

Strathclyde Electrophysiology Software

# **WinEDR V3.4.3**

Electrophysiology Disk Recorder

(c) John Dempster, University of Strathclyde 1996-2014

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# **WinEDR V3.4.3**

## Electrophysiology Disk Recorder

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## Introduction > Main Features of WinEDR

WinEDR is a data acquisition and analysis program for handling signals from electrophysiological experiments. These may include whole-cell patch clamp experiments, single- and two-microelectrode voltage-clamp studies, or simple membrane potential recordings. Whole-cell signals are produced by the summation of currents through the (usually) large population of ion channels in the cell membrane, and thus consist of relatively smooth current or potential waveforms. The amplitude and time course of such signals contain information concerning the kinetic behaviour of the underlying ion channels, and other cellular processes, which can be extracted by the application of a variety of waveform analysis techniques.

WinEDR provides, in a single program, the data acquisition and experimental stimulus generation features necessary to make a digital recording of the electrophysiological signals, and a range of waveform analysis procedures commonly applied to such signals. WinEDR acts like a multi-channel digital tape recorder, collecting series of signals and storing them in a data file on magnetic disk. Its major features are

### Recording

- 1-16 analogue input channels.
- Continuous sampling-to-disc at rates up to 100 kHz.
- Stimulus generator.
- Real-time event frequency, resistance, capacity and fluorescence ratio measurement.

### Analysis

- Single-channel transition detection and amplitude/dwell time analysis.
- Event detection and frequency analysis
- Noise analysis
- Miniature synaptic current, ion channel current fluctuation and single-channel current simulations.

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## Introduction > Conditions of Use

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 > Hardware Requirements

To run WinEDR you will require an IBM PC-compatible personal computer with at least 16Mbyte of RAM, a 66MHz 80486 (or better) CPU, and the Microsoft Windows 95, 98, NT V4, 2000, XP (32 bit), Vista or Windows 7 (32 or 64 bit) operating systems. (Note. Windows 7 (64 bit) support not available for Digidata 1200 and 1320 interfaces.)

A laboratory interface unit is required to perform analog-digital (A/D) and digital-analog (D/A) conversion of the signals and stimulus waveforms. The following families of laboratory interfaces are supported:

- Cambridge Electronic Design 1401, 1401-plus, Micro 1401, Power 1401.
- National Instruments laboratory interface cards.
- Axon Instruments Digidata 1200, 1320 or 1440.
- Instrutech ITC-16 or ITC-18.
- Biologic VP500.
- Tecella Pico, Triton, Triton Plus.

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## Getting Started > Installing WinEDR

To install the WinEDR program on your computer:

- 1) Go to the web page <http://spider.science.strath.ac.uk/sipbs/page.php?page=software> and click the WinEDR Vxxx Setup File option to download the WinEDR installation program (WinEDR\_Vxxx\_Setup). Store this file in a temporary folder (e.g. c:\temp) on your computer.
- 2) Start the installation program by double-clicking the program **WinEDR\_Vx.x.x\_Setup**. The setup program creates the folder **c:\ WinEDR** and installs the WinEDR programs files within it. (You can change the disk drive and location of the WinEDR folder if you wish).
- 3) To start WinEDR, click the Microsoft Windows **Start** button and select **WinEDR Vx.x.x** from the **WinEDR** group in the **Programs** menu.
- 4) Install the laboratory interface unit with the appropriate device driver software and support library supplied with the device ([See Laboratory Interfaces](#)).
- 5) Configure WinEDR to work with laboratory interface.
- 6) Attach analog input/output signal cables between amplifier and laboratory interface (see section 4).

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## Getting Started > Laboratory Interfaces > National Instruments Interface Cards

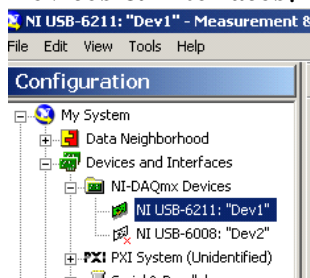
National Instruments Inc. ([www.ni.com](http://www.ni.com))

WinEDR is compatible with most National Instruments multifunction data acquisition cards or devices, including M, X, and E series cards, the Lab-PC/1200 series and USB devices. The PCI-6221 PCI card (with BNC-2110 I/O box and 2m SHC68-68-EPM cable) or the USB-6221-BNC device is currently recommended.

The National Instruments NIDAQ interface library must be installed before WinEDR can use the interface card. Most modern cards (X, M and E series) are supported via the NIDAQ-MX library. Older cards (Lab-PC/1200 series) require the 'Traditional' NIDAQ library (V4.9 or earlier) to be installed. WinEDR supports both types of library.

### Software installation

- 1) Install the NIDAQ library from the disks supplied with interface card, following the instructions supplied by National Instruments.
- 2) Install the interface card in an expansion slot (or attach a USB device).
- 3) Reboot the computer.
- 4) Run National Instruments' **Measurement & Automation Explorer** program. You should find the card listed under **Devices & Interfaces**. Note the Device number (Dev1, Dev2 etc.) of the card.



- 5) Right-click over the device and select **Self Test** to check that the device is functioning correctly.
- 6) If the tests check out OK, run WinEDR and select from the main menu

### Setup Laboratory Interface

to open the Laboratory Interface Setup dialog box



then select **National Instruments (NIDAQ-MX)** from the list of laboratory interface options (except for Lab-PC or 1200 series cards in which case use **National Instruments (NIDAQ Trad)**).

- 7) Select the device number of the card listed in the NI Devices & Interfaces list from the **Device** list (usually **Dev1** if only one card is installed).

