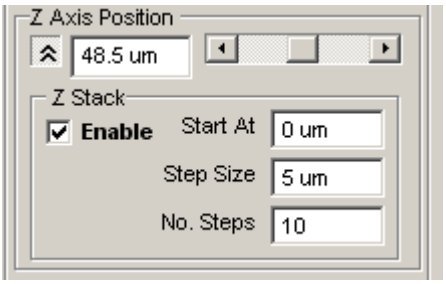
The spectrum options define the spectral sequence applied when the Spectrum excitation option is selected in the Live Images window.



To define a spectral sequence, enter the beginning and end (in nm) of the range of wavelengths to be applied into the **Range** box. Enter the amount (in nm) that the wavelength is to be shifted after each frame is acquired in the **Step Size** box. Enter the width (in nm) of the passband to be used in the **Bandwidth** box.

Recording > Images + Signals > Z Axis Position

When a lens/stage positioner is available, the **Z Axis Position** group of settings defines the lens focal plane (Z) position.



The plane of focus can be shifted up our down using the **Z Axis Position** slider or moved to a specific position by entering the position (in microns) in the **Z Axis Position** box and pressing the return key.

Z Stack

The **Z Stack** option can be used to record a set of images at a series of focal planes.

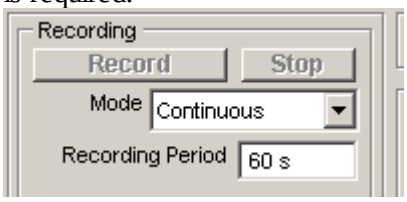
To record a Z stack, enter the position of the initial image plane in the **Start A**t box, the number of planes to be acquired in the **No. Steps** box and the distance between planes in the **Step Size** box. Tick the **Enable** option to enable the Z stack recording.

Recording > Images + Signals > Recording Image & Signals

To record a series of images (and analogue signals):

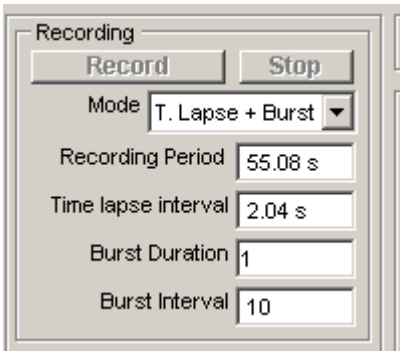
1. Create a new data file to hold the recording by selecting
2. **File**
   New Data File
3. and entering a file name in the **New Data File** dialog box.
4. Enter the duration of the recording into the **Recording Period** box.
5. Select the Recording mode:

   Select the **Continuous** option if continuous recording at a rate defined by the camera **Exposure Interval** setting is required.



   Select the **Time Lapse** option to record images intermittently at intervals defined by the **Time Lapse Interval** box.

   Select the **T. Lapse + Burst** option to both record images intermittently at intervals defined by the **Time Lapse Interval** box and to acquire high speed bursts of images at the rate defined by the camera **Exposure Interval.** Enter the duration of the high speed burst into the **Burst Duration** box and the interval between bursts into **Burst Interval.**

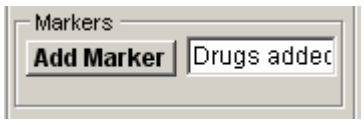


6. Enter any experiment identification information into the **Expt.** box.
7. Click the **Record** button to begin recording.

During recording, the number of frames acquired is indicated in the status line at the bottom of the program window. Recording is terminated when the required number of frames have been acquired or when the **Stop** button is clicked.

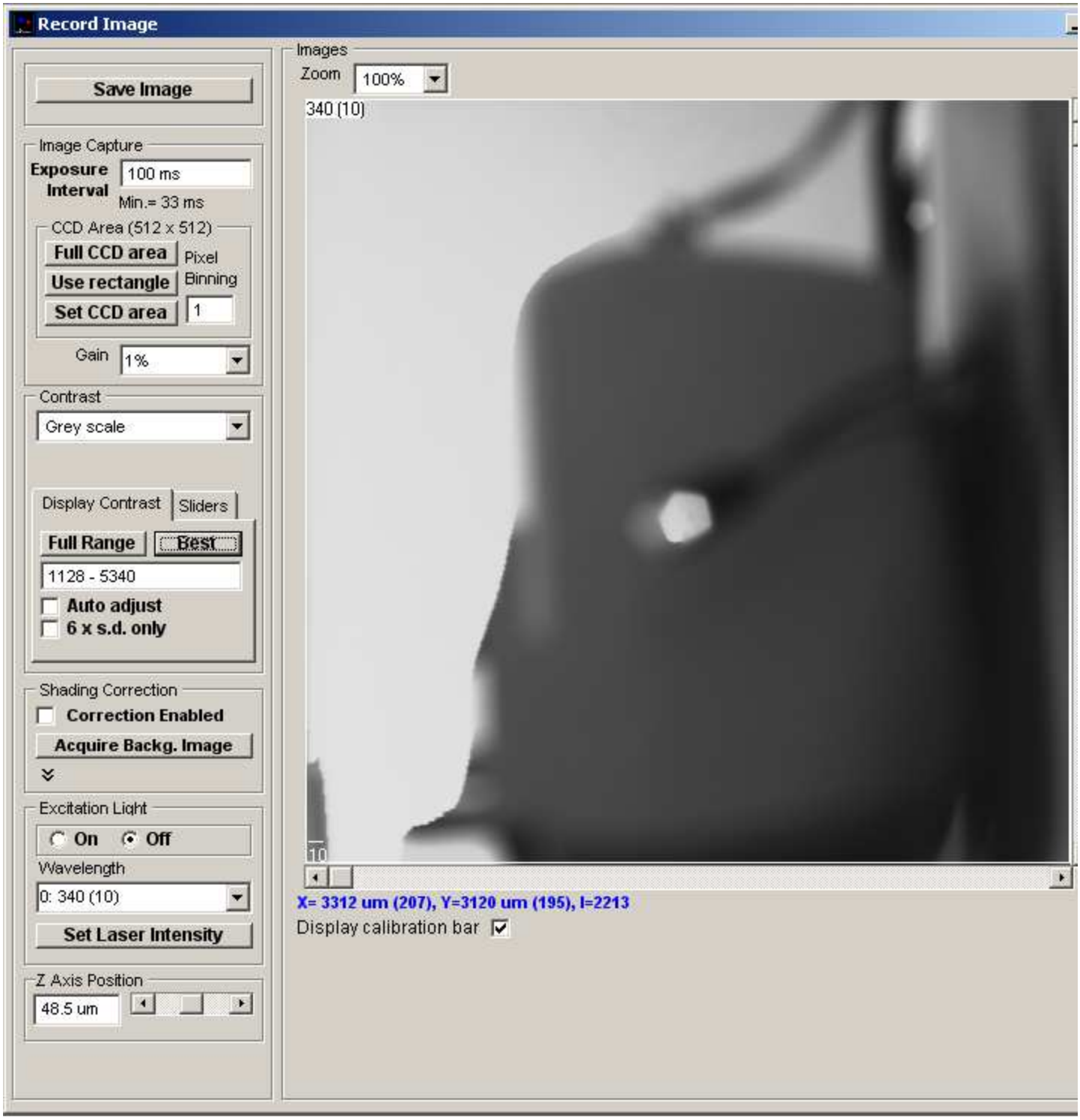
Markers

To add marker information to the file during the recording, enter the text into the Markers text box and click the **Add Marker** button.


Markers
Add Marker
Drugs added

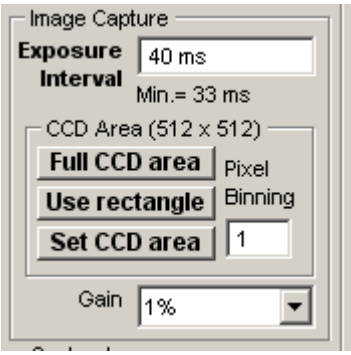
Recording > **Image** > **Record Image**

To open a window displaying live camera images, Select
Record
Image
to display the window.



Recording > Image > Image Capture

The Image Capture group of settings define the size and location of the image to be captured and the time interval between frames



Exposure Interval

Enter the required time interval (in ms) between successive image frames into the **Exposure Interval** box. (The shortest valid inter-frame interval is indicated below the entry box. This will vary depending upon capture region size and CCD binning factor.)

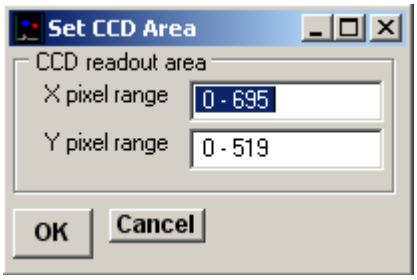
CCD Area

If the camera supports CCD sub-region readout, the CCD area controls allow you to acquire the image from a defined sub-region within the CCD.

To use the full imaging area of the CCD, click the **Full CCD Area** button.

To acquire the image from a CCD sub-area defined on screen, use the mouse to drag in the edges of the capture rectangle superimposed on the live displayed image until it encompasses the region to be acquired, then click the **Use rectangle** button.

To define the CCD sub-area in terms of its X and Y pixel coordinates within the CCD, click the Set **CCD Area** button and enter the required X and Y pixel range into the Set CCD Area dialog box which pops up.



Binning

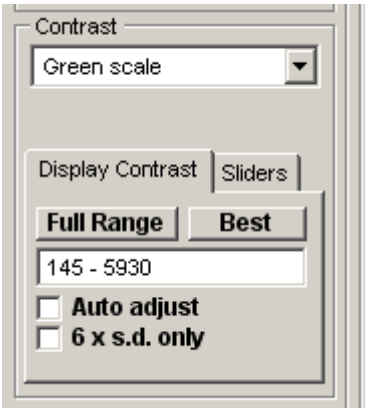
If the camera supports CCD pixel binning, to bin together blocks of pixels to increase image intensity and/or allow faster frame capture rates, enter a value greater than one into the **Pixel Binning** box, then press the <Enter> key. (A value of 2 bins together 2x2 blocks of pixels, increasing image intensity by 4).

Gain

Set the camera CCD readout amplifier gain by selecting a gain factor from the **Gain** list. (With EMCCD cameras the gain is expressed as a percentage of the maximum electron multiplying gain).

Recording > Image > Display Contrast (Image)

The display colour mapping options determines how pixel intensities within images are displayed on screen.



The **Grey Scale** option maps image intensity as shades of grey. **Green, Blue** and **Red Scale** maps intensities into shades of these colours. **False Colour** maps intensities in a rainbow of colours, ranging from minimum to maximum in the sequence - black, blue, green, yellow, red, white.

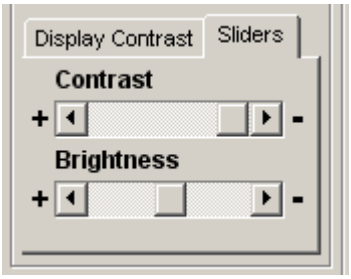
The **Zoom** option can be used to magnify or reduce the region of the image displayed within the image panel. Zoom factors of 25% - 800% are currently supported. The magnified region of the image displayed within the image panel can be panned horizontally and vertically within the image.

The range of pixel intensities mapped into the display colour range is displayed in the **Display Contrast** tab. To achieve optimal image contrast, click the **Best** button to adjust the intensity range to the range of pixel intensities within the image. Click the **Full Range** button to set the display to the full range of intensities supported by the camera. The display can be set to a specific intensity range, by entering the required range into the intensity range display box and pressing the **<Enter>** key. When the **Auto adjust** option is ticked, the display contrast is automatically adjusted to the optimal setting as frames as acquired.

The **6 x s.d. only** option is ticked the optimal contrast range is set to 3 standard deviations on either side of the mean image intensity, and to the minimum and maximum intensities within the image when unticked.

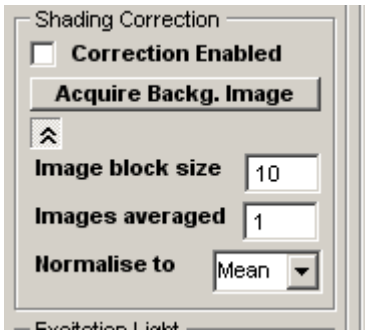
When the **All image panels** option is ticked the contrast is set for all image panels of multi-wavelength files simultaneously. Unticked, the contrast can be adjusted for individual panels, by clicking on a panel to select it, then clicking the **Best** or **Full Range** button.

Display contrast can also be adjusted manually by selected the **Sliders** tab and adjusting the **Brightness** and **Contrast** slider bars to achieve the desired image contrast.



Recording > Image > Shading Correction

The shading correction function can be used to cancel out uneven illumination intensity across the image by subtracting a smoothed background image.



To apply shading correction:

1. Set the of number pixels (*n x n*) in a smoothing block in the **Image block size** box (default 10).

2. Set the the number of images to be averaged in the **Images Averaged** box (default 1).

3. Select the grey level to which the corrected image is normalised from in the **Normalise to** list. Select **Mean, Min. or Max.** to normalise to the mean, minimum or maximum grey level within the uncorrected image, or **Zero** to normalise about zero (default **Mean**).

4. Click the **Acquire Backg. Image** button to acquire a background image.

5. Select the **Correction Enabled** option to apply the shading correction to live images. Shading correction using the background image can be toggled on and off as required.

Recording > **Image** > **Z Stage/Focus Control**

When a lens/stage positioner is available, the **Z Axis Position** group of settings defines the lens focal plane (Z) position.

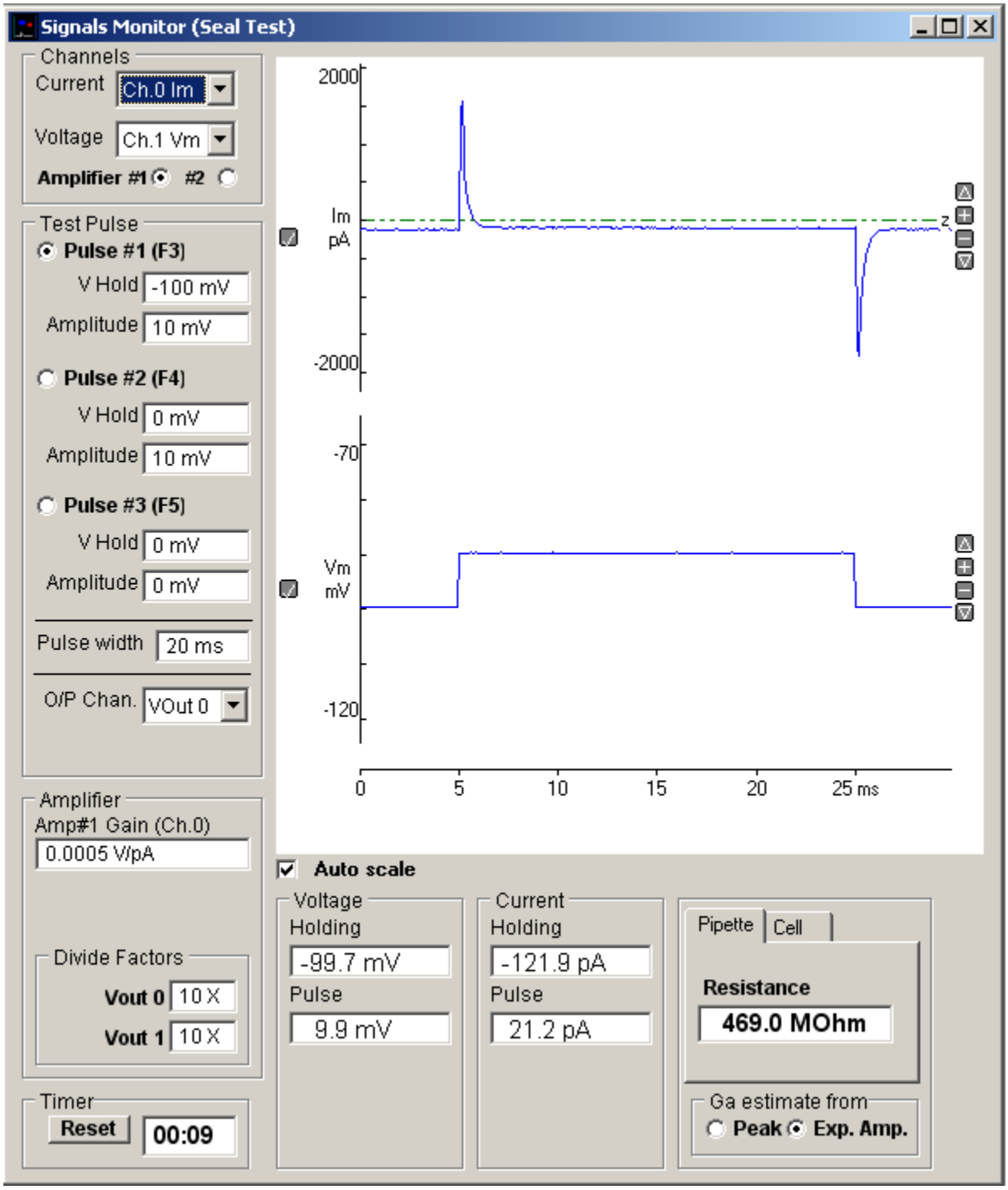
The plane of focus can be shifted up our down using the **Z Axis Position** slider or moved to a specific position by entering the position (in microns) in the **Z Axis Position** box and pressing the return key.

Recording > Signals Monitor (Seal Test) > Signals Monitor (Seal Test)

The Signals Monitor (Seal Test) window provides a monitor of analogue signals, allows the application of seal test pulses to assist the formation of a giga-seal and computes pipette resistance, cell conductance and capacity. Select

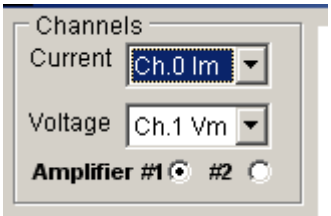
Record
Signals Monitor (Seal Test)

to open the Signals Monitor (Seal Test) window. An oscilloscope monitor showing the current signal on each input channel is displayed.



Current and voltage channels

The Channels group shows the analogue input channels containing the patch clamp current and voltage signals.