- 8) Set the **A/D Input** mode. If you are using a BNC-2110 or BNC-2090 input/output box, select **Differential**. (**Note.** The **SE/DI** switches on a BNC 2090 panel must be set to **DI** ) If you are using a Lab-PC or 1200 series card, set the A/D Input mode to **Single Ended (RSE)**.

### *Signal input / output connections*

Signal input and output from National Instruments PCI cards are made via 68 pin sockets on the rear of the card attached to BNC socket input/output panels (BNC-2110 or BNC-2090 ) or screw terminal panels by appropriate shielded cables available from National Instruments.



### **National Instruments X, E & M Series cards**

<b>National Instruments X, E &amp; M Series cards</b>		
<b>Analog Input</b>	<b>I/O Panel</b>	<b>Screw terminal panel</b>
Ch. 0	ACH0	68, 67+62 (signal,ground)
Ch. 1	ACH1	33, 67+62
Ch. 2	ACH2	65, 67+62
Ch. 3	ACH3	30, 67+62
Ch. 4	ACH4	28, 67+62
Ch. 5	ACH5	60, 67+62
Ch. 6	ACH6	25, 67+62
Ch. 7	ACH7	57, 67+62
<b>Analog Output</b>		
Ch. 0	DACOUT 0	22,55
Ch. 1	DACOUT 1	21,55
<b>Trigger Inputs</b>		
Ext. Sweep Trigger	PFI0/TRIG1	11,44
Ext. Stimulus Trigger	PFI0/TRIG1 (NIDAQ-MX) PFI1/TRIG2 (NIDAQ)	11,44 (See Note 1) 10.44
<b>Digital Output</b>		
Ch. 0	DIO0	52,53

Ch. 1	DIO1	17,53
Ch. 2	DIO2	49,53
Ch. 3	DIO3	47,53
Ch. 4	DIO4	19,53
Ch. 5	DIO5	51,53
Ch. 6	DIO6	16,53
Ch. 7	DIO7	48,53

Note 1. An active-high TTL pulse on this input triggers the start a stimulus program which has been set up with the External Stimulus Trigger = Y option. The trigger signal is applied to **PF10** when the NIDAQ-MX interface library is in use (**Laboratory Interface Card = National Instruments (NIDAQ-MX)**) and **PF11** when the Traditional NIDAQ interface library (for Lab-PC/1200 cards only) is in use (**Laboratory Interface Card = National Instruments (NIDAQ)**)

### Lab-PC/1200 Series Cards

The input/output connections for 50 pin Lab-PC and 1200- series boards are tabulated below.

Lab-PC/1200 Cards		
Analog Inputs	I/O Panel	Screw terminal panel
Ch. 0	ACH0	1,9 (signal,ground)
Ch. 1	ACH1	2,9
Ch. 2	ACH2	3,9
Ch. 3	ACH3	4,9
Ch. 4	ACH4	5,9
Ch. 5	ACH5	6,9
Ch. 6	ACH6	7,9
Ch. 7	ACH7	8,9
Analog Outputs		
Ch. 0	DAC 0	10,11
Ch. 1	DAC 1	12,11 (See Note 1)
Trigger Inputs		
Ext. Sweep Trigger	EXTTRIG	38,50 (See Note 1)
Ext. Stimulus Trigger	PB7	29,50 (See Note 2)
Digital Synch. Input	PC6	36, 13 (See Note 1)
Digital Outputs		
Ch. 0	PA0	14,13

Ch. 1	PA1	15,13
Ch. 2	PA2	16,13
Ch. 3	PA3	17,13
Ch. 4	PA4	18,13
Ch. 5	PA5	19,13
Ch. 6	PA6	20,13
Ch. 7	PA7	21,13

NOTE 2 Analog output channel 1 (**DAC1**) is used to synchronise the start of the A/D conversion and D/A waveform generation and must be connected to **EXTTRIG** for WinEDR waveform generation functions to operate. In addition, if stimulus protocols containing digital outputs are required **DAC1 & EXTTRIG** must also be connected to the digital synchronisation input **PC6**.

Note. 2. Lab-PC/1200 series boards need the pulse to be 10 ms in duration or longer.

### ***Troubleshooting***

National Instruments cards can be used with a number of different types of input/output panels ( BNC 2090, BNC 2110 or CB-68 terminal panel) and can also be configured to handle the analog input channels in a number of different ways (differential, referenced single ended and non-referenced single ended). Some combinations of settings can lead to input signals drifting or going off scale.

**Differential mode (DIFF):** Analog input channels are paired together and subtracted (e.g. Ch.0 – Ch.7, Ch.1 – Ch.8 etc.).

**Referenced single ended mode (RSE):** Analog input channels are used individually and measured relative to signal ground of the computer.

**Non-referenced single ended mode (NRSE):** Analog input channels are used individually and measured relative to the electrical ground of the device being measured.

When using the BNC-2110 I/O box, the USB-6221-BNC or USB-6229-BNC USB interface device the WinEDR A/D Input Mode **must** be set at **Differential**.

When using the BNC-2090 I/O box with its SE/DI switches set to DI (the default setting) the WinEDR A/D Input Mode should be set to **Differential**.

When using the BNC-2090 I/O box with its SE/DI switches set to SE and the RSE/NRSE switch set to NRSE the WinEDR A/D Input Mode should be set at **Single Ended (NRSE)**

## Getting Started > Laboratory Interfaces > Axon Instruments Digidata 1200

Axon Instruments Inc.

(now owned by Molecular Devices, [www.moleculardevices.com](http://www.moleculardevices.com))

The Digidata 1200, 1200A and 1200B interface boards fully supports all WinEDR features. They have a 330 kHz maximum sampling rates and 4 programmable input voltage ranges (10V, 5V, 2.5V, 1.25V). Inputs to and outputs from the board are via BNC connectors on an I/O box, connected to the board via a shielded ribbon cable. In order to use WinEDR with a Digidata 1200, the following computer system resources must be available for use by the Digidata 1200.

- I/O port address 320-33F (Hex)
- DMA channels 5 and 7

### *Software Installation*

- 1) Install the Digidata 1200 card into an ISA computer expansion slot, and attach it to its BNC I/O panel using the shielded ribbon cable supplied with the card.
- 2) Install the WinEDR Digidata 1200 driver software for your Windows operating system, by running the appropriate installation batch file.

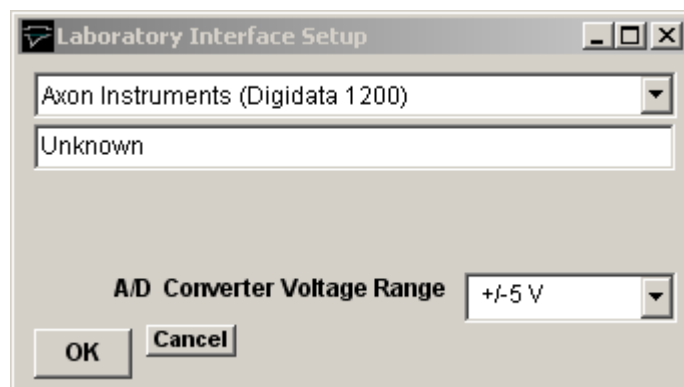
If you are running Windows 95, 98 or Me, select **WinEDR\Digidata 1200 Drivers\Install Digidata 1200 driver (Win95/98/Me)** from the Programs menu.

If you are running the Windows NT, 2000 or XP, select **WinEDR\Digidata 1200 Drivers\Install Digidata 1200 driver (Win NT/2000/XP)**. (Note. WinEDR does not use the standard Axon Instruments Digidata 1200 device driver).

- 3) Reboot the computer.
- 4) Run WinEDR and select from the main menu

### **Setup Laboratory Interface**

to open the Laboratory Interface Setup dialog box



then select **Axon Instruments (Digidata 1200)** from the list of laboratory interface options.

### *Signal input / output connections*

Signal input and output connections are made via the BNC sockets on the front and rear of the Digidata 1200 I/O box.

<b>Digidata 1200</b>		
<b>Analog Input</b>	<b>I/O Panel</b>	<b>Notes</b>
Ch. 0	Analog In 0	
Ch. 1	Analog In 1	
Ch. 2	Analog In 2	
Ch. 3	Analog In 3	
Ch. 4	Analog In 4	
Ch. 5	Analog In 5	
Ch. 6	Analog In 6	
Ch. 7	Analog In 7	
<b>Analog Output</b>		
Ch. 0	Analog Out 0	
Ch. 1	Analog Out 1	
<b>Trigger Inputs</b>		
Ext. Sweep Trigger	Gate 3	
Ext. Stimulus Trigger	Gate 3	See Note 1
<b>Digital Output</b>		
Ch. 0	Digital Out 0	See Note 2
Ch. 1	Digital Out 1	
Ch. 2	Digital Out 2	
Ch. 3	Digital Out 3	

Note 1. An active-high TTL pulse on this input triggers the start a stimulus program which has been set up with the External Stimulus Trigger = Y option.

Note 2. WinEDR only supports digital output lines 0-3 of the Digidata 1200.

## ***Troubleshooting***

There are two known problems which will prevent WinEDR from recording from a Digidata 1200's analog input channels.

**I/O port conflict.** The Digidata 1200 default I/O port addresses span the range 320H-33AH. These settings conflict with the default MIDI port setting (330H) of Creative Labs. Sound-Blaster 16 and similar sound cards. There are a number of solutions to this problem.

- 1) Change the Sound-Blaster MIDI port setting to a value higher than 33AH.
- 2) Remove the Sound-Blaster card (or disable it using the BIOS setup if it is built in to the computer motherboard).

**DMA channel conflicts.** WinEDR requires DMA channels 5 and 7 to support the transfer of data to/from PC memory

and the Digidata 1200. Many sound cards also make use of DMA 5 and can interfere with the operation of the Digidata 1200.



## Getting Started > Laboratory Interfaces > Cambridge Electronic Design 1401 Series

Cambridge Electronic Design Ltd. ([www.ced.co.uk](http://www.ced.co.uk))

The CED 1401 series consists of an external microprocessor-controlled programmable laboratory interface units attached to the PC via a digital interface card or USB. There are 4 main types of CED 1401 in common use - CED 1401, CED 1401-plus, CED Micro-1401 and CED Power-1401. They are all fully supported WinEDR with the exception that only 4 analog input channels are available on the Micro1401 and that the maximum sampling rate and number of samples/sweep for the standard CED 1401 is substantially less than the others.

### Software installation

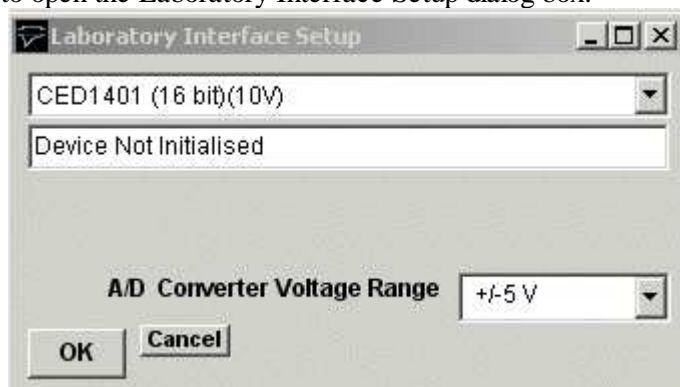
Before WinEDR can use these interface units, the CED 1401 device driver (CED1401.SYS), support library (USE1432.DLL), and a number of 1401 command files stored in the directory \1401 must be installed on the computer.