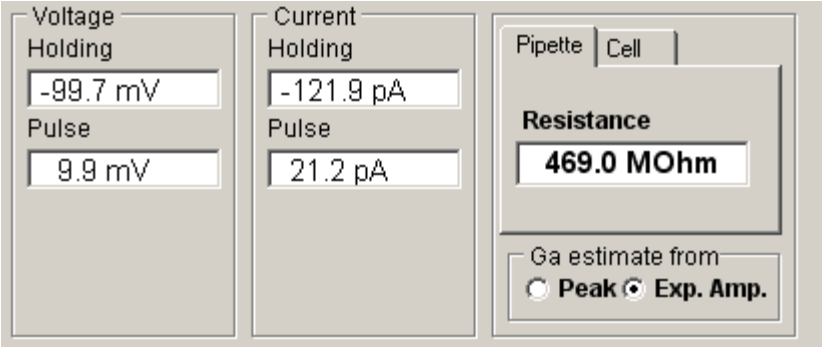
When two patch clamp amplifiers are configured the seal test pulse and cell resistance calculation can be switched between amplifiers by selecting **Amplifier #1** or **Amplifier #2**.

Display scaling

The vertical display magnification is automatically adjusted to maintain a visible image of the test pulse within the display area. Automatic scaling can be disabled by un-checking the **Auto scale** check box allowing the vertical magnification for each channel to be expanded to a selected region by moving the mouse to the upper limit of the region, pressing the left mouse button, drawing a rectangle to indicate the region and releasing the mouse button. The vertical magnification can also be adjusted using the **+** **-** buttons at the right edge of each plot.

Recording > Signals Monitor (Seal Test) > Current & Voltage Readout

A readout of the cell membrane holding current and voltage, and test pulse amplitude, appears at the bottom of the monitor window.

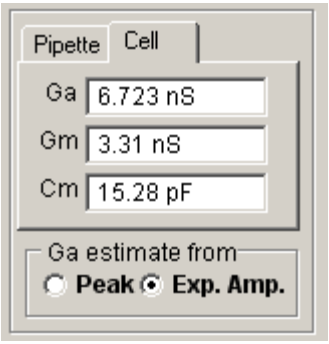


During initial formation of a giga-seal, the **Pipette** option displays pipette resistance, computed from

$$R_{pipette} = \frac{V_{pulse}}{I_{pulse}}$$

where V_{pulse} and I_{pulse} are the steady-state voltage and current pulse amplitudes.

The **Cell** option displays the cell membrane conductance, G_m, capacity, C_m, and access conductance, G_a,



computed from

$$G_a = \frac{I_0}{V_{pulse}}$$

$$G_m = \frac{I_{pulse}}{\left(V_{pulse} - \frac{I_{pulse}}{G_a}\right)}$$

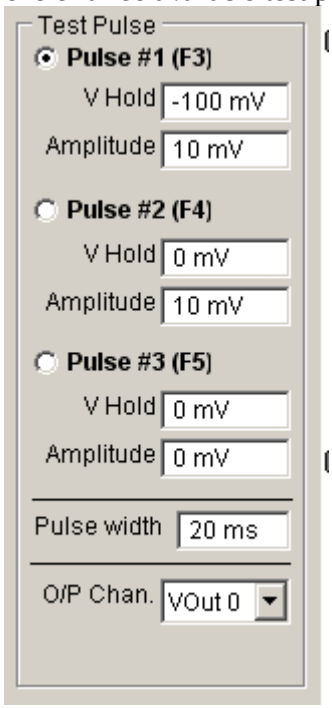
$$C_m = \tau\left(G_a + G_m\right)$$

where I_0 is the initial current at the peak of the capacity transient and is the exponential time constant of decay of the capacitance current (See Gillis, 1995, for details). **Note.** If G_a, G_m and C_m are to be estimated correctly, the patch clamp's pipette series resistance compensation and capacity current cancellation features must be turned off.

A good test, to check if WinFluor is set up with the correct input/output connections and channel scaling factors, is to attach the model cell supplied with most voltage/patch clamps, and observe the holding potential and current, test pulse amplitude and cell parameters correspond with the known values of the model.

Recording > Signals Monitor (Seal Test) > Holding Voltage and Test Pulses

You can control the holding voltage applied to the cell and the amplitude and duration of a test voltage pulse by selecting one of three available test pulses (Pulse #1, #2, #3)



The size of each pulse is set by entering an appropriate value for holding voltage and pulse amplitude into the **V Hold** or **Amplitude** box for each pulse.

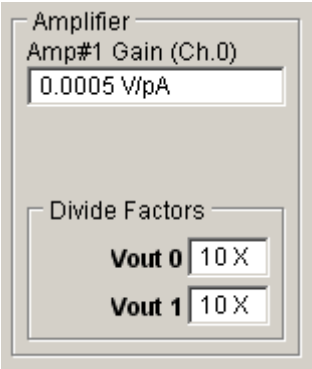
The width of all pulses is defined by the **pulse width** box

You can switch between pulses by pressing the function key associated with each pulse (Pulse #1 = F3, Pulse #1 = F4, Pulse #1 = F5).

The voltage stimulus output to which the pulse is is indicated in the **O/P Chan** list.This is normally set automatically to output channel connected to the patch clamp command voltage input.

Recording > Signals Monitor (Seal Test) > Patch Clamp Amplifier Settings

The patch clamp current gain (in voltage-clamp mode) and voltage gain (current-clamp mode) is indicated in the **Amplifier** group box.



When using a patch clamp with gain telegraph support the Gain value is automatically updated when the gain setting is changed on the patch clamp front panel.

The voltage output channel (**Vout 0** or **Vout 1**) connected to the patch clamp command voltage input is displayed in the V Command **O/P to** list. The patch clamp command voltage division factor used to scale the stimulus voltage output to obtain the correct voltage at the cell is indicated in the **Divide Factor** box.

The **Gain**, **O/P to** and **Divide Factor** settings are set automatically, when a specific amplifier supported by WinFluor (e.g.Amplifier = Axopatch 1D, Axopatch 200 etc.) has been selected as the Amplifier in in Setup Patch Clamp/Analogue Inputs dialog box. When an unsupported amplifier is in used (Amplifier = None or Manual Gain Entry) appropriate settings must be entered by user.

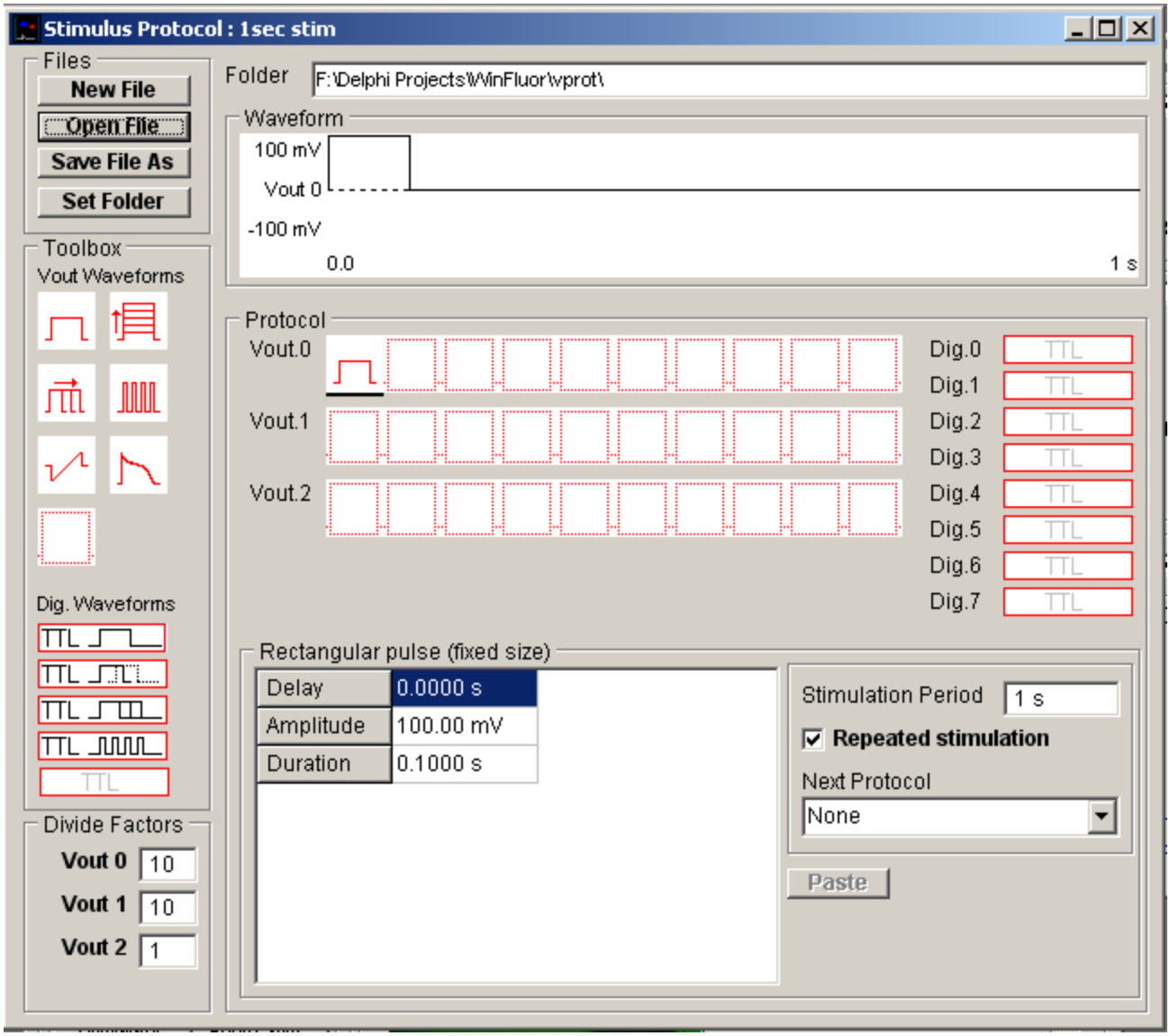
Stimulus Protocols > Creating Stimulus Protocols

Stimulus protocols can consist of voltage waveforms on up to 3 voltage output channels (Vout 0-2) and 5V TTL digital waveforms on up to 8 TTL digital output channels (Note. Number of available DAC and digital channels depends on laboratory interface type and available output lines).

To create a new (or edit an existing) stimulus protocol file, select

Setup
Stimulus Protocol Editor

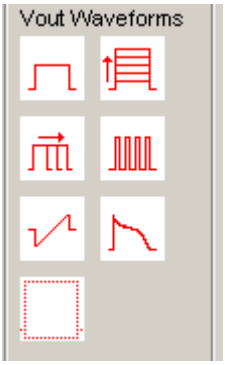
to open the stimulus protocol editor. A diagram of the output waveforms appears in the **Waveform** display box.



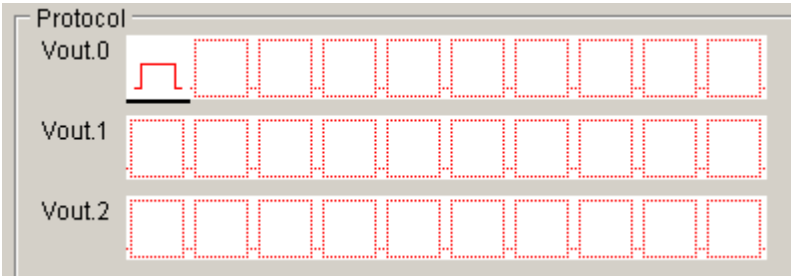
To begin, click the **New File** button to create a blank protocol or **Open File** to load an existing protocol.

Voltage waveform stimuli

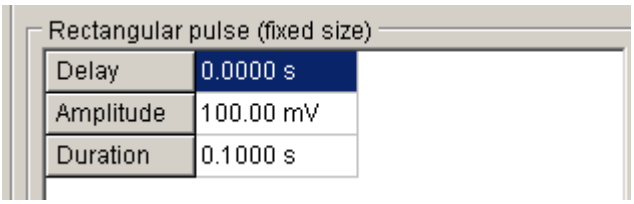
Voltage waveforms are constructed by dragging **Vout** waveform elements from the **Toolbox**



and dropping them into the selected **Vout.0**, **Vout.1** or **Vout.2** voltage waveform sequence within the protocol.



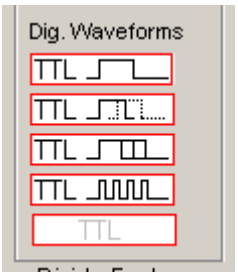
A voltage waveform can consist of a sequence of up 10 separate waveform elements. The amplitude and duration for each element is defined in its **parameters table** which can be made to appear by clicking the element in the waveform sequence.



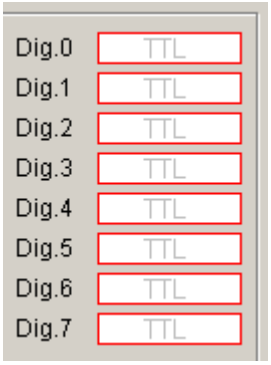
Seven different waveform elements are available (rectangular pulse; family of pulses incrementing in amplitude; family of pulses incrementing in duration; train of pulses; series of pulse trains, varying in frequency; ramp; user-defined waveform) Details of each waveform shape and its parameters are defined in Table ?.1

Digital stimulus waveforms

To create a digital stimulus waveform, drag a digital stimulus element from the Toolbox



and drop it into the selected digital output channel.

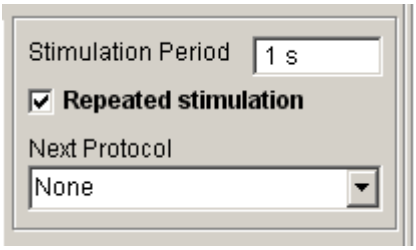


Each digital output channel controls the on (5V) / off (0V) state of a digital output line. The duration and

inverted/non-inverted signal polarity for each protocol is defined in its parameters table which can be made to appear by clicking on the element in the protocol list

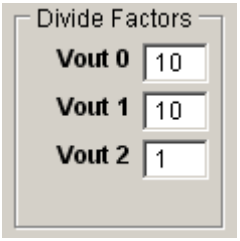
Repeated and linked protocols

Waveform protocols can be made to repeat by ticking the **Repeated Stimulation** option and entering a repetition time in **the Stimulation Period** box. Multiple stimulus protocols can also be linked together by selecting the name of another protocol in the **Next Protocol** list (When linked protocols are in use, the time set in Stimulation Period determines the time interval between protocols).



Divide factors

Most voltage and patch clamp amplifiers divide down their command voltage input signals by some factor. Enter the scaling factor into the **Divide Factor** box. This factor is used to scale the stimulus voltage output to the voltage output channel to obtain the correct voltage at the cell. (**NOTE**. The voltage divide factors for Vout 0 and Vout 1 set automatically when amplifiers supported by WinFluor have been selected as Amplifiers #1 and #2.)



Saving a stimulus protocol

On completion, a stimulus protocol can be saved for use, by clicking the **Save File As** button and providing a name for the protocol file.

Stimulus protocol folder

Protocol files are stored in the folder **c:\winfluor\vprot** (the default location) and appear in the stimulus protocols list in the live recording window. To change the folder used to store protocol files, click the **Set Folder** button to open the folder selection dialog box and create or select another folder.

Rectangular voltage pulse of fixed size

This is a simple pulse, which does not vary in amplitude and duration between records. It has 3 parameters.

Initial Delay: the delay period before the pulse begins.

Amplitude: the pulse amplitude (mV).

Duration: the duration of the pulse.

This element can be used to provide series of stimuli of fixed size or, in combination with other elements, to provide fixed pre-conditioning pulses.

Family of rectangular pulses varying in amplitude

This is a rectangular voltage pulse whose amplitude is automatically incremented between recording sweeps. It has 5 parameters.

Initial delay: the delay period before the pulse begins.

Start at Amplitude: the amplitude of the first pulse in the protocol sequence.

Increment by: the increment to be added to the pulse amplitude between records.

Number of increments: the number of steps in the sequence.

Pulse duration: the duration of the pulse.

This element is typically used to explore the voltage-sensitivity of ionic conductances, by generating records containing the whole-cell membrane currents evoked in response to a series of voltage steps to different membrane potentials.

Family of rectangular voltage pulses varying in duration

This is a rectangular voltage pulse whose duration can be automatically incremented between recording sweeps. It has 5 parameters.

Initial delay: the delay period before the pulse begins.

Amplitude: the amplitude of the pulse.

Pulse duration: the duration of the pulse.

Increment by: the increment to be added to the pulse duration between records.

Number of increments: the number of steps in the sequence.

This element is most commonly used as a variable duration preconditioning pulse in 2 or 3 step protocols for investigating inactivation kinetics of Hodgkin-Huxley type conductances.

	**Train of rectangular voltage pulses** This is a train of rectangular voltage pulses of fixed size. It is defined by 5 parameters: **Initial delay:** the delay period before the series of pulses begin. **Amplitude:** the amplitude of each pulse in the series. **Duration:** the duration of each pulse. **Pulse interval:** (within train) determines the time interval between pulses. **Number of pulses:** defines the number of pulses in the train. This element can be used to produce a series of stimuli to observe the effect of repeated application of a stimulus at a high rate. It can also be used to produce a train of pre-conditioning stimuli for a subsequent test waveform.
	**Voltage ramp** This element produces a linear voltage ramp between two voltage levels. It is defined by 4 parameters **Initial delay:** the delay period before the series of pulses begin. **Start at amplitude:** the voltage level at the start of the ramp. **End at amplitude:** the voltage level at the end of the ramp. **Ramp duration:** the time taken for the voltage to slew between the start and end amplitudes. Voltage ramps provide a means of rapidly generating the steady state current-voltage relationship for an ionic conductance. (Note that, the ramp generated by the computer is not truly linear, but consists of a staircase of fine steps. These steps can be smoothed out, by low-pass filtering the voltage stimulus signal before it is fed into the patch clamp.)

Digitised analogue waveform

Digitised analogue waveforms which have been previously acquired by WinFluor (or synthesised by another program) can be used as a waveform element.

To insert a digitised waveform into the protocol:

Select the source of the waveform and copy it to the Windows clipboard. Waveforms may be copied from a WinWCP signal record (using the Edit/Copy Data menu option) or from a spreadsheet or similar program.

Drag a digitised analogue waveform icon from the toolbox and drop it into the protocol list.

Insert the waveform into the protocol by clicking the 

button. The waveform appears in the waveform display and its data points appear in the parameters table.

The parameters table consists of:

Initial delay defines the delay period before the series of pulses begin.

A list of data points for the analogue waveform. The waveform can be altered by modifying this list.

There are a number of limitations when using the digitised waveform element.

Only one digitised waveform element is permitted per protocol.

Digitised waveforms must consist of less than 1000 data points.

The sampling interval of the digitised waveform must be greater than 0.1 msec.

If digitised waveforms are created with a spreadsheet, the data points must be formatted as a pair of columns containing time (msecs) in the first and amplitude (mV) in the second. E.g.

T_0 V_0
T_1 V_1
…etc

	**Digital pulse (fixed duration)** This produced a digital pulse on the selected output line of fixed duration. It is defined by 4 parameters. **Initial delay** defines the delay before the start of the pulse. **Duration** defines the duration of the digital pulse. **Invert Signal** defines whether the digital pulse is an OFF-ON or an ON-OFF pulse. If set to No, the digital line is initially OFF (0V) and switches to ON (5V) during the pulse. If set to Yes, the digital line is initially ON (5V) and switches to OFF (0V) during the pulse. The digital pulse element can be used to switch open or close valves controlling the flow of solutions over a cell. Multiple digital outputs can be used to simultaneously open one valve while another is closed.
	**Family of digital pulse (varying in duration)** This produced a digital pulse on the selected output line, with a duration which is incrementable between records. It is defined by 5 parameters. **Initial delay** defines the delay before the start of the pulse. **Starting duration** defines the duration of the first pulse in the protocol. **Increment by** defines the amount that the duration is incremented between records. **Number of increments** defines the number of increments in the protocol. (Note that, if there are any voltage waveform elements in use within the protocol, the number of increments defined here must be the same.) **Invert Signal** defines whether the digital pulse is an OFF-ON or an ON-OFF pulse. If set to No, the digital line is initially OFF (0V) and switches to ON (5V) during the pulse. If set to Yes, the digital line is initially ON (5V) and switches to OFF (0V) during the pulse.
	**Train of digital pulses** This produces a series of digital pulses of fixed intervals and of fixed duration. It is defined by 5 parameters. **Initial delay** defines the delay before the start of the first pulse in the series. **Pulse duration** defines the duration of the each pulse in the series. **Inter-pulse interval** defines the time interval between pulses in the series. **Number of pulses** defines the number of pulses in the series. **Invert Signal** defines whether the digital pulse is an OFF-ON or an ON-OFF pulse. If set to No, the digital line is initially OFF (0V) and switches to ON (5V) during the pulse. If set to Yes, the digital line is initially ON (5V) and switches to OFF (0V) during the pulse. This element can be used to apply a rapid train of stimuli to a cell.

Viewing/Measuring Recorded Images > Regions of Interest

Regions of interest (ROIs) are used to define specific areas within each image for measurement, required for intensity time course plots, pixel intensity histograms and image region export functions.

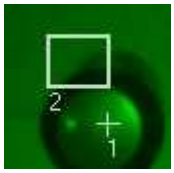
Up to 50 ROIs can be defined within an image. Four shapes of ROI are available

- Point : A single pixel within the image
- Line : A line of pixels
- Rectangle : A rectangular block of pixels
- Ellipse : An elliptical block of pixels
- User defined line
- User defined region

Adding a Region of Interest

To add a new ROI to an image, select the shape of ROI to be added from the ROI shape list.

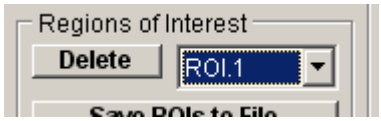
Drag the ROI on to the image and adjust its position and boundaries.



Existing ROIs can be moved/resized by clicking on them.

Removing Regions of Interest

To remove an ROI from the image, select the ROI number from the deletion list and click the **Delete** button. To remove all ROIs click **Delete All**.



Saving/Loading Regions of Interest

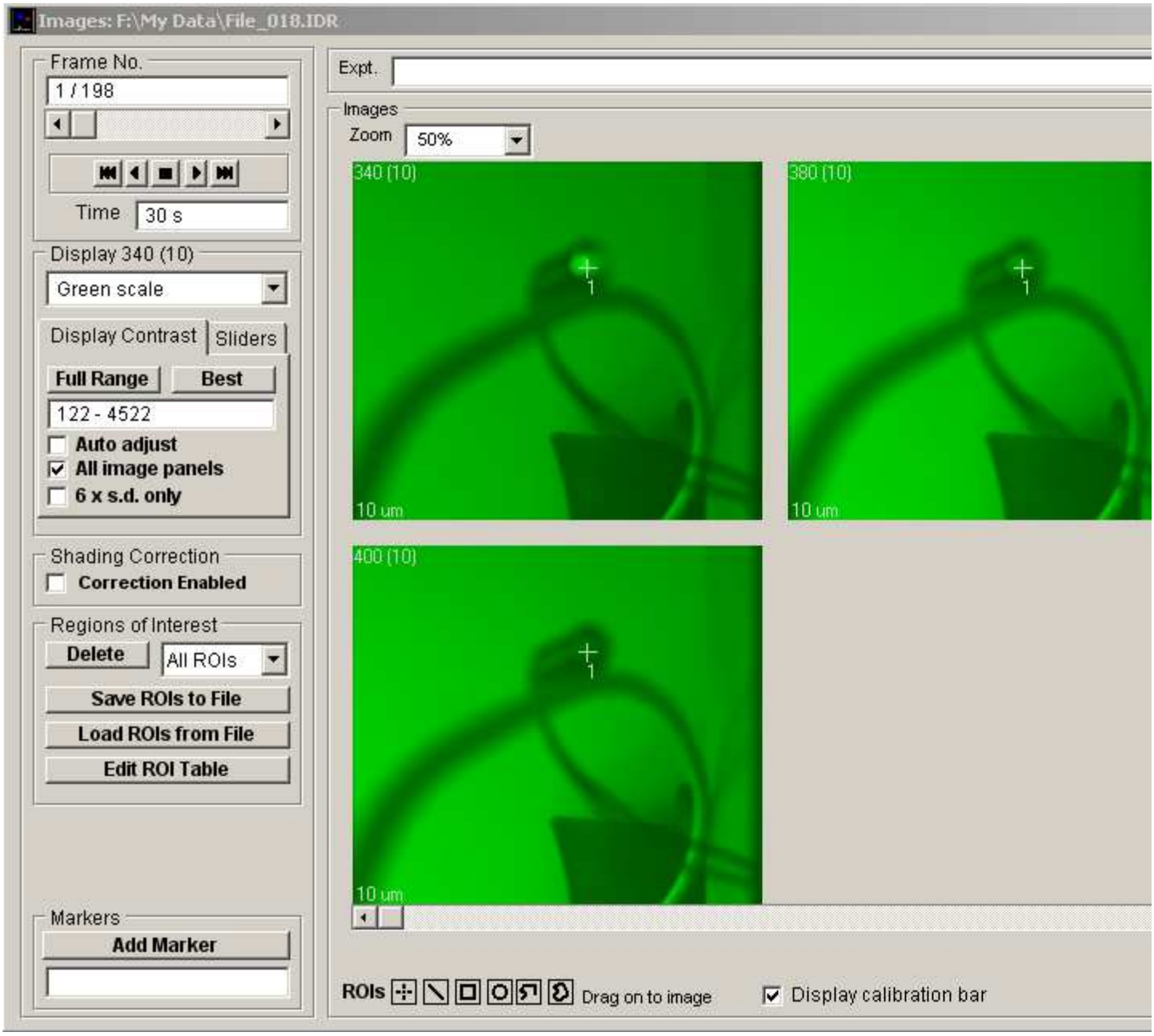
The current set of ROIs can be saved to a ROI settings file by clicking the **Save ROIs to File** button and entering a file name. Settings can be reloaded from an ROI settings file by clicking the **Load ROIs from File** button. (Note. ROI settings files have a .ROI extension.)

Viewing/Measuring Recorded Images > Viewing Image Recordings

To open and view a WinFluor data file containing a series of stored images & signals, select

File
Open Data File

to open the Open Data File dialog box, then select the required (*.IDR) file.



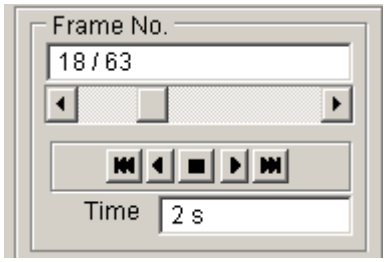
A graph of the time course of pixel intensity from a selected region of interest (ROI) within the displayed image (one for each image pane in multi-wavelength sequence) is displayed in the time course window. Analogue signals (if they have been acquired) are also displayed in this area.

Display Calibration Bar

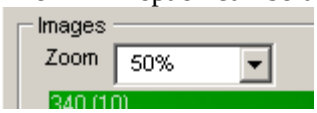
Checking this option displays a calibration bar at the bottom-left of each display pane. (The size of the calibration bar and lens magnification from which it is derived is defined in the Camera Settings dialog box.)

Selecting/displaying frames

Individual image frames can be selected for display using the frame selection slider bar or by directly entering the required frame number into the **Frame No.** display box. Frames can be played forwards and backward in sequence using the playback control buttons.



The **Zoom** option can be used to magnify or reduce the region of the image displayed within the image panel.



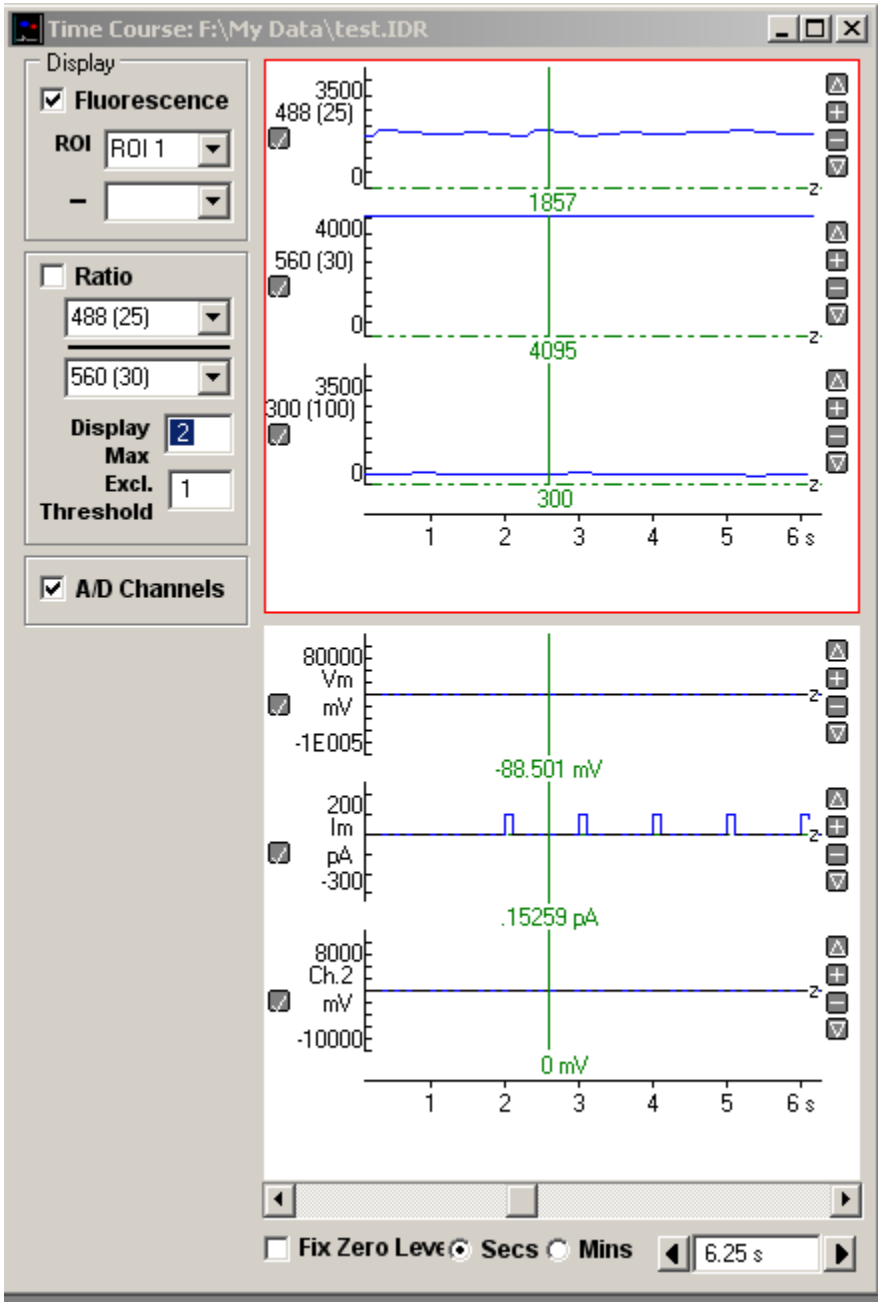
Zoom factors of 25% - 800% are currently supported. The magnified region of the image displayed within the image panel can be panned horizontally and vertically within the image.

Adding markers

Marker text can be added to each image by entering text into the marker text box and clicking the **Add Marker** button. The marker text appears at the bottom-right of the image and also on the time course plot. Up to 20 marker text labels can be added to each file. (Marker text can also be edited by opening the data file Properties box (File/Properties) and editing the text in the Markers table.

Viewing/Measuring Recorded Images > Time Course Window

The Time Course Plots window displays the time course of the fluorescence intensity at a selected region of interest in the image and/or analogue signals.



Fluorescence

Select the **Fluorescence** option to display the time course of the fluorescence intensity of a selected the region of interest (**ROI.1** 2 etc.) Once defined, the fluorescence intensity within the ROI can be selected for display in the time course plot by selecting it in **Fluorescence ROI** list. in the time course window. The difference between 2 ROIs can be plotted by selecting an ROI in the **- ROI** list.

Ratio

Select the **Ratio** option to display the time course of the ratio of intensities from a selected pair of wavelength image at the selected ROIs. The **Display Max** box sets the upper limit of the ratio display range. The **Excl. Threshold** box defines the minimum fluorescence signal level required for computation of a ratio. (If either of the two fluorescence signals fall below this value, the ratio is set to 0).

A/D Channels

Select the **A/D Channel** option to display analogue signal channels (if they are being acquired).

Display

The duration of the time course plot can be set by entering a value (in seconds) into the duration box at the bottom-right of the graph. Clicking the left and right arrow buttons halves and doubles the display duration.

The vertical magnification of each time course plot can be expanded to a selected region by moving the mouse to the upper limit of the region, pressing the left mouse button, drawing a rectangle to indicate the region and releasing the mouse button. The vertical magnification can also be adjusted using the + - buttons at the right edge of each plot.

Displayed traces can be printed (**File / Print Graph**) or copied to the Windows clipboard as data (**Edit / Copy Graph Data**) or as an image (**Edit / Copy Graph as Image**). (A plot is selected for printing/copying by clicking on it. Selected plot is outlined in red.)

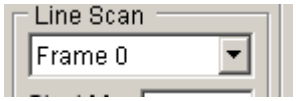
Viewing/Measuring Recorded Images > Viewing Line Scan Recordings

To open and view a WinFluor data file containing a series of stored line scan images & signals, select

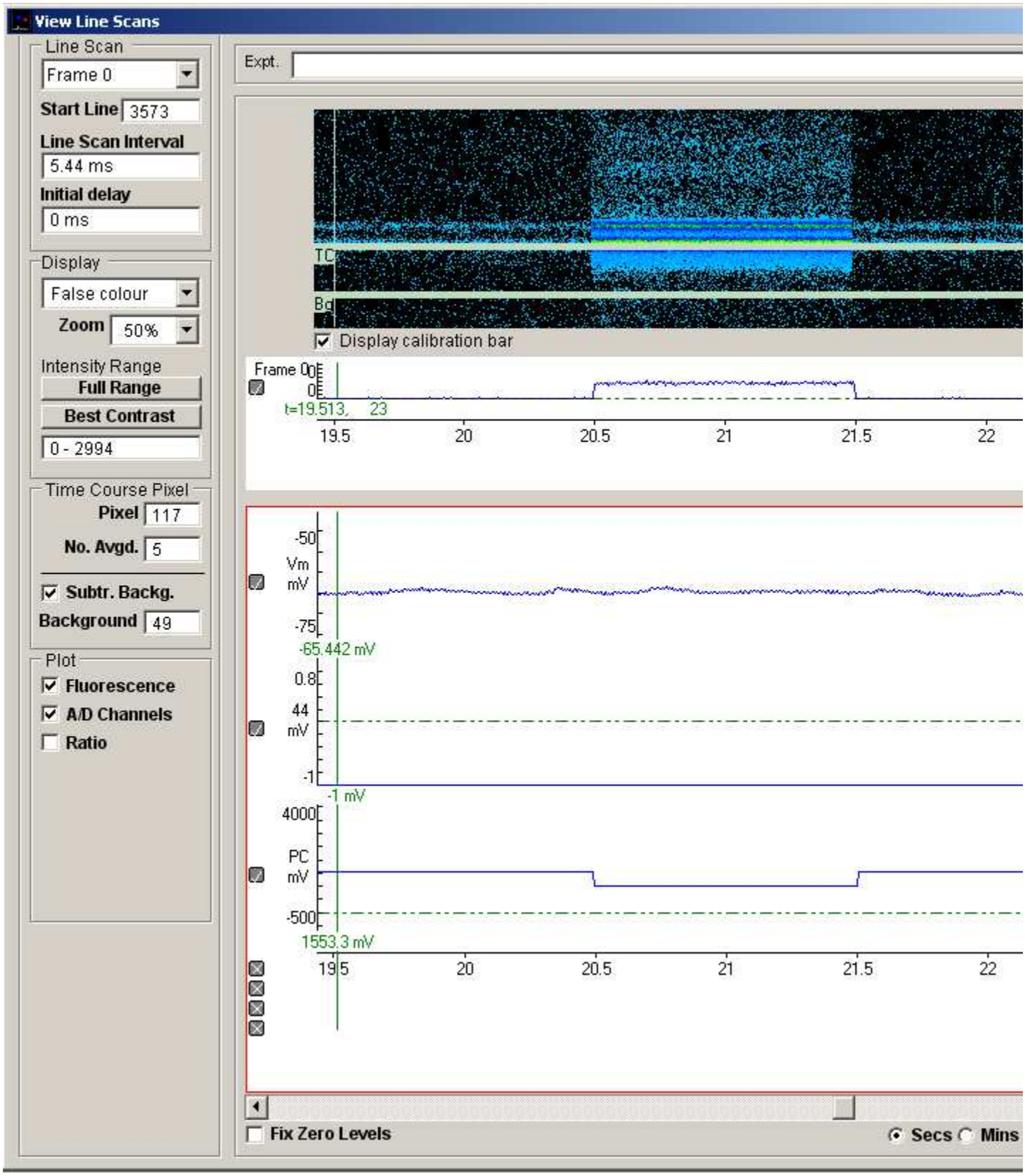
File
Open Data File

to open the Open Data File dialog box, then select the required (*.IDR) file.