The installation procedure is as following, but see CED documentation for details.

- 1) Install the CED interface card in a PC expansion slot and attach it to the CED 1401 via the ribbon cable supplied (or attach to USB port for USB versions).
- 2) Download the CED 1401 Standard Windows Installer program (WINSUPP.EXE) from the CED web site (<http://www.ced.co.uk/upu.shtml>) and run it to install the CED1401.SYS device driver and 1401 commands.
- 3) Ensure that the CED 1401 is switched on, and then reboot your computer.
- 4) Test the CED interface by running the program.  
**c:\1401\utils\try1401w.exe**  
and clicking the button  
**Run Once**
- 5) If the tests check out OK, run WinEDR and select from the main menu

### Setup/Laboratory Interface

to open the Laboratory Interface Setup dialog box.



If you have a CED 1401 with standard analog  $\pm 5V$  output voltage range, select **CED 1401 (16bit)(5V)** from the list of laboratory interface options. If you have a CED 1401 with a  $\pm 10V$  output voltage range, select **CED 1401 (16bit)(10V)**.

### Signal input / output connections

Analog signal I/O connections are made via BNC sockets on the front panel of the CED 1401 units.

<b>CED 1401 Series</b>
------------------------

Analog Input	I/O Panel	Notes
Ch. 0	ADC Input 0	
Ch. 1	ADC Input 1	
Ch. 2	ADC Input 2	
Ch. 3	ADC Input 3	
Ch. 4	ADC Input 4	ex. Micro1401
Ch. 5	ADC Input 5	ex. Micro1401
Ch. 6	ADC Input 6	ex. Micro1401
Ch. 7	ADC Input 7	ex. Micro1401
<b>Analog Output</b>		
Ch. 0	DAC Output 0	
Ch. 1	DAC Output 1	
Sync. Out	DAC Output 2	CED 1401 Only (See Note 1)
<b>Trigger Inputs</b>		
Ext. Sweep Trigger	Event Input 4 Trigger In	CED 1401, CED 1401-plus Micro 1401, Power 1401
Ext. Stimulus Trigger	Event Input 0	
Digital Gate I/P	Event Input 2	
<b>Digital Output</b>		<b>25 way D socket</b>
Ch. 0	Digital Out 8	17,13 (signal,ground)
Ch. 1	Digital Out 9	4,13
Ch. 2	Digital Out 10	16,13
Ch. 3	Digital Out 11	3,13
Ch. 4	Digital Out 12	15,13
Ch. 5	Digital Out 13	2,13
Ch. 6	Digital Out 14	14,13
Ch. 7	Digital Out 15	1,13

#### NOTE1 STANDARD CED 1401 ONLY

Events Inputs 2, 3, and 4 must be connected together and connected to DAC Output 2, to synchronise A/D sampling, D/A waveform generation and digital pulse output for WinEDR's Seal Test option and recording with stimulus pulse protocols.

Note 2. A TTL pulse on the **Ext. Sweep Trigger** input triggers the start of a recording sweep when **Ext Trigger** sweep trigger mode has been selected.

Note 3. An active-high TTL pulse on the **Ext. Stimulus Trigger** input triggers the start a stimulus program which has been set up

with the **External Stimulus Trigger = Y** option.

### ***Troubleshooting tips***

Verify that the CED 1401 is working correctly, before investigating problems using WinEDR. Use the TRY1401W program to test the CED 1401.

WinEDR uses the commands, ADCMEMI.CMD, MEMDACI.CMD and DIGTIM.CMD with the CED 1401; ADCMEM.GXC, MEMDAC.GXC and DIGTIM.GXC with the CED 1401-plus; and ADCMEM.ARM, MEMDAC.ARM and DIGTIM.ARM with the CED Micro-1401. All three commands must be available within the \1401 directory.

### **Power 1401**

The digital pattern output command DIGTIM appears to behave differently on different versions of the Power 1401 resulting in the the digital output waveforms in a WinEDR stimulus protocol being produced incorrectly (On/Off levels are inverted and pulse durations are incorrect). This problem can be corrected by setting the **CEDPOWER1401DIGTIMCOUNTSHIFT** entry in the WinEDR **lab interface.xml** file in c:\WinEDR to 0.

Stop the WinEDR program (if it is running) and open the file **c:\WinEDR\lab interface.xml** with the Notepad text editor and search for the entry:

```
CEDPOWER1401DIGTIMCOUNTSHIFT>1</CEDPOWER1401DIGTIMCOUNTSHIFT
```

Change it to:

```
CEDPOWER1401DIGTIMCOUNTSHIFT>0</CEDPOWER1401DIGTIMCOUNTSHIFT
```

and save the file.

### **Standard CED 1401**

The performance of the original CED 1401 interface unit is very limited compared to later models. The number of samples/record is limited to a total of 8192 and the achievable sampling rate when stimulus pulses are being generated is limited to a maximum of around 20 kHz divided by the number of channels.

In some circumstances, the sampling rate set by WinEDR can exceed the capabilities of the standard 1401, resulting in samples between mixed up between channels. This problem can be resolved by reducing the number of samples per record or by increasing the duration of the recording sweep.

The CED 1401 ISA card default I/O port addresses are at 300H. Check that these do not conflict with other cards within the computer. The CED 1401 also makes use of DMA channel 1 and an IRQ channel (IRQ2). These may also conflict with other cards.

Some standard 1401 appear to fail the DMA (direct memory access) test in TRY1401W and this also causes problems when running WinEDR. If this error occurs, disable the DMA channel, by clicking on the **CED 1401** icon within the Windows Control Panel and un-checking the **Enable DMA transfers** check box.

## Getting Started > Laboratory Interfaces > Axon Instruments Digidata 1320

Axon Instruments Inc. (now owned by Molecular Devices, [www.moleculardevices.com](http://www.moleculardevices.com))

The Digidata 1320 Series (1320A, 1322) interfaces consist of self-contained, mains-powered digitiser units with BNC I/O sockets attached to the host computer via a SCSI (Small Computer Systems Interface) interface card and cable. A number of versions are available including the 1320A and 1322A. The 1322A supports sampling rates up to 500 kHz (16 bit resolution) on up to 16 channels. It has a fixed input and output voltage range of 10V and supports 4 digital output channels..

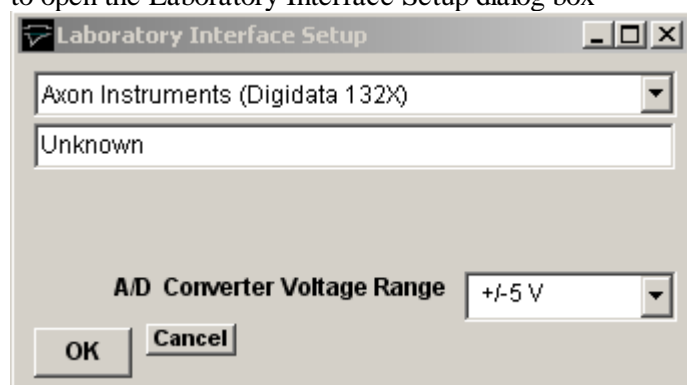
### Software Installation

WinEDR uses Axon's standard software library (AxDD132x.DLL) for the Digidata 1320 Series. Details for steps (1)-(5) can be found in Axon's Digidata 1320 Series Operator's Manual.

- 1) Install the Axon SCSI card in a PCI expansion slot.
- 2) Attach the Digidata 1320 to the SCSI card and switch on the computer and 1320.
- 3) Install the AxoScope software supplied with the Digidata 1320.
- 4) Reboot the computer.
- 5) Run AxoScope to ensure that the software installed OK.
- 6) Run WinEDR and select from the main menu:

### Setup Laboratory Interface

to open the Laboratory Interface Setup dialog box



then select **Axon Instruments (Digidata 132X)** from the laboratory interface list.

### Signal input / output connections

Signal input and output connections are made via the BNC sockets on the front of the Digidata 1320 Series digitiser unit.

Digidata 132X Series		
Analog Input	I/O Panel	Notes
Ch. 0	Analog In 0	
Ch. 1	Analog In 1	
Ch. 2	Analog In 2	

Ch. 3	Analog In 3	
Ch. 4	Analog In 4	
Ch. 5	Analog In 5	
Ch. 6	Analog In 6	
Ch. 7	Analog In 7	
<b>Analog Output</b>		
Ch. 0	Analog Out 0	
Ch. 1	Analog Out 1	
<b>Trigger Inputs</b>		
Ext. Sweep Trigger	Trigger In Start	
Ext. Stimulus Trigger	Trigger In Start	See Note 1
<b>Digital Output</b>		
Ch. 0	Digital Out 0	See Note 2
Ch. 1	Digital Out 1	
Ch. 2	Digital Out 2	
Ch. 3	Digital Out 3	

Note 1. An active-high TTL pulse on this input triggers the start a stimulus program which has been set up with the External Stimulus Trigger = Y option.

Note 2. The Digidata 1320 Series only supports 4 digital output lines.

### ***Troubleshooting***

When multiple analog input channels are being sampled and the sampling interval is greater than 10 ms, samples get mixed up between channels. This problem can be seen to occur also with AxoScope, suggesting a bug in the Digidata 1320 firmware or AXDD132X.DDL library. The only limited solution at present is to increase the number of samples per record to ensure that the sampling interval is less than 10 ms.

## Getting Started > Laboratory Interfaces > Molecular Devices Digidata 1440A

Molecular Devices Corporation ([www.moleculardevices.com](http://www.moleculardevices.com))

The Digidata 1440A interface consists of self-contained, mains-powered digitiser unit with BNC I/O sockets attached to the host computer via a USB 2.0 port. The 1440A supports sampling rates up to 250 kHz (16 bit resolution) on up to 16 channels. It has a fixed input and output voltage range of 10V and supports 4 analog output channels and 8 digital output channels.

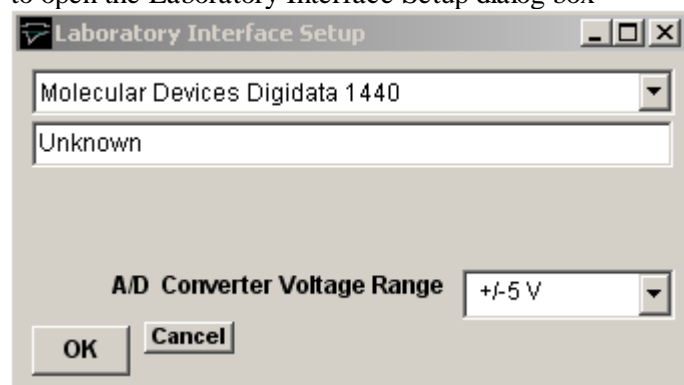
### Software Installation

WinEDR uses Axon's standard software library (AxDD1400.DLL) for the Digidata 1400 Series. Details for steps (1)-(5) can be found in Axon's Digidata 1440A Manual.