The line scans time series is displayed in the top panel of the **View Line Scans** windows with the line oriented vertically and time horizontally. For multi-wavelength files containing more than one line scan image acquired at different emission wavelengths, the displayed line scan image can be selected from the **Line Scan** list.



A graph of the time course of pixel intensity from a selected point (TC) on the line is displayed in a time course plot below the image. Analogue signals (if they are being acquired) are also displayed in the analogue signal plot.



Display Calibration Bar.

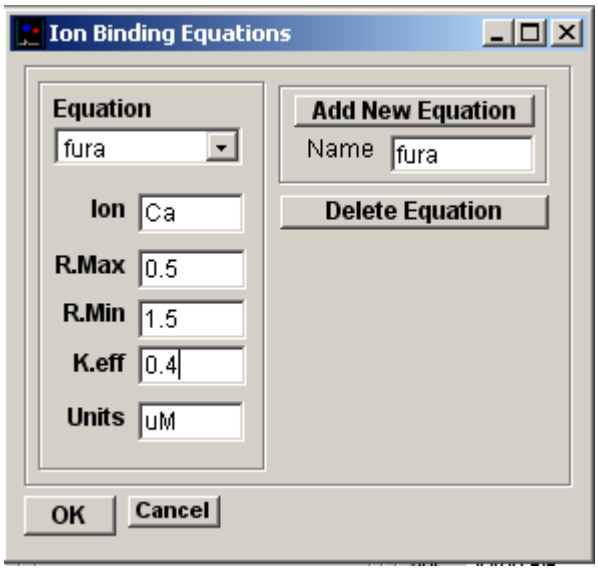
Checking this option displays a calibration bar at the left edge of the line scan image.

Viewing/Measuring Recorded Images > Ion Binding Equations

The parameters for up to 10 ratiometric ion binding equations can be entered and stored using the **Ion Binding Equations** setup dialog box. To enter/edit an equation, select

Setup
Ion binding equations

To display the setup box.



To add a new equation:

1. Enter the name of the ion-binding fluorophore in the **Name** box and click the **Add New Equation** button.
2. Enter the name of the binding ion in the **Ion** box.
3. Enter the maximum fluorescence intensity ratio achieved at high concentrations of the binding ion in the **R.Max** box.
4. Enter the minimum fluorescence intensity ratio achieved at high concentrations of the binding ion in the **R.Min** box.
5. Enter the effective binding coefficient for the ion in the **K.Eff** box.
6. Enter the units to be used to express ionic concentration (e.g. nM, uM, mM, M) in the **Units** box.
7. Click the **OK** button to save the equation.

Equations stored within the program can be displayed/edited by selecting the required equation from the **Equation** list and if necessary modifying any of the parameters. A selected equation can be deleted by clicking the **Delete Equation** button.

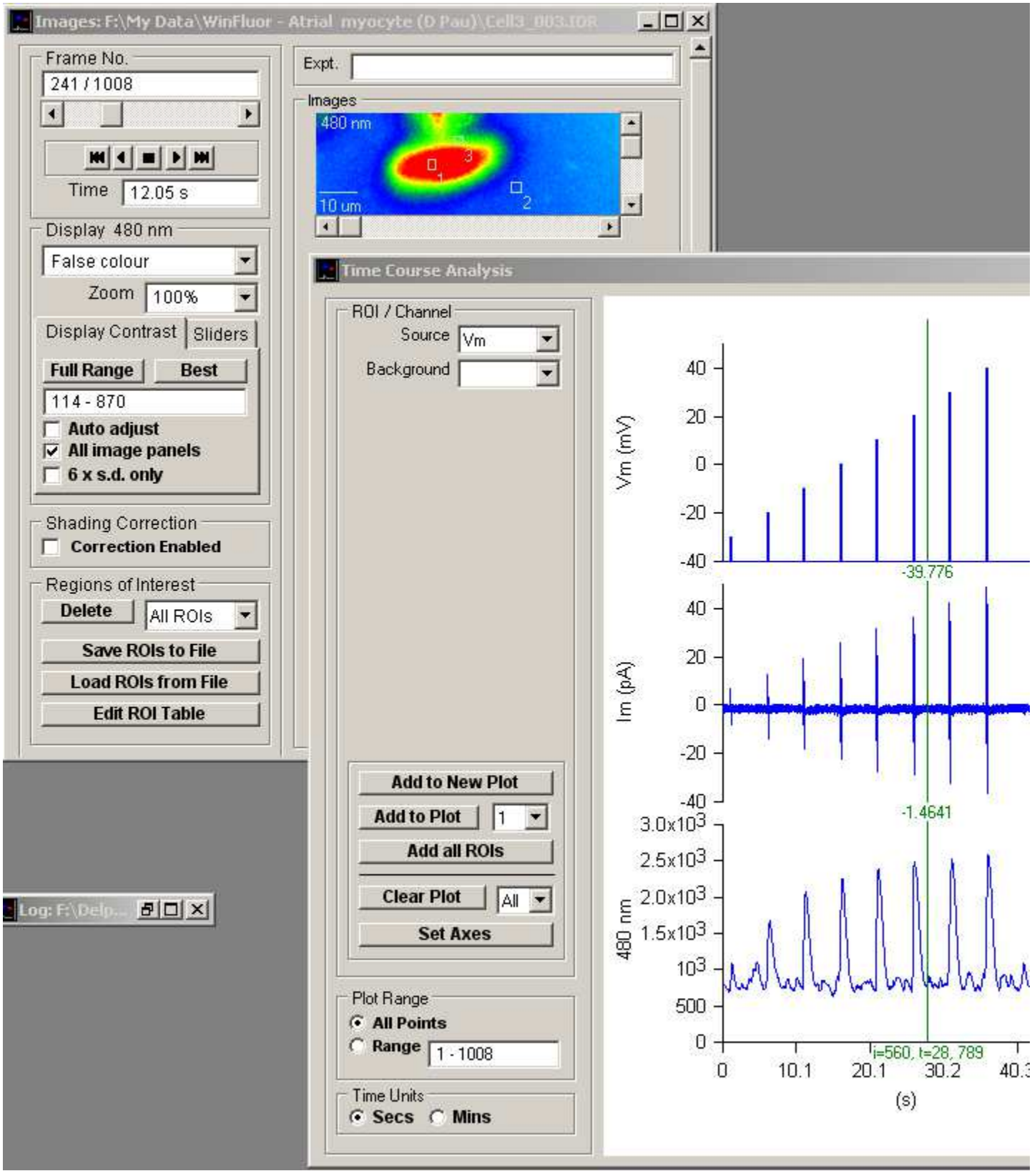
Analysis > Time Course Analysis > Time Course Analysis

The time course analysis window plots graphs of the time course of the mean image intensity within the regions of interest defined in the **Images** window. Relative changes in intensity (F/F0 ratios) can be computed and also intensity ratios and ionic concentration plots for multi-wavelength recordings.

Select

Analysis
Time Course Analysis

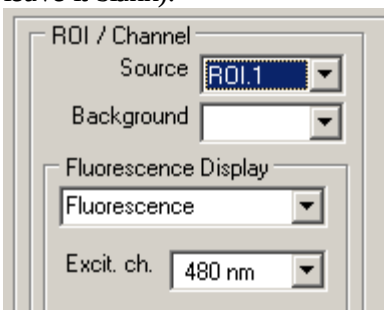
to open the time course analysis window.



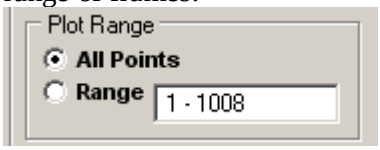
Analysis > Time Course Analysis > Plotting Time Courses

To plot the time course of changes in image intensity:

1. Define one or more regions of interest within the image displayed in the **Images** window.
2. Select a region of interest (or analogue signal) to be plotted from the **Source** ROI/Channel list. [Optional] To subtract the intensity of a background region of interest, select an ROI from the **Background** ROI list (otherwise leave it blank).



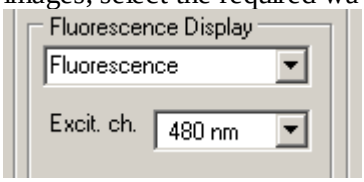
3. Select the range of frames to be plotted either by selecting **All Frames** or selecting **Range** and specifying a range of frames.



4. Select the time units for the plot (**Secs or Mins**).

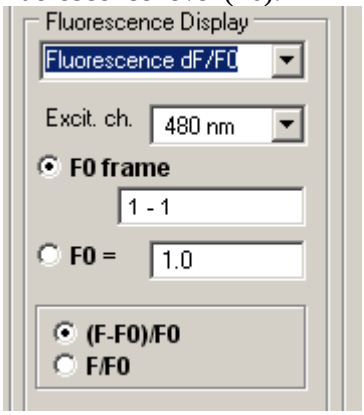
5. The **Fluorescence Display** option determines how the fluorescence signal from the ROI selected in Source is displayed: in terms of either the raw fluorescence intensity (in pixel grey scale units), the fractional change relative to a standard level, the ratio at two different wavelengths, or the computed ion concentration.

   Select the **Fluorescence** option to display the raw fluorescence signal. If the file contains multi-wavelength images, select the required wavelength from the list.



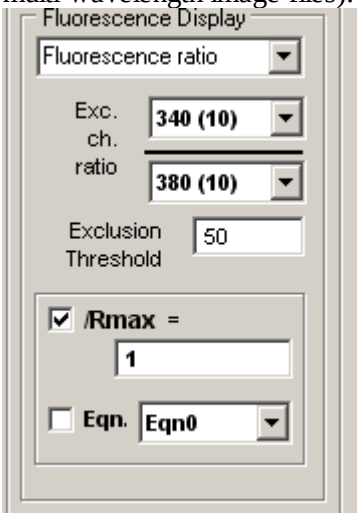
   or

   Select the **Fluorescence dF/F0** option to display the fluorescence signal as a fraction of a predefined baseline fluorescence level (F0).



   Select the **F0 frame** option to define the baseline fluorescence level as the average fluorescence between the

range of frames defined in the adjacent range box, or select the **F0=** option and enter the value into the F0= box. Select the **(F-F0)/F0** option to plot the fractional difference relative to F0 or **F/F0** to plot the fraction of F0.
or
Select the **Fluorescence ratio** option to the display fluorescence ratio of two wavelengths (only available for multi-wavelength image files).



Select the wavelengths to be ratioed from the **Exc. ch. ratio** wavelength selection lists. Enter the lowest acceptable intensity level for the denominator wavelength in the **Exclusion Threshold** box. (Ratios with denominators below this level are set to zero.) To display the ratio as a fraction of a maximum ratio, tick the **/Rmax=** option and enter the maximum in the **/Rmax=** box. To display ion concentration computed from the fluorescence ratio, tick the **Eqn.** option and select an equation.

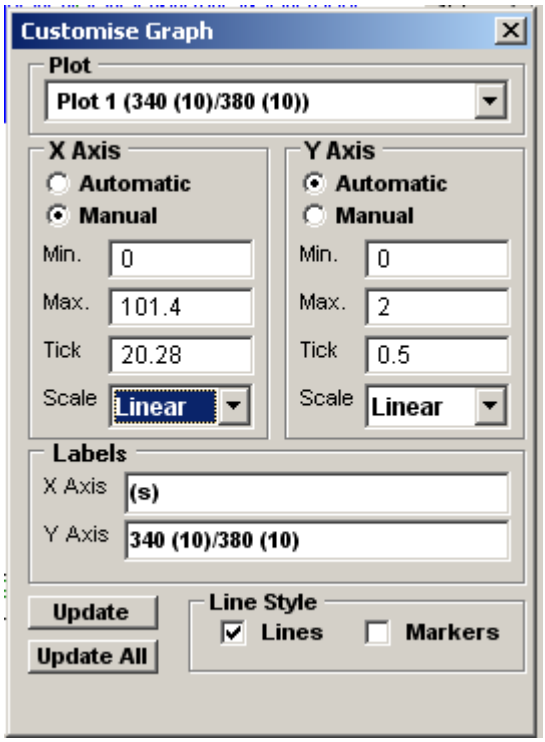
6. Click the **Add to New Plot** button to create a new Y axis and to plot the time course graph of the ROI selected in the Source list OR click the **Add all ROIs** button to add all ROIs (except that selected as the background subtraction ROI) to the plot.

   If a plot already exists, you can add the new time course graph to an existing Y axis by selecting its Axis No. from the list and clicking the **Add To Plot** button instead.)

The displayed plot can be printed (**File / Print Graph**) or copied to the Windows clipboard as data (**Edit / Copy Graph Data**) or as an image (**Edit / Copy Graph as Image**). (A plot is selected for printing/copying by clicking on it. Selected plot is outlined in red.)

Analysis > Time Course Analysis > Changing Plot Axes & Labels

The X and Y axis range and labels of the graphs can be modified by clicking the **Set Axes** button to open **the Customise Graph** dialog box.



Select the graph (when more than one exists in the plot) to be modified from the **Plot** list.

Axis limits and tick spacing are initially set to default values based upon the range of the data. The axis limits can be changed by entering new values for into **Min, Max**. and **Tick** (spacing) boxes for the X and Y axes. An axis can be made **Linear** or **Logarithmic** by selecting the option from its **Scale** list.

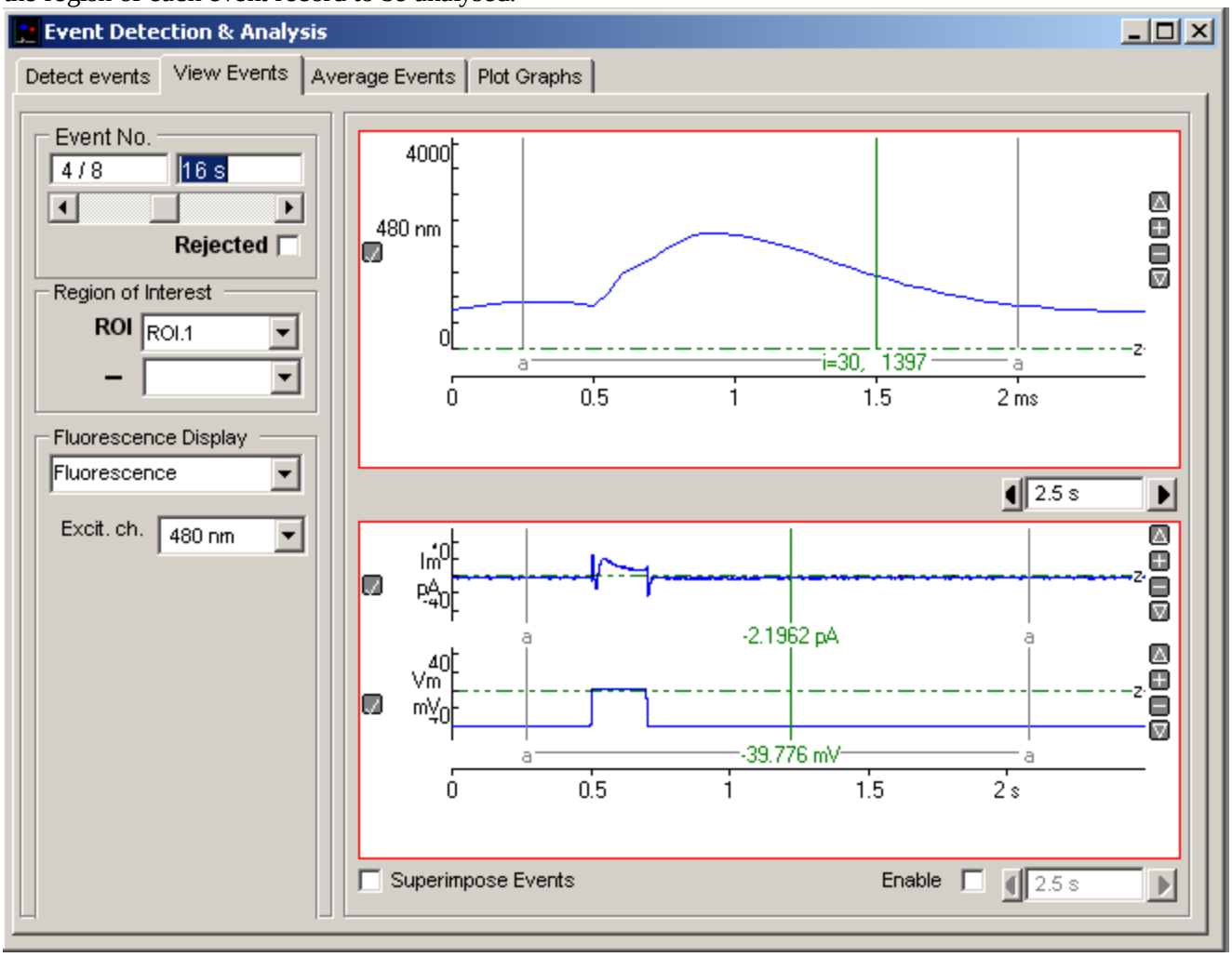
The X and Y axis labels can be changed by editing the entries in the **X Axis** and **Y Axis** labels boxes. X-Y graphs can be plotted as a line, unconnected markers, or both, by ticking the **Lines**, and/or **Markers** tick boxes.

Click the **Update** button to update the graph after changes have been made or Update All to update all graphs (when more than one exists within the plot).