- 1) Install the AxoScope (or PCLAMP ) software supplied with the Digidata 1440.
- 2) Reboot the computer.
- 3) Attach the Digidata 1440A to a USB port and turn it on.
- 4) Run WinEDR and select from the main menu

#### Setup Laboratory Interface

to open the Laboratory Interface Setup dialog box



then select **Molecular Devices (Digidata 1440)** from the laboratory interface list.

### Signal input / output connections

Signal input and output connections are made via the BNC sockets on the front of the Digidata 1440A digitiser unit.

Digidata 1440A		
Analog Input	I/O Panel	Notes
Ch. 0	Analog Input 0	
Ch. 1	Analog Input 1	
Ch. 2	Analog Input 2	
Ch. 3	Analog Input 3	

Ch. 4	Analog Input 4	
Ch. 5	Analog Input 5	
Ch. 6	Analog Input 6	
Ch. 7	Analog Input 7	
<b>Analog Output</b>		
Ch. 0	Analog Out 0	
Ch. 1	Analog Out 1	
Ch. 2	Analog Out 2	
Ch. 3	Analog Out 3	
<b>Trigger Inputs</b>		
Ext. Sweep Trigger	Start	
Ext. Stimulus Trigger	Start	See Note 1
<b>Digital Output</b>		
Ch. 0	Digital Output 0	
Ch. 1	Digital Output 1	
Ch. 2	Digital Output 2	
Ch. 3	Digital Output 3	
Ch. 4	Digital Output 4	
Ch. 5	Digital Output 5	
Ch. 6	Digital Output 6	
Ch. 7	Digital Output 7	

Note 1. An active-high TTL pulse on this input triggers the start of a stimulus program which has been set up with the External Stimulus Trigger = Y option.

## Getting Started > Laboratory Interfaces > Instrutech ITC-16/18

Instrutech Corp [www.instrutech.com](http://www.instrutech.com) (now handled by Heka Elektronik GmbH)

The Instrutech ITC-16 and ITC-18 interfaces are self-contained, 19" rack-mountable, mains-powered digitiser units with BNC I/O sockets attached to the host computer via a digital interface card and cable. Both the ITC-16 and ITC-18 support 8 analog input channels, 4 analog outputs and 8 digital outputs.

Note. The Instrutech ITC-1600, LIH8+8 and USB-18 are not currently supported by WinEDR.

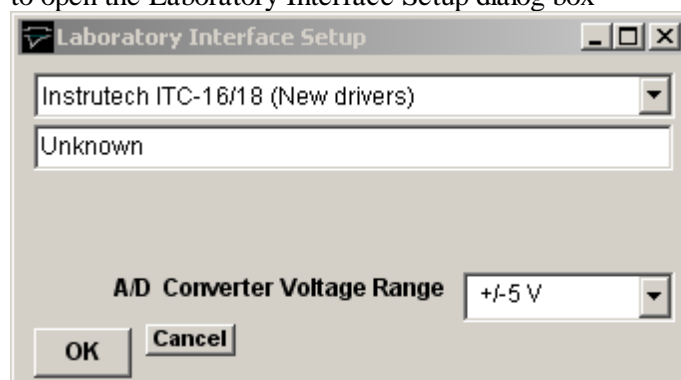
### *Software installation*

WinEDR uses the Instrutech device interface libraries for the ITC-16/18 family. Details for steps (1)-(3) can be found in the Instrutech Data Acquisition Interface user manual.

- 1) Install the Instrutech interface card in an expansion slot.
- 2) Attach the ITC-16 or ITC18 unit to the card.
- 3) Install the Instrutech Device Driver software supplied with the card (or downloaded from [www.instrutech.com](http://www.instrutech.com))
- 4) Reboot the computer.
- 5) Run the Instrutech test program installed with the device driver to test whether the software installed OK.
- 6) Run WinEDR and select from the main menu

### **Setup Laboratory Interface**

to open the Laboratory Interface Setup dialog box



then select **Instrutech ITC-16/18 (New driver)** from the laboratory interface options list. Note, on systems with Instrutech's older device driver software installed, it may be necessary to select either **Instrutech ITC-16 (Old Driver)** or **Instrutech ITC-18 (Old Driver)** depending upon which interface unit is installed.

### *Instrutech ITC-16/18: I/O Panel Connections*

Signal input and output connections are made via the BNC sockets on the front of the ITC-16/18 unit.

Instrutech ITC-18		
Analog Input	I/O Panel	Notes
Ch. 0	ADC Input 0	
Ch. 1	ADC Input 1	
Ch. 2	ADC Input 2	



Ch. 3	ADC Input 3	
Ch. 4	ADC Input 4	
Ch. 5	ADC Input 5	
Ch. 6	ADC Input 6	
Ch. 7	ADC Input 7	
<b>Analog Output</b>		
Ch. 0	DAC Output 0	
Ch. 1	DAC Output 1	
Ch. 2	DAC Output 2	
Ch. 3	DAC Output 3	
<b>Trigger Inputs</b>		
Ext. Sweep Trigger	Trig In	
Ext. Stimulus Trigger	Trig In	See Note 1
<b>Digital Output</b>		
Ch. 0	TTL Output 0	
Ch. 1	TTL Output 1	
Ch. 2	TTL Output 2	
Ch. 3	TTL Output 3	

Note 1. An active-high TTL pulse on this input triggers the start of a stimulus program which has been set up with the External Stimulus Trigger = Y option.