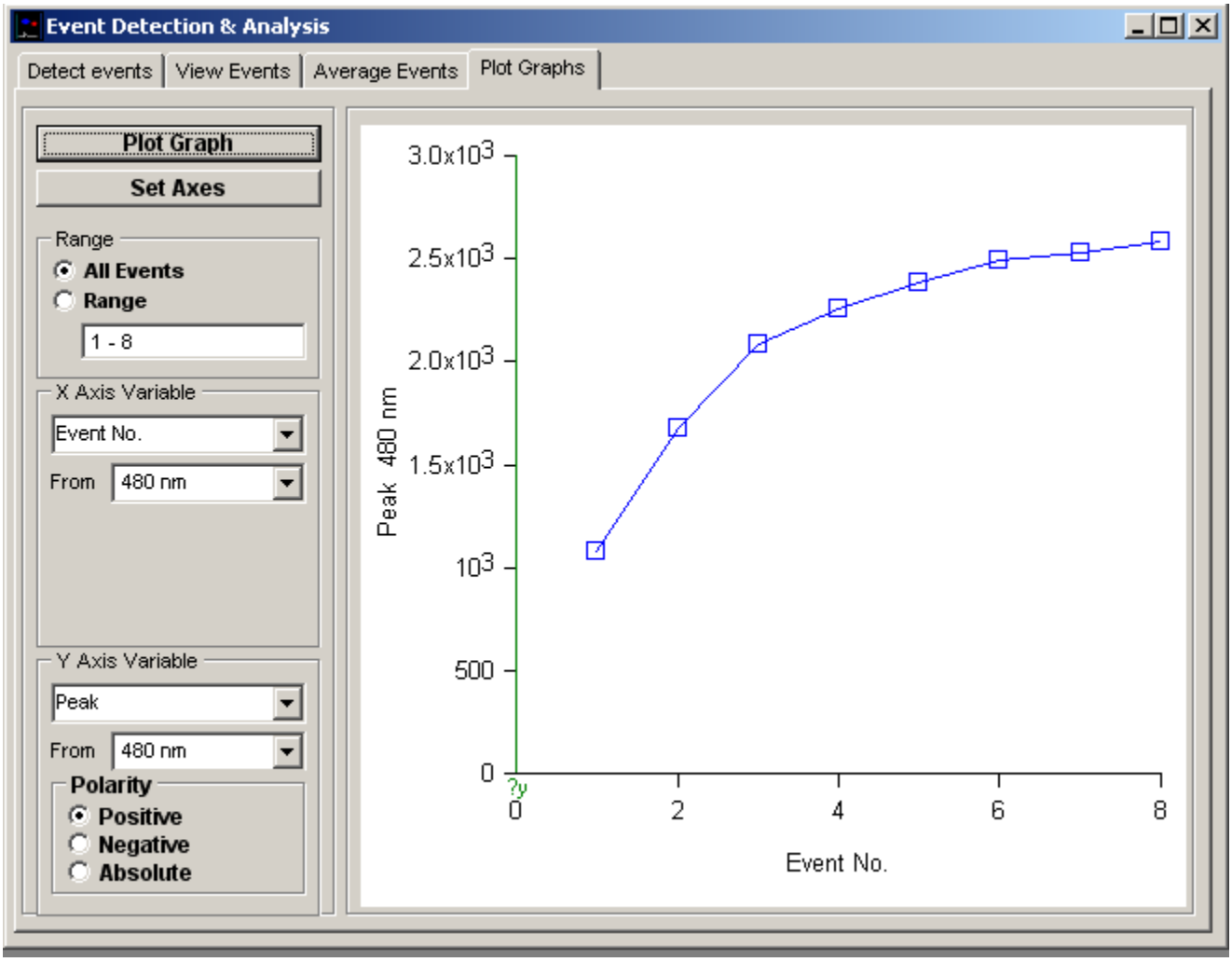
Analysis > Event Detection & Analysis > Plotting Waveform Measurements

A series of waveform measurements can be computed from the detect event records and plotted on the Plot Graphs page.

1. Before plotting a graph, switch to the **View Events** page and use the waveform measurement cursors to define the region of each event record to be analysed.



2. If you want to measure a specific point on each event waveform (Display Cursor measurement) drag the green measurement cursor to the point to be measured.
3. The pair of analysis region cursors (grey (a) cursors linked by a horizontal bar) define the region of the displayed event record to be measured. Points outside this region are ignored for peak and time course measurements. They are initially set to include the whole record. Move these cursors, if you want to limit analysis to a specific region of the record.
4. If you want to exclude an event from a measurement plot tick the event's **Rejected** box.
5. Change to the **Plot Graphs** page.



6. Select the range of events to be plotted either by selecting **All Events** or by selecting **Range** and entering the range of events. (Note. Events marked as **Rejected** are not included in the plot.)
7. Select the type of measurement (see Table.1 below) and the signal to be measured and plotted on the X axis of the graph from the **X Axis variable** lists.
8. Select the type of measurement (see Table.1 below) and the signal to be measured and plotted on the Y axis of the graph from the **Y Axis variable** lists.
9. Click the **Plot Graph** button to plot the selected graph.
10. To modify the graph axes and labels, click the **Set Axes** button.

The displayed graph can be printed (**File / Print Graph**) or copied to the Windows clipboard as data (**Edit / Copy Graph Data**) or as an image (**Edit / Copy Graph as Image**).

Table 1. Waveform Measurements	
Event No.	Sequence number of event in detected event list.
Time	Time (since start of recording) that event was detected. (s)
Interval	Time between event and previous event (s)
Frequency	Event frequency (reciprocal of Interval)

Cursor	Average of block of points around display cursor.
Peak	Peak amplitude within defined analysis region (positive, negative or level can be selected)
Area	Integral of signal within analysis region.
T.rise	Time taken to rise from 10% to 90% of signal peak amplitude.
T(90%)	Time taken to fall from peak to 10% of peak.
Tau(decay)	Exponential decay time constant.
Duration	Event duration
Baseline	level at start of display window.

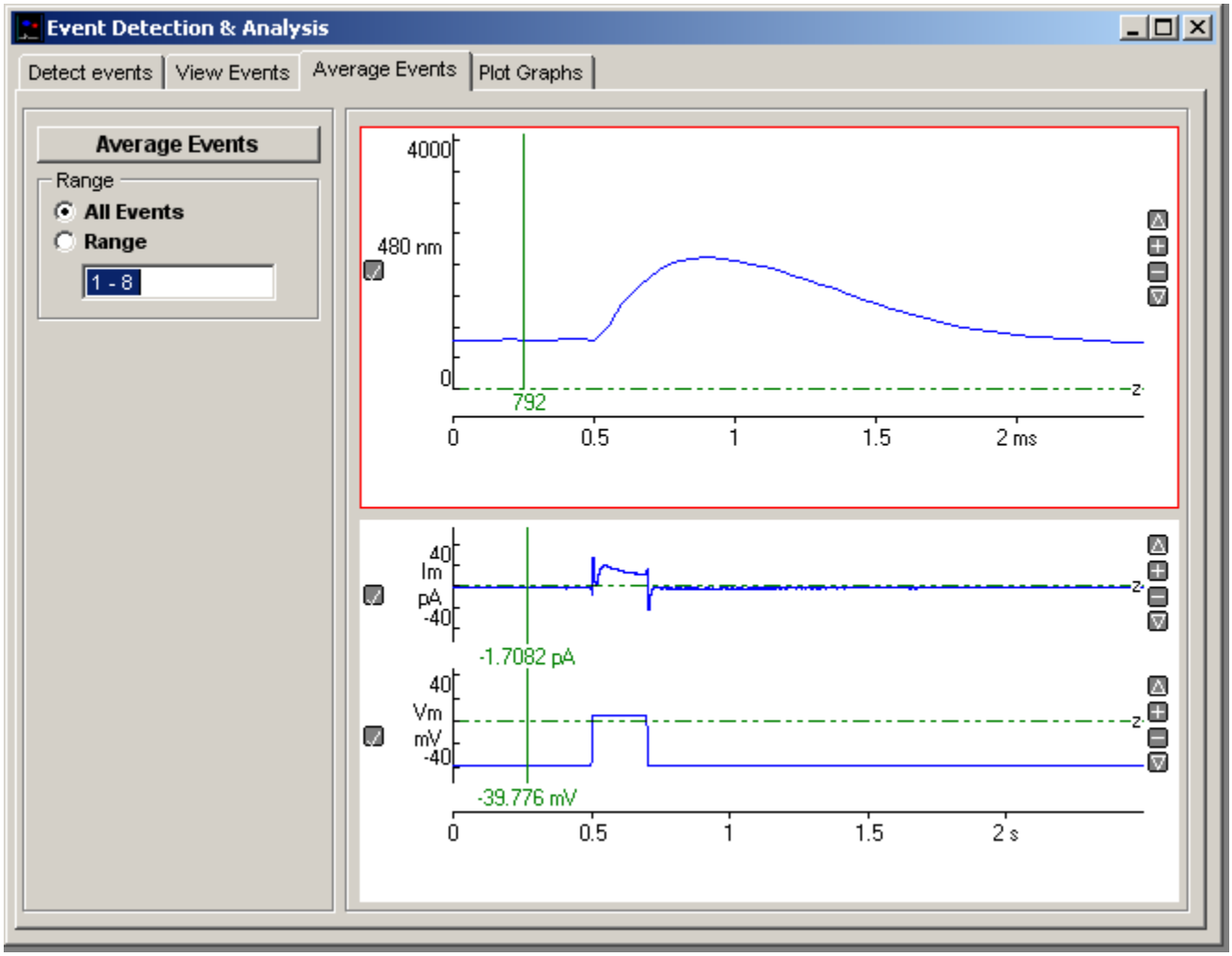
Analysis > Event Detection & Analysis > Averaging Events

Series of detected events can be combined into a single ensemble average event, by averaging corresponding fluorescence and analogue sample points, lined up relative to the detection point.

To average a series of events:

1. Select the **Average Events** page of the Event Detection & Analysis window.
2. Select the range of events to be averaged either by select **All Events** OR by selecting **Range** and entering the range of events. (Note. Events marked as **Rejected** are not included in the average.)
3. Click the **Average Events** button.

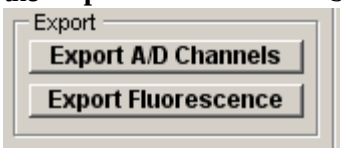
Displayed traces can be printed (**File / Print Graph**) or copied to the Windows clipboard as data (**Edit / Copy Graph Data**) or as an image (**Edit / Copy Graph as Image**). (A plot is selected for printing/copying by clicking on it. Selected plot is outlined in red.)



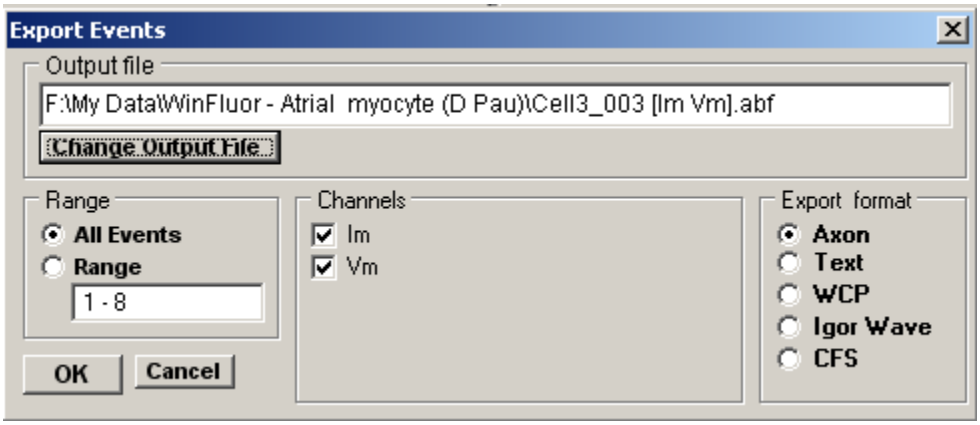
Analysis > Event Detection & Analysis > Exporting Detected Events

Detected event fluorescence and analogue channel waveform records can be exported to WinWCP, Axon PCLAMP, Igor Binary Wave and CED CFS format files and in ASCII tabular form. To export a series of detected records:

1. Click the **Export A/D Channels** button to export the analogue data channels of one or more event records or the **Export Fluorescence** button to export the currently displayed event fluorescence waveform.



2. Select the format of the export file: **Axon** for Axon Instruments ABF format, **WCP** for Strathclyde WinWCP format, **Text** for ASCII text files, **Igor Wave** for IGOR Pro Igor Binary Wave (IBW) format or **CFS** for CED Filing System format.

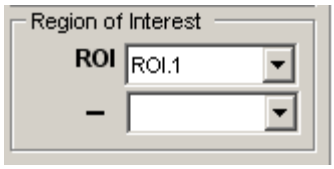


3. Select the range of events to be exported either by select **All Events** or by selecting **Range** and entering the range of events. (Events marked as **Rejected** are not exported.)
4. [Optional] Click the **Change Output File** button to change the name or destination folder of the export file.
5. [Optional] If more one signal channel is available, select the channels to be exported.
6. Click the **OK** button to export

Analysis > Event Detection & Analysis > Fluorescence Display

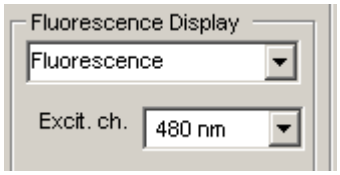
The **Fluorescence Display** options determines how the fluorescence signal is displayed: in terms of either the raw fluorescence intensity (in pixel grey scale units), the fractional change relative to a standard level, the ratio at two different wavelengths, or the computed ion concentration.

Region of Interest: The region of interest within the image from which the fluorescence signal is derived can be selected from the **ROI** list. A background fluorescence signal can be subtracted from the displayed signal by selected a background ROI from the **-** list. (not applicable to line scan files).



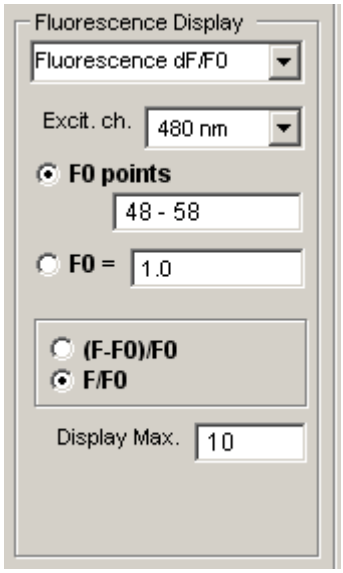
Fluorescence

Select the **Fluorescence** option to display the average fluorescence intensity signal computed over the selected ROI. If the file contains multiple excitation-wavelength channels, select the required wavelength from **Excit. Ch.** list.



Fluorescence dF/F0

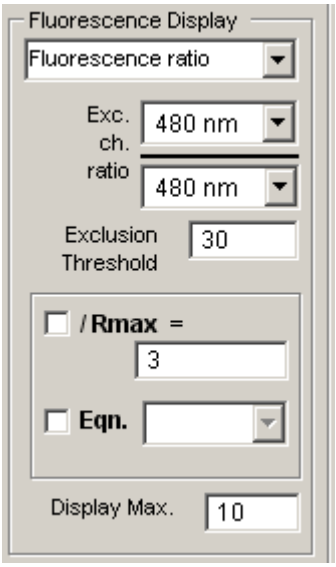
Select the **Fluorescence dF/F0** option to display the fluorescence signal as a fraction of a predefined baseline fluorescence level (F0).



Select the **F0 frame** option to define the baseline fluorescence level as the average fluorescence between the range of frames defined in the adjacent range box, or select the **F0=** option and enter the value into the F0= box. Select the **(F-F0)/F0** option to plot the fractional difference relative to F0 or **F/F0** to plot the fraction of F0. Enter the upper limit of the display range into the **Display Max.** box.

Fluorescence ratio

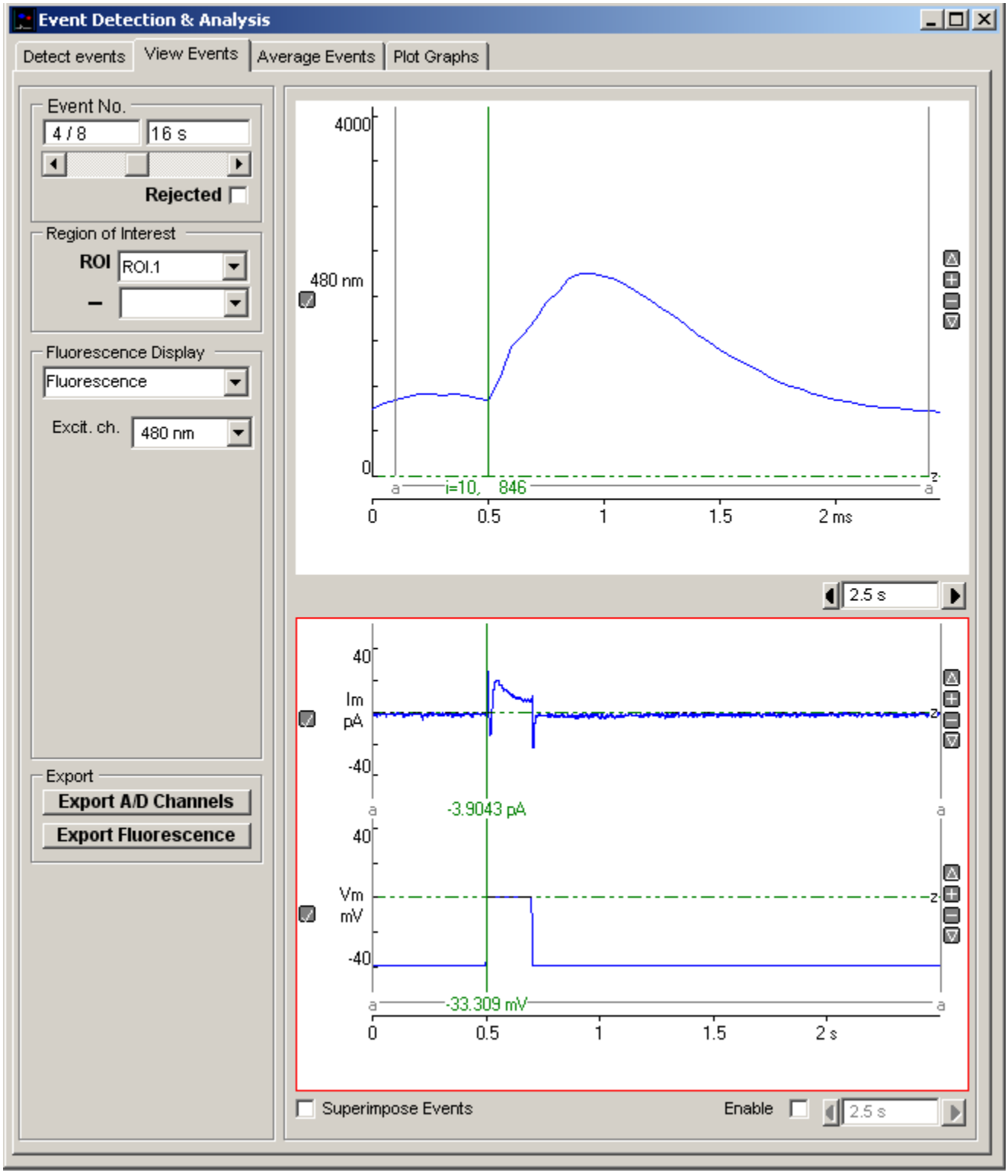
Select the **Fluorescence ratio** option to the display fluorescence ratio of two wavelengths (only available for multi-wavelength image files).



Select the wavelengths to be ratioed from the **Exc. ch. ratio** wavelength selection lists. Enter the lowest acceptable intensity level for the denominator wavelength in the **Exclusion Threshold** box. (Ratios with denominators below this level are set to zero.) To display the ratio as a fraction of a maximum ratio, tick the **/Rmax=** option and enter the maximum in the **/Rmax=** box. To display ion concentration computed from the fluorescence ratio, tick the **Eqn.** option and select an equation.

Analysis > Event Detection & Analysis > Viewing Detected Events

The set of events detected within a data file can be viewed by selecting the **View Events** page in the Event Detection & Analysis window.



Individual events can be displayed using the **Event No.** slider bar or by entering the number of the event in the **Event No.** box and pressing the return key.

The selected fluorescence signal is display in the top display panel and the analogue channels (if any) in the bottom panel. The duration of each signal display panel can be adjusted separately using the **Duration** box at the right hand edge of each display.

Events can be marked as rejected by ticking the **Rejected** option. Rejected records are excluded from averages, export lists, or waveform measurement plots.

Displayed events can be printed (**File / Print Graph**) or copied to the Windows clipboard as data (**Edit / Copy Graph Data**) or as an image (**Edit / Copy Graph as Image**). (A plot is selected for printing/copying by clicking on it.

Selected plot is outlined in red.)

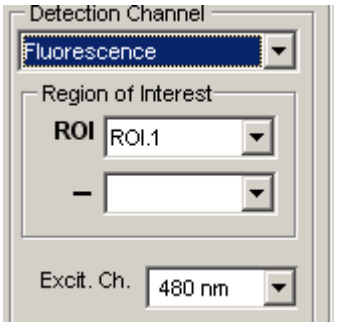
Analysis > Event Detection & Analysis > Detecting Events

To detect events:

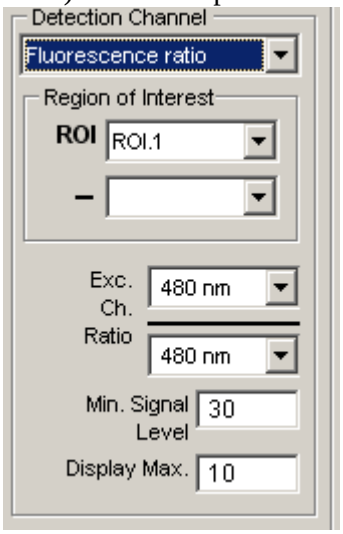
1. Select the **Detect Events** page in the Event Detection & Analysis window.
2. Select the signal channel to be scanned for events from the **Detection Channel** list which displays the available analogue and fluorescence channels

   Analogue channels:
   Select the channel to be scanned from the list.

   Fluorescence channels:
   Select the region of interest to be used from the **ROI** list (and optionally a background subtraction ROI from the – list). If more than one excitation wavelength is available, select the excitation wavelength channel from the **Excit. Ch.** list.

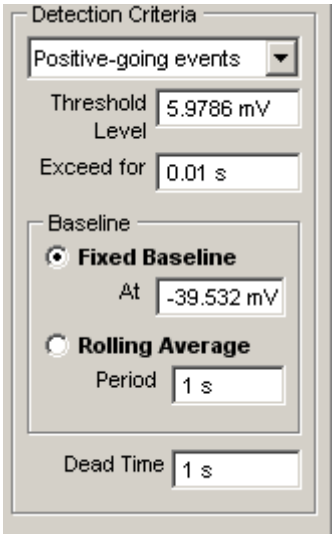


   Fluorescence ratios:
   Select the region of interest to be used from the **ROI** list (and optionally a background subtraction ROI from the – list). Select the pair of excitation wavelength channels to be ratioed from the **Exc. Ch. Ratio** lists.



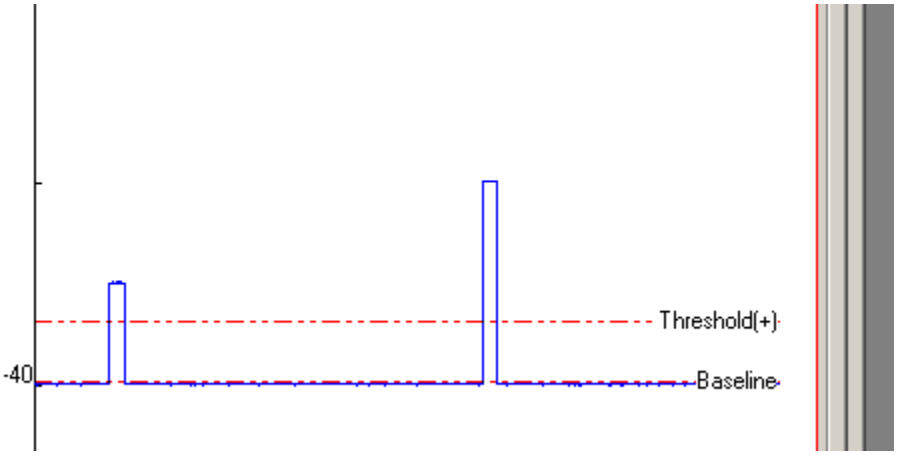
3. Set the upper limit of the ratio display range in the **Display Max**. box. Enter the minimum signal level that each fluorescence signal channel must exceed to be included in the ratio into the **Min. Signal Level** box. The ratio is set to zero if either signal within the ratio falls below this level.

4. Enter the event detection criteria.



Polarity: Set the polarity of the signals to be detected, selecting **Positive-going events** for signals which rise to a positive peak relative to the detection baseline, **Negative-going events** for signals which rise to a negative peak. Select **Positive or Negative** for to detect both positive- and negative-going signals.

Threshold: Set the signal baseline and detection threshold levels using the horizontal **Baseline** and **Threshold** display cursors.



Scroll through the displayed detection channel signal to locate and display the smallest signal to be detected. Place the **Baseline** cursor on the signal baseline before the event. Place the **Threshold** cursor at a level which is crossed by the signal and which exceeds the signal background noise. (Note, a pair of (+) and (-) thresholds are displayed when the **Positive or Negative** detection option is selected.)

Exceed For: Enter the period of time that the signal has to remain above the threshold before detection takes place into the **Exceed For** box.

Baseline: Select the **Fixed Baseline** option to maintain the baseline and thresholds at a fixed level throughout the event detection scan. Select the **Rolling Average** option to make the baseline follow changes in the signal baseline level averaged over the period of time set by the **Period** box

Dead Time: Enter the period of time after detection of an event before another event can be detected into the **Dead Time** box. (To avoid detecting an event more than once, the dead time value should be longer than the duration of the event being detected but shorter than the time interval between events.)

5. Click the **Detect Events** button to detect events within the data file using these criteria.

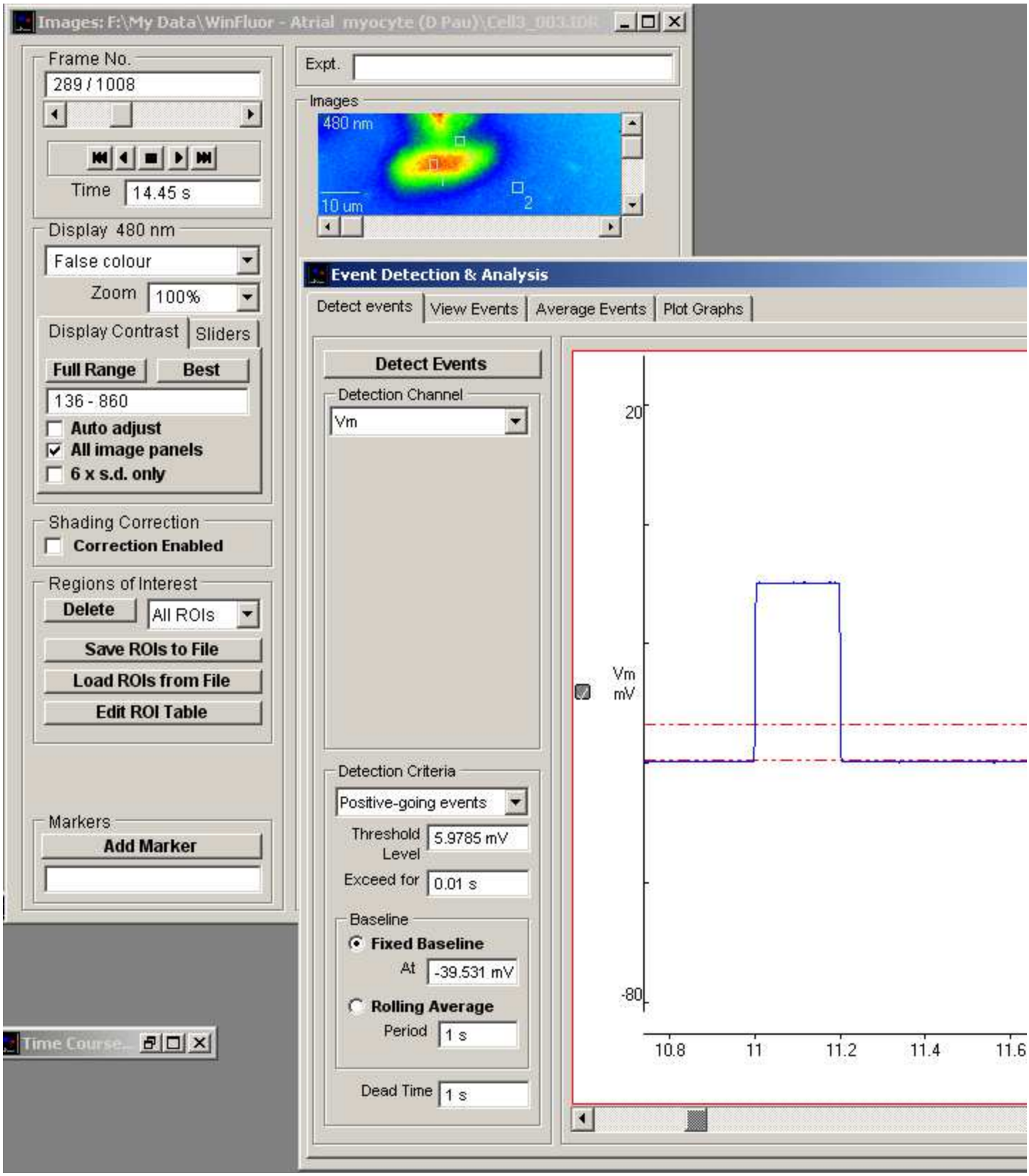
Analysis > Event Detection & Analysis > Event Detection & Analysis

The event detection and analysis window allows discrete events (voltage steps, current pulses, fluorescence waves) to be located within a continuous recording. Detected event waveforms can be superimposed, averaged and a range of waveform measurements computed and plotted.

Select

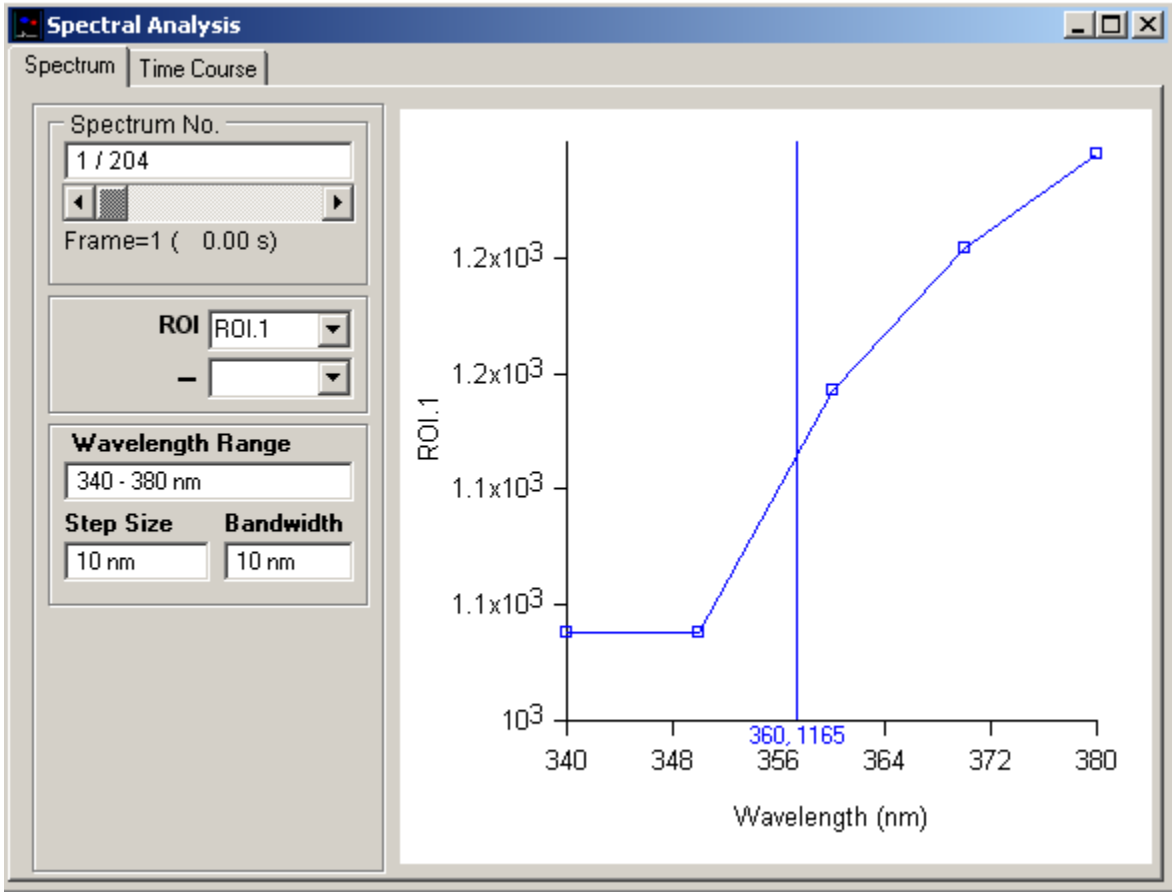
Analysis
Event Detection & Analysis

to open the event detection & analysis window.



Analysis > Spectral Analysis > Plotting Fluorescence Excitation Spectra

To analyse spectral series data files, recorded using the **Spectrum** excitation light option, select
Analysis
Spectral Analysis
to open the spectral window. Each file is divided into a series of spectral blocks, each block containing a series of images with the excitation light wavelength incremented from the lower to upper limit of the spectrum. The spectral analysis modules calculates and displays the intensity from defined regions of interest within the image vs. excitation light wavelength throughout the time series.

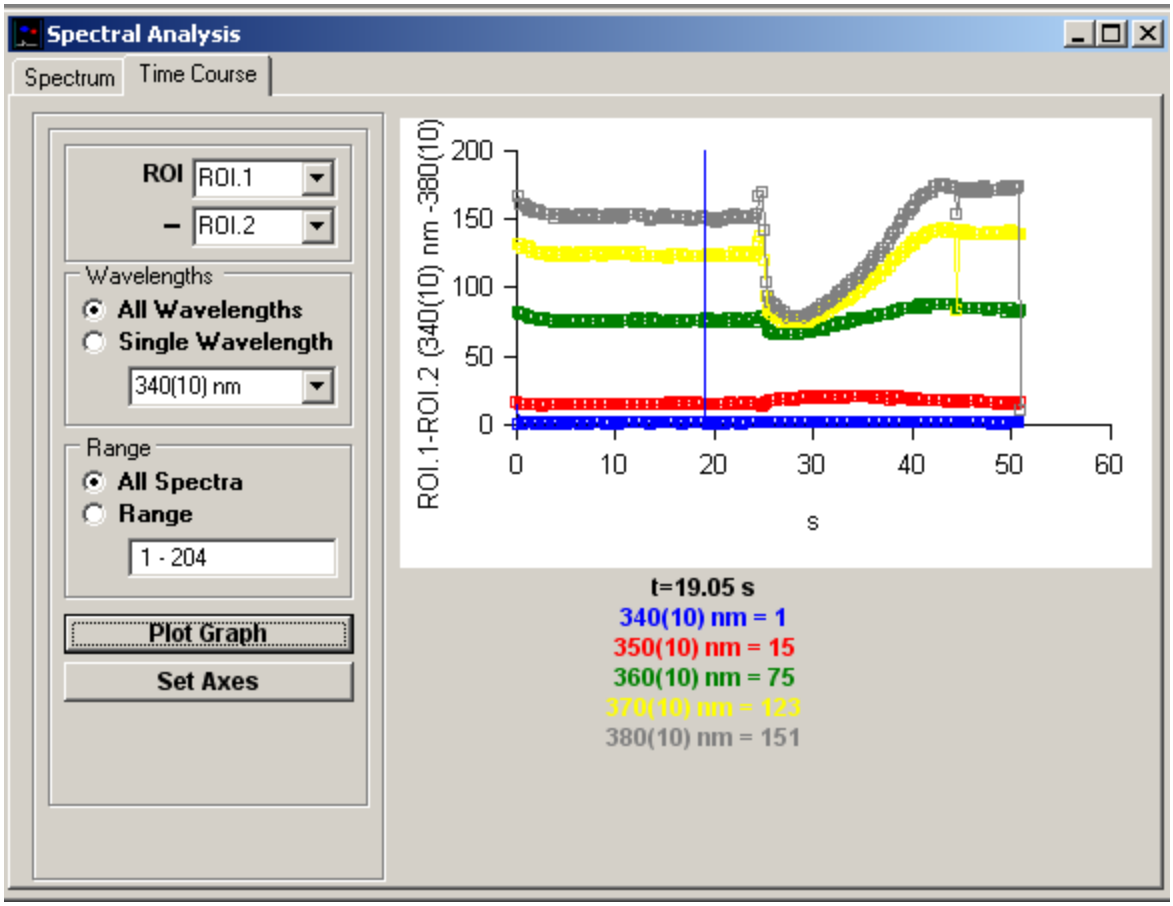


To display the excitation spectrum for a selected region of interest:

1. In the Images window, place one or more ROIs over regions of interest on cells where the intensity spectra are to be computed and displayed.
2. Place an ROI on an image region not containing cells to provide a measure of background intensity.
3. Select the **Spectrum** page in the **Spectral Analysis** window.
4. Select a region of interest for display in the **ROI** list.
5. Select a background region to be subtracted in the subtraction ROI list (optional).
6. Individual spectra throughout the image series file can selected using using **Spectrum No.** slider bar. The Frame number and time of the start of the spectrum within the file is also indicated.

The displayed graph can be printed (**File / Print Graph**) or copied to the Windows clipboard as data (**Edit / Copy Graph Data**) or as an image (**Edit / Copy Graph as Image**).