### ***Instrutech ITC-16/18: Troubleshooting***

WinEDR requires Instrutech's combined device driver library ITCMM.DLL (released late 2001). It may not work with earlier libraries.

## Getting Started > Laboratory Interfaces > Biologic VP500

Bio-Logic - Science Instruments SA ([www.bio-logic.fr](http://www.bio-logic.fr))

The Biologic VP500 is a computer-controlled patch clamp with a built-in laboratory interface unit, attached to the host computer via a GPIB interface bus. It is supported under Windows 95/98, NT and 2000. The VP500 patch clamp functions (gain, filtering, capacity compensation, etc.) can be controlled from a virtual front panel within WinEDR.

The current implementation of the WinEDR software supports

- 2 analog input channels (membrane current and voltage)
- Command voltage output

### *Software installation*

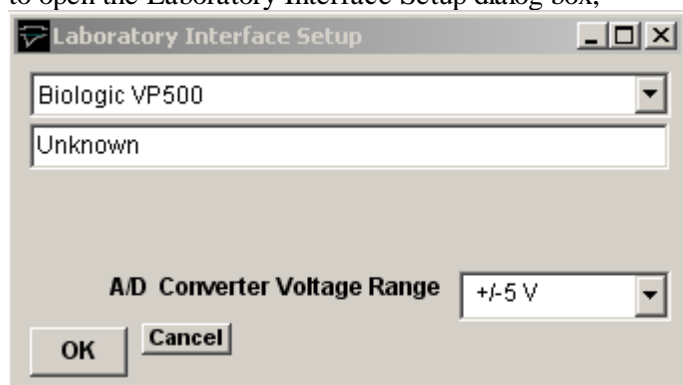
WinEDR uses Biologic's BLVP500.DLL library (supplied with WinEDR) to control and acquire data from the VP500.

- 1) Install the National Instruments NIDAQ software, supplied with the GPIB interface card and reboot.
- 2) Install the GPIB card into the host computer and reboot.
- 3) Check using the National Instruments Measurement & Automation Explorer program that the GPIB has been detected and is functioning correctly.
- 4) Run WinEDR and select from the main menu

#### **Setup**

#### **Laboratory Interface**

to open the Laboratory Interface Setup dialog box,



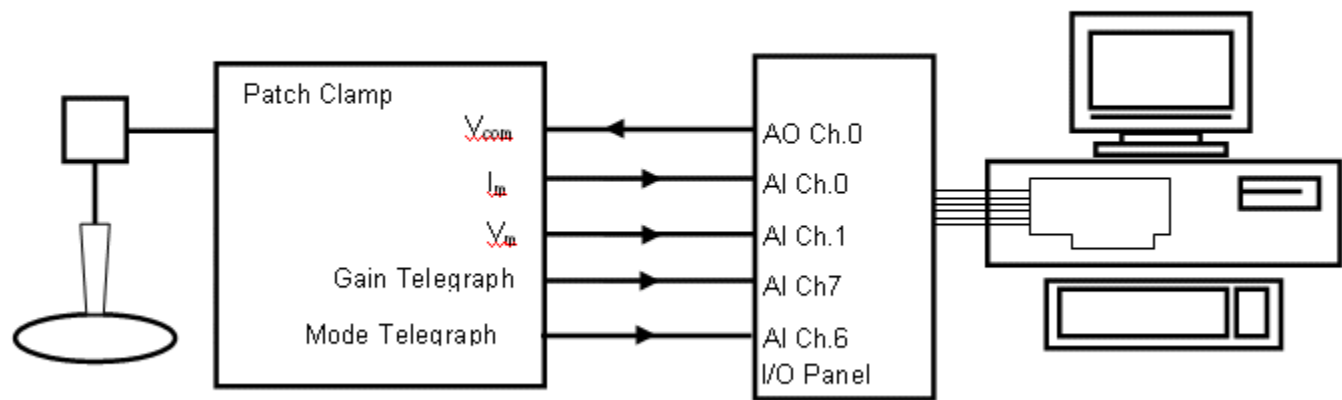
then select **Biologic VP500** from the laboratory interface options list.

### *Biologic VP500: I/O Panel Connections*

No I/O panel connections are necessary. All connections between patch clamp and laboratory interface are internal to the VP500.

Getting Started > Amplifiers > Patch/Voltage-clamp Amplifiers

One of the most common WinEDR applications is recording from and controlling a whole-cell patch- or voltage-clamp experiment. Two analog channels are normally recorded by WinEDR (membrane current and voltage), and computer-generated voltage pulses are applied to the patch clamp command voltage input to stimulate the cell.



WinEDR supports up to 4 patch/voltage-clamp amplifiers and, for each amplifier in use, up to 5 analog signal connections must be made between the amplifier and laboratory interface.

A pair of laboratory interface analog input channels (designated the **primary** and **secondary** input channels) are required to record the membrane current ( $I_m$ ) and membrane voltage ( $V_m$ ) outputs from the amplifier.

An analog output must be connected to the amplifier command voltage input ( $V_{com}$ ), to provide current/voltage stimulus waveforms. Two additional analog inputs may be required to receive the amplifier gain (GAIN) and voltage/current clamp mode (MODE) telegraph signals (Note. Some patch clamps do not support gain and/or mode telegraphs, others communicate gain/mode information via USB or other communications lines.)

WinEDR supports many of the commonly used models of patch- and voltage-clamp amplifiers and is able to read gain and mode telegraph signals allowing the current and voltage signal channels factors to be scaled correctly.

The required primary and secondary input channel, command voltage output, gain and mode telegraph signals connections for the amplifiers currently supported by WinEDR are shown in the table on the following page.