Analysis > Spectral Analysis > Plotting Fluorescence Time Course at Selected Wavelengths



To display the time of intensity at selected regions of interest:

1. Select the **Time Course** page in the **Spectral Analysis** window.
2. Select a region of interest for plotting in the **ROI** list and (optional) a background region to be subtracted in the **-** list .
3. Wavelengths: Select the **All Wavelengths** option to plot the time course for all wavelengths in the spectrum or **Single Wavelength** and select a single wavelength from the list.
4. Range: Select the **All Spectra** option to include all spectral time points in the plot or select **Range** and enter a selected range of spectra within the file.
5. Click the **Plot Graphs** button to plot the time course(s).
6.

The displayed graph can be printed (**File / Print Graph**) or copied to the Windows clipboard as data (**Edit / Copy Graph Data**) or as an image (**Edit / Copy Graph as Image**).

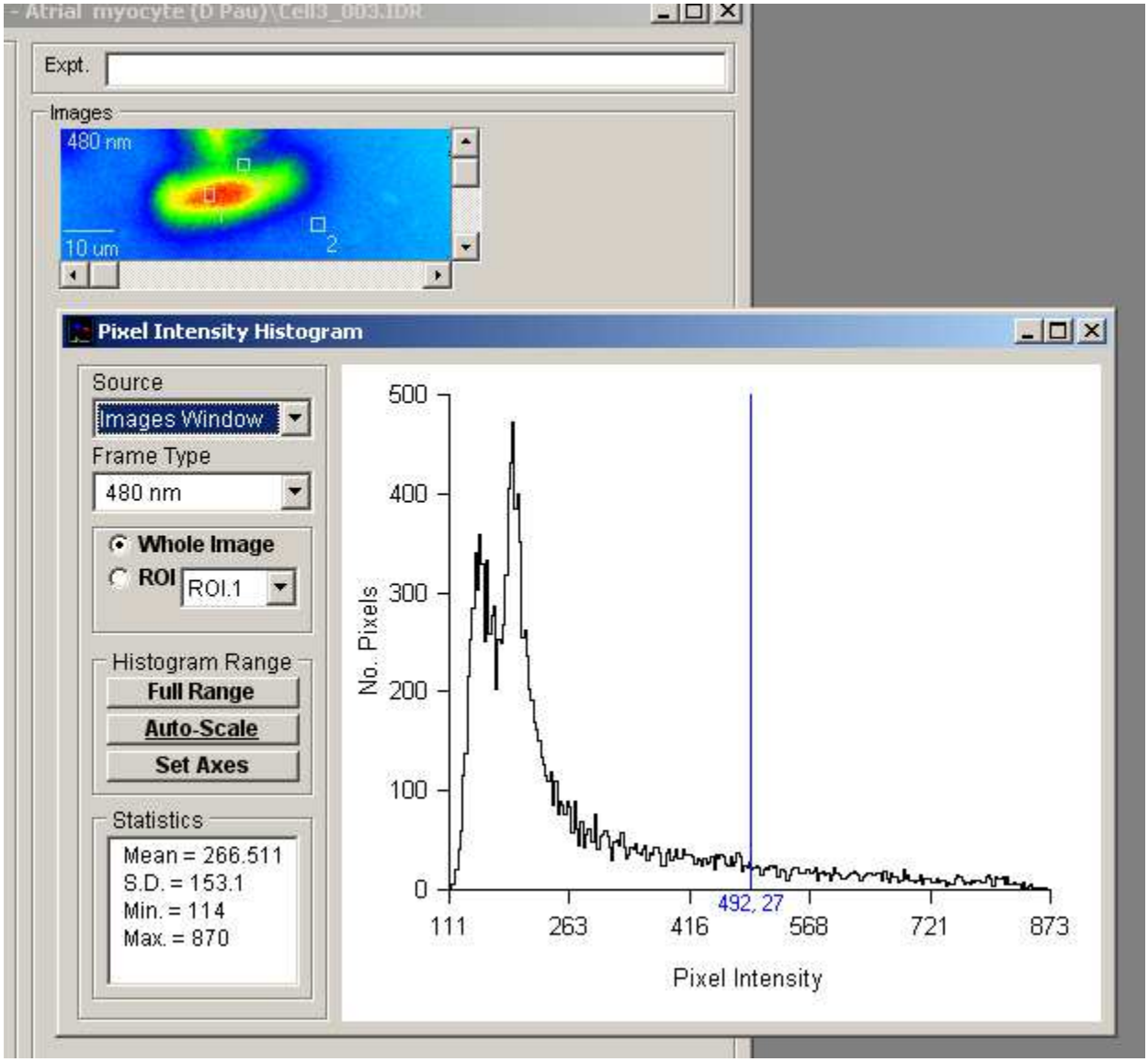
Analysis > Pixel Intensity Histogram > Pixel Intensity Histogram

The pixel intensity histogram window displays the distribution of pixel intensity values within the image or a region of interest within the image. Intensity histograms can be plotted both from live images and those recorded on file. The mean, minimum, maximum and standard deviation of the image intensity values is also displayed.

Select

Analysis
Pixel Intensity Histogram

to open the Pixel Intensity Histogram display window.



Select the source of the images to be plotted in the histogram from the **Source** list which contains a list of currently active image display windows. **Images Window** selects images stored on the currently open data file. **Record Window** selects the live image.

If the image source is a multi-wavelength sequence, select the wavelength frame to be displayed from the **Frame Type** list.

Select the **Whole Image** option to display the intensity histogram for all pixels within the the image. To restrict the histogram to a region of interest within the image, select the **ROI** option, and choose an ROI number from the list. (Note. The ROI option is not available when the image source is live images.)

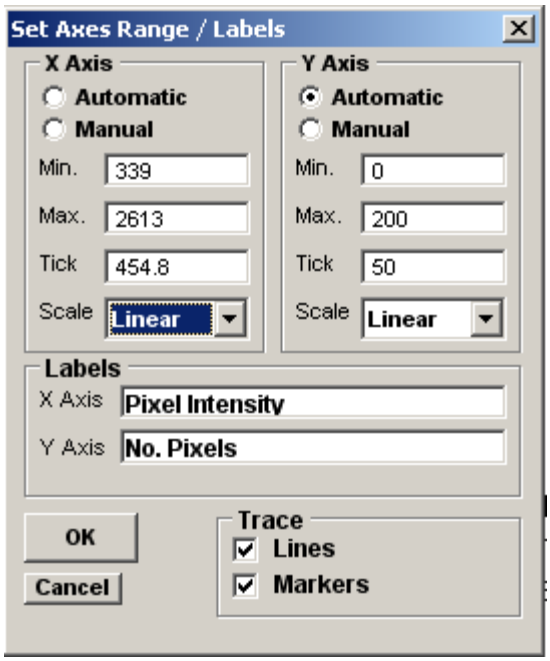
Click the **Full Range** button to set the range of intensities plotted in the histogram to full intensity range of the camera. Click the **Auto-Scale** button to restrict the intensity range to the min. – max. range of values actually within the image. The histogram can also be set to a user-defined range by entering values into the **Low** and **High** histogram range boxes, then pressing the **<Enter>** key.

Customising graph axes and labels

The X and Y axis range and labels of the histogram graph can be customised by clicking the

Set Axes

button to open **the Customise Histogram** dialog box.



Axis limits and tick spacing are initially set to default values based upon the range of the data. The axis limits can be changed by entering new values for into **Min, Max**. and **Tick** (spacing) boxes for the X and Y axes. An axis can be made **Linear** or **Logarithmic** by selecting the option from its **Scale** list. The X and Y axis labels can be changed by editing the entries in the **X Axis** and **Y Axis** labels boxes. The histogram **Bin Style** can be set to empty, solid or hatched boxes in a variety of colours. When Full Borders option is ticked a solid rectangular border is drawn round each bin.

Click the **Update** button to update the graph after changes have been made or Update All to update all graphs (when more than one exists within the plot).

Analysis > Line Profile > Displaying a Line Profile

The line profile module plots image intensity along the length of a linear ROI defined within an image stored on file. It can oper

Select
Analysis
Line Profile

to open the Line Profile display window.

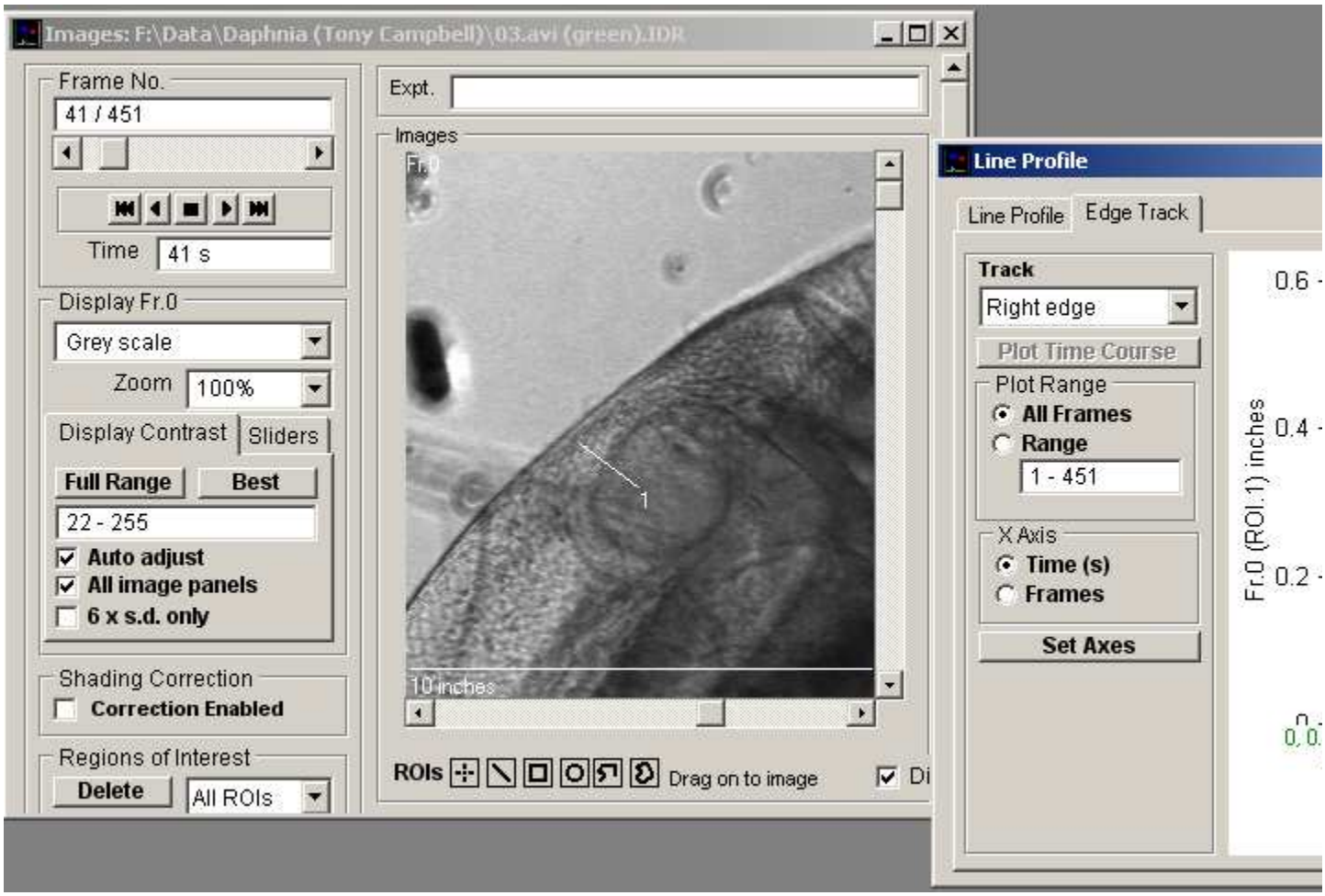
To display a line profile:

1. Define a **Line** or multi-segment line ROIs within the image displayed in the **Images** window.
2. Frame Type: If the file contains multi-wavelength images, select the wavelength to be displayed from the **Frame Type** list.
3. Select the region of interest from the list of available ROIs displayed in the **ROI** list.
4. Line Width: Define the number of pixels (at right angles to each pixel point on the line) to be averaged to produce the line profile in the **Line Width** box.
5. Axes Range: Select the **Auto Scale** option to automatically scale the plot axes range or **Manual** to specify a fixed range, define using **Set Axes**.

The displayed plot can be printed (**File / Print Graph**) or copied to the Windows clipboard as data (**Edit / Copy Graph Data**) or as an image (**Edit / Copy Graph as Image**).

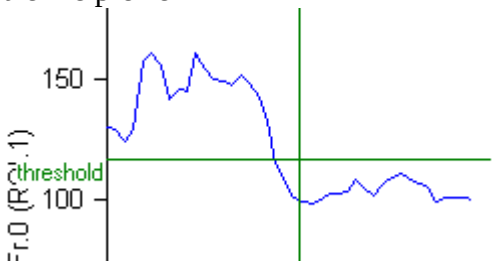
Analysis > Line Profile > Tracking Edges

The Edge Track facility within the Line Profile module can be used to track frame by frame changes in the position of the left or right edges or width of a line profile.



To track the edge of line profile that has been set up on the Line Profile page:

1. On the Line Profile page, use the horizontal **Threshold** cursor to set the tracking point on the selected edge of the line profile



2. Track: Select the part of the profile to be tracked: **Left Edge** or **Right Edge** for the right or left hand end edges of the profile or **Width** to track the distance between the left and right edges.
3.
4. Plot Range: Select the range of frames within the data file to be tracked. Select the **All Frames** option to track all image frames in the file or select **Range** and enter the sub-range to be used.
5.
6. X Axis: Select **Time(s)** to plot the edge vs time or **Frames** to plot vs. frame number.
7.
8. Click **Plot Time Course** to plot the edge track graph.

The X and Y axis range and labels of the graphs can be modified by clicking the **Set Axes** button to open **the Customise Graph** dialog box.

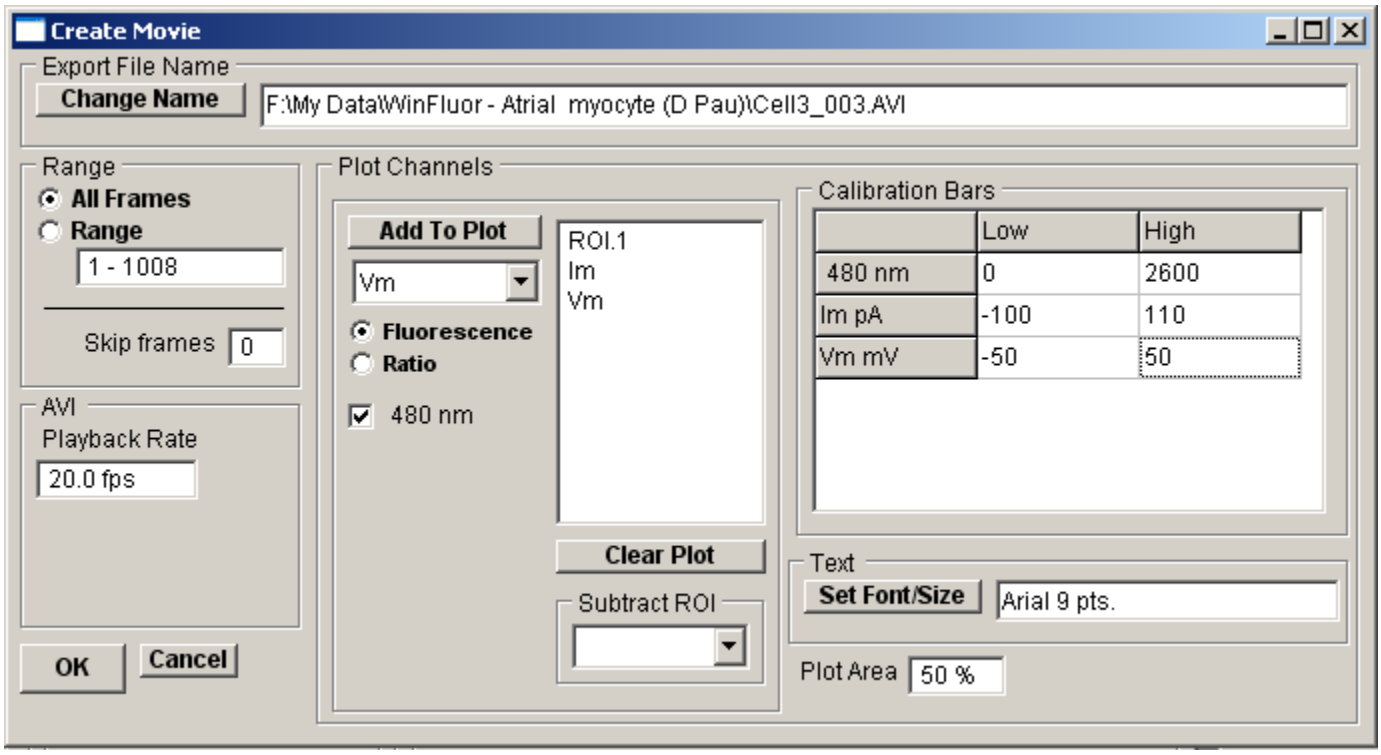
The displayed plot can be printed (**File / Print Graph**) or copied to the Windows clipboard as data (**Edit / Copy Graph Data**) or as an image (**Edit / Copy Graph as Image**).

File Import/Export > Creating Movies

To create an AVI format movie showing images and plots of fluorescence, fluorescence ratio and/or analogue signal time course, select

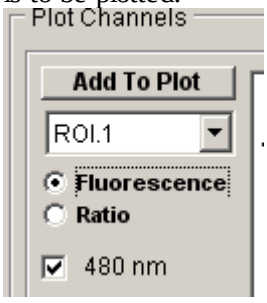
File
Create Movie

to open the window.

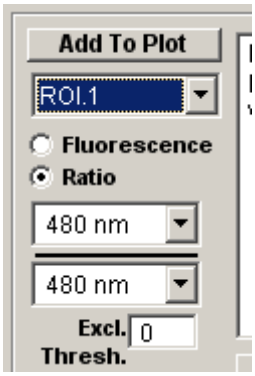


To create a movie:

1. Range: Select **All Frames** to plot a movie using all images in the data file or **Range** and enter a sub-range of image frames. Set the number of frames within the data file to be skipped to reduce the size of the AVI movie file in the **Skip frames** box (optional).
2. Plot Channels: Select the **Fluorescence** option to plot raw fluorescence time course from the region of interest selected in the ROI list. If a background ROI has been defined and is to be subtracted from the fluorescence plots, select it from the **Subtract ROI** list.For files containing multi-wavelength images, select which wavelength is to be plotted.

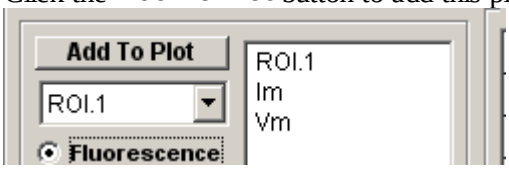


or select **Ratio** to plot the ROI fluorescence ratio from a selected pair of wavelengths in a multi-wavelength data file.



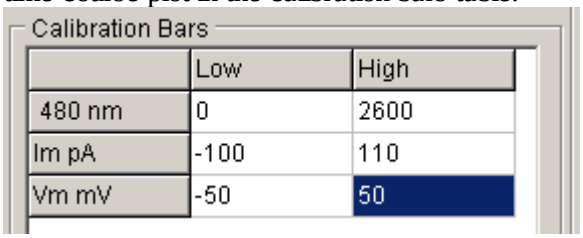
Select the wavelengths to be ratioed from the two wavelength selection lists. Enter the lowest acceptable intensity level for the denominator wavelength in the **Excl Thresh.** box. (Ratios with denominators below this level are set to zero.)

3. Click the **Add To Plot** button to add this plot to the Movie.



ROIs and analogue channels selected for plotting are indicated in the plot list. (The plot list can be cleared by clicking the **Clear Plot** button.)
Repeat steps 1.- 4. for additional plots to be added to the movie. (a movie can contain up to 4 plots.)

4. Calibration Bars: Once all ROI/analogue channels have been added, define the upper and lower limits of each time course plot in the calibration bars table.



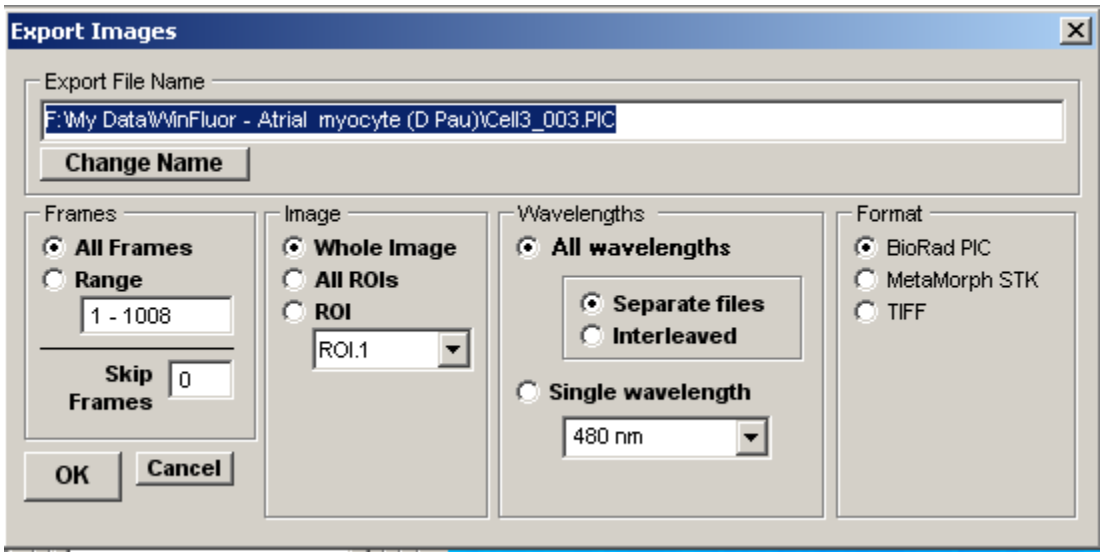
5. Set the percentage of the height of the movie allocated to the time course plots in to **Plot AreaSize** box (optional).
6. Text: Click the **Set Font** button to change the font size and typeface used to label the time course plots (optional) .
7. AVI: Set the AVI movie playback rate by entering the desired rate (frames per second) in the **Playback rate** box (optional) .
8. (Optional) Click the **Change Name** button to change the name of the AVI file to be created.
9. Click the **OK** button to create the movie.

File Import/Export > Exporting Images

To export the images stored in a WinFluor data file, select

File
Export
Image Series

to open the window.



To export a series of images:

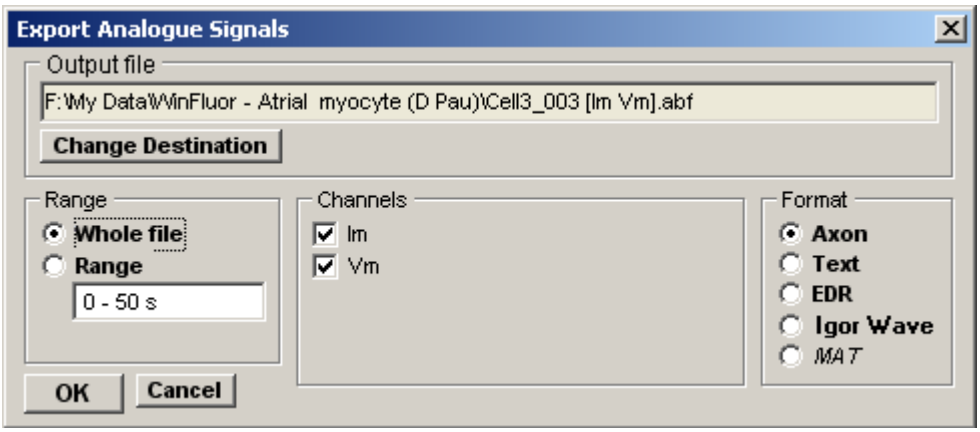
1. Format: Select the format of the export file: **BioRad PIC, MetaMorph STK**, or **TIFF** format.
2. Frames: Select the **All Frames** option to export all images in the data file or **Range** and enter a selected the range of frames. Enter the number of frames to be skipped for each frame exported in the **Skip frames** box (optional) .
3. Image: Select the **Whole Image** option to export the whole image or **All ROIs** to export a series of rectangular sub-images defined by the currently defined regions of interest or **ROI** to export the single ROI image selected from the ROI list.
4. Wavelengths: For multi-wavelength data files, select **All wavelengths** to export all wavelength image types, selecting **Separate files** to store each wavelength types in a separate file or **Interleaved** to store them in a single file. To export one wavelength type only, select **Single wavelength** and select the wavelength from the list.
5. Export File Name: (Optional) Select **Change Name** to change the name of the export file.
6. Click the **OK** button to export the images.

File Import/Export > Exporting Analogue Signals

To export analogue signals, select:

File
Export
Analogue Signals

to open the window.



To export the data from one or more analogue signal channel:

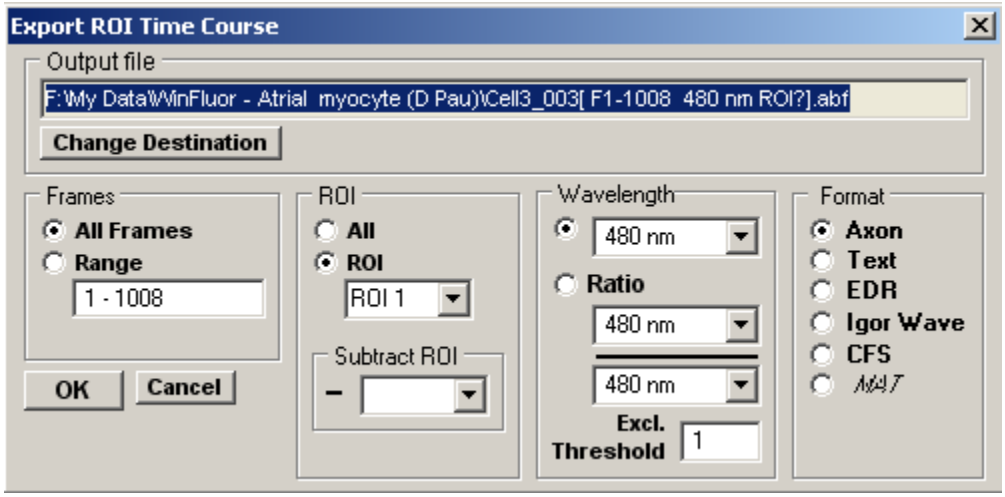
1. Format: Select the format of the export file: Axon for the Axon ABF file format (readable by PCLAMP), **EDR** for the Strathclyde WinEDR file format or **Text** to export the data as tables of tab-delimited ASCII text, **Igor Wave** to export the data to an Igor binary wave format file.
2. Range: Select the **Whole file** option to export all digitised signal data the file OR **Range** and enter a selected time period to be exported.
3. Channels: Select the analogue signal channels to be exported.
4. Click the **OK** button to export the signals.

File Import/Export > Exporting ROI Time Courses

To export the fluorescence time course from selected ROI within an image series, select:

File
Export
ROI Time Course

to open the window.



To export the fluorescence time courses:

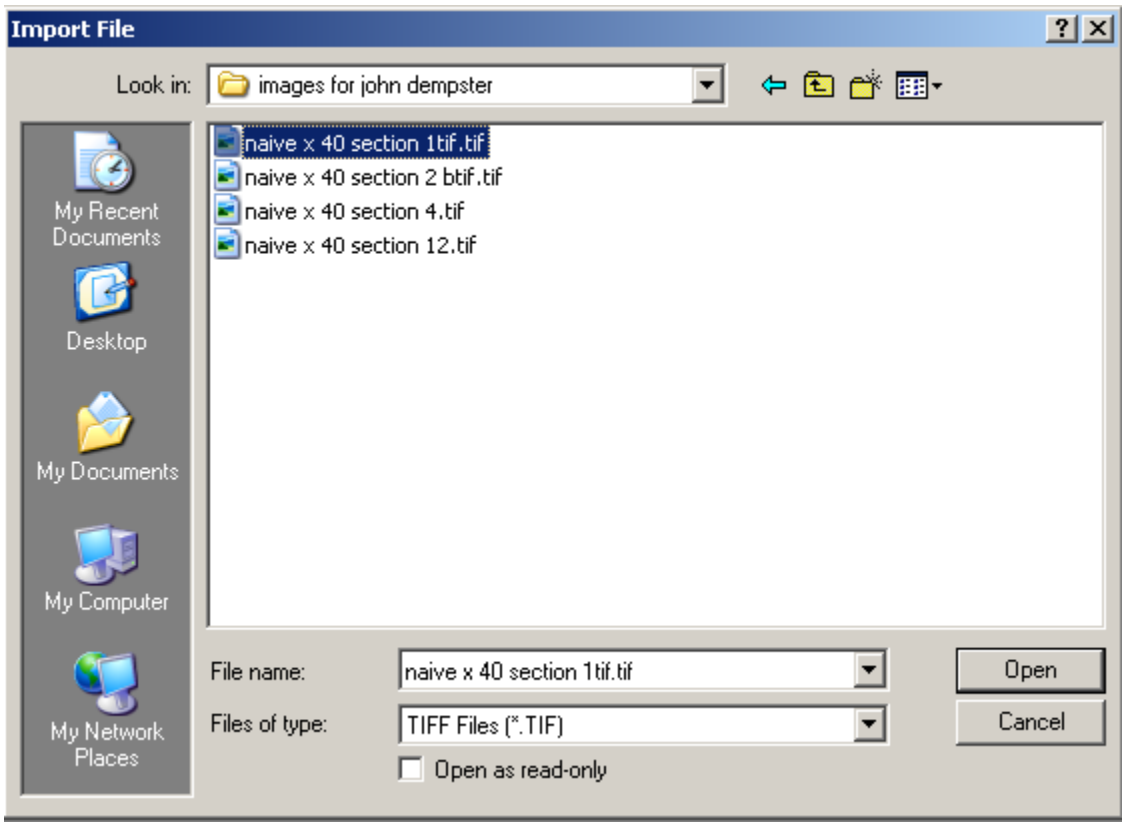
1. Export Format: Select the format of the export file: Axon for the Axon ABF file format (readable by PCLAMP), **EDR** for the Strathclyde WinEDR file format or **Text** to export the data as tables of tab-delimited ASCII text, **Igor Wave** to export the data to an Igor binary wave format file, or **CED CFS** format.
2. Frames: Select the **All Frames** option to export all frames or **Range** and enter a selected frame range to be exported.
3. ROI: Select the ROI(s) to be exported. Select **All** to export all ROI time courses or **ROI** to export the ROI selected in the adjacent list.
4. Wavelength: For multi-wavelength file, select the specific wavelength to be exported or **Ratio** to export the ratio of two wavelengths. (Enter the lowest acceptable intensity level for the ratio denominator wavelength in the **Excl. Threshold** box. Ratios with denominators below this level are set to zero.)
5. Click the **OK** button to export the ROI time courses.

File Import/Export > Importing Images

To import a series of images into a WinFluor data file, select:

File
Import Images

To open to the dialog box.



To import an image series:

1. Select the type of file to be imported from **Files of Type** list.
2. Find and select the file to be imported.
3. Click the **Open** button to import the file.
4.

Data file format which can currently be imported are:

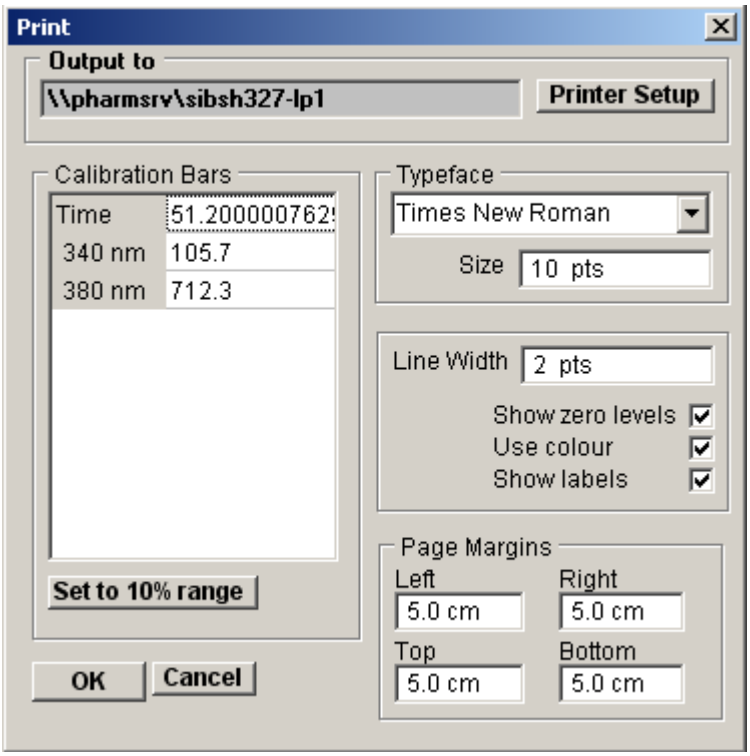
PIC	PIC files (as produced by BioRad confocal and 2P microscopes)
STK	STK MetaMorph STK format files
TIF	TIF Multi-page Tagged Image File Format (TIFF) files
ICS	Image Cytology Standard format (including the version produced by the Nikon C2 confocal microscope).

Printing & Copying Graphs > Printing Graphs

Hard copies of graphs displayed in a plotting window can be produced by clicking on the graph plot (to ensure that it the active window) and selecting

File
Print

To open the dialog box.



1. The printer selected for output is indicated in the Output to box and can be changed by clicking the **Printer Setup** button to open the Printer Setup dialog box.
2. The size and position of the graph on the page can be adjusted by changing the **Left, Right, Top** and **Bottom** page margin settings.
3. The typeface used to label the axes can be selected from the **Typeface** list and the size of the text set by the **Size** field. The thickness of the lines on the graph and the size of data point marker is set by the **Line width** and **Marker size** fields. Lines are printed in colour (as on the display screen) If the **Use colour** option is ticked.
4. Clicking the **OK** button plots the graph on the printer.

Printing & Copying Graphs > Copying a Graph as Data to the Clipboard

The data points of graph(s) displayed in a plotting window can be copied to the Windows clipboard for pasting into other programs, by clicking on the window (to ensure that it the active window) and selecting.

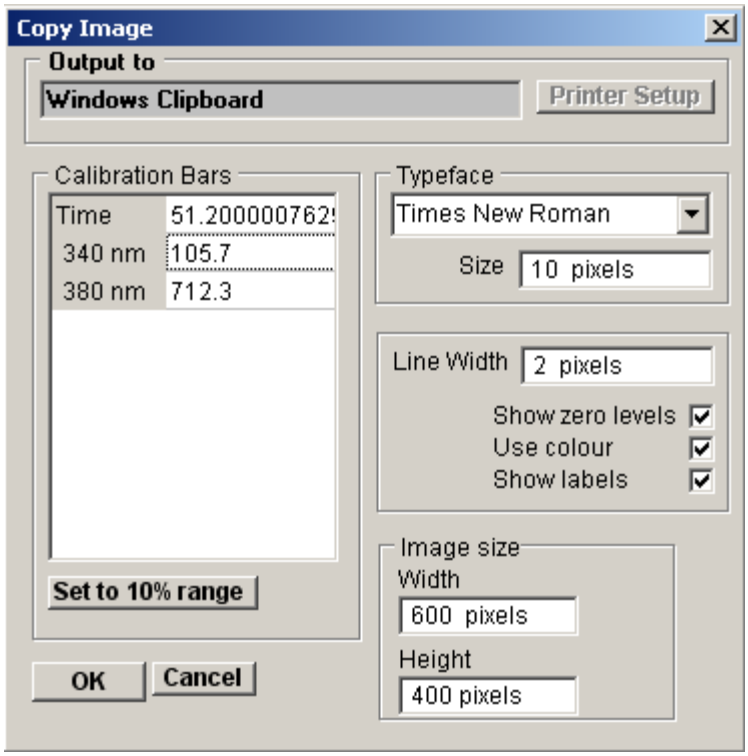
Edit
Copy Graph as Data

Printing & Copying Graphs > Copy a Graph as an Image to the Clipboard

An image of the graph(s) displayed in a plotting window can be copied to the Windows clipboard for pasting into other programs, by clicking on the window (to ensure that it the active window) and selecting

Edit
Copy Graph Image

to open the dialog box.



1. The dimensions of the bit map, which will hold the image, can be set using the **Width** and **Height** image size boxes. The more pixels used in the bit map the better the quality of the image.
2. The typeface used to label the axes can be selected from the **Typeface** list and the size of the text set by the **Size** field. The thickness of the lines on the graph and the size of data point marker is set by the **Line width** and **Marker size** fields.
3. Clicking the **OK** button copies the image to the clipboard.