[Next](#)

## Getting Started > Amplifiers > Signal Connections Table

The following table shows signal connections the required for the WinEDR-supported amplifiers.

Amplifier outputs/input in panel (A) are connected to the laboratory interface input/outputs in the corresponding column in panel (B).

A)	Amplifier Inputs/Outputs				
Type of Amplifier	Primary Channel	Secondary Channel	MODE	GAIN	Vcom
Manual gain entry	Im Out	Vm Out	-	-	Vcommand In
Axopatch 1D	Scaled Output	10 Vm	-	Gain Telegraph	Ext. Command
Axopatch 200	Scaled Output	10 Vm (VC mode) Im (CC mode) <b>See Note.1</b>	Mode Telegraph	Gain Telegraph	Ext.Command (front switched)
Multiclamp 700A	Scaled Output	Raw Output	-	- <b>See Note 2</b>	Ext.Command
Multiclamp 700B	Primary Output	Secondary Output	-	- <b>See Note 2</b>	Ext. Command
RK400	Iout	10 x Vm	-	-	Voltage Command Input
VP500	-	-	-	-	-
Heka EPC-7	Current Monitor	Vcomm Monitor	-	-	Stim Input X10
Heka EPC-8	Current Monitor	Vcomm Monitor	-	See Note 3.	Stim Input X10
Heka EPC-800	Current Monitor	Vcomm Monitor	Mode Telegraph	Gain Telegraph	Stim Input X10
Cairn Optopatch	Gain Out	Command X10 Out	Pin 9 Pin 2 Gnd 37 way D socket	Gain Telegraph Out	Command /10 In
Warner PC501A	Im	Vm x10	-	Gain Telegraph	CMD In
Warner PC505B	Im	Vm x10	-	Gain Telegraph	Command In
Warner OC725	I Monitor	Vm x10	-	Gain Telegraph	Command In ÷10
NPI SEC05LX	Current Output	Potential Output	-	Curr. Sensitivity Monitor	VC Command Input /10
AM Systems 2400	Output	X10 Vm	Mode Telegraph	Gain Telegraph	External ÷50
Tecella	-	-	-	-	-

B)	<b>Default Laboratory Interface Inputs/Outputs</b>				
Amplifier #1	AI Ch.0	AI Ch.1	AI Ch.6	AI Ch.7	AO Ch.0
Amplifier #2	AI Ch.2	AI Ch.3	AI Ch.14	AI Ch.15	AO Ch.1
Amplifier #3	AI Ch.4	AI Ch.5	AI Ch.13	AI Ch.12	AO Ch.2
Amplifier #4	AI Ch.6	AI Ch.7	AI Ch.10	AI Ch.11	AO Ch.3

**Note 1.** When the Axopatch 200 is switched from voltage- to current-clamp mode, the Scaled Output signal to the primary channel (AI Ch.0 for Amplifier #1) changes from membrane current to voltage. To retain a current signal, the secondary channel (AI Ch.1 for Amplifier #1) of WinEDR must be switched manually from the Axopatch 200 10 Vm to the Axopatch 200 Im output (on the rear panel).

**Note 2.** Axon Multiclamp amplifiers support two separate amplifier channels (1 & 2). When using amplifier Ch.1, select Multiclamp 700A/B as Amplifier #1 and connect the Ch.1 Scaled and Raw/Secondary outputs to AI Ch.0 and AI Ch.1, respectively, and AO Ch.0 to Ch.1 EXT COMMAND. When using both channels, select Multiclamp 700A/B also as Amplifier #2 and connect the Ch.2 Scaled and Raw/Secondary outputs to AI Ch.2 and AI Ch.3 and AO Ch.1 to Ch.2 EXT COMMAND. (Axon Multiclamp amplifiers can only be selected as WinEDR Amplifiers #1 and #2.)

The Axon Multiclamp Commander software must be started up and running **before** WinEDR is started.

**Note 3.** The Heka EPC-8 gain and mode telegraphs are TTL digital signals provided via a 50 way IDC ribbon cable on the rear of the EPC-8. The signals in the table below must be connected to the digital inputs of the laboratory interface.

Heka EPC-8 Digital gain telegraph connections						
	Gain 0	Gain 1	Gain 2	Range 0	Range 1	Gnd
EPC-8 50 way IDC socket	5	7	9	1	17	19
Digital Input Channel	0	1	2	3	4	Gnd

[Next](#)

## Getting Started > Amplifiers > Configuring Amplifier Support in WinEDR

To configure amplifier support select

### Setup

#### Input Channels & Amplifiers

to open the Input Channels & Amplifiers Setup dialog box.

**Input Channels & Amplifiers Setup**

**Input Channels** | **Amplifiers**

**Amplifier #1** | **Amplifier #2** | **Amplifier #3** | **Amplifier #4**

**Axopatch 200**

**Input Channels**  
☒ VClamp ☐ IClamp

**Primary channel (Scaled Output)**  
Scale factor: **0.0005 V/pA** Units: **pA**  
On analog input: **AI Ch.0**

**Secondary channel (10 Vm)**  
Scale factor: **0.01 V/mV** Units: **mV**  
On analog input: **AI Ch.1**

**Voltage-clamp command channel**  
Scale factor: **0.02 V/V** Output: **AO Ch.0**

**Current-clamp command channel**  
Scale factor: **2E-009 A/V** Output: **AO Ch.0**

**Telegraph channels**  
Gain: **AI Ch.7**  
Voltage/current clamp mode: **AI Ch.6**

**Load Settings** **Save Settings** **Default Settings**

Select the **Amplifiers** tab and choose the amplifier to be configured (**Amplifier #1** if only one amplifier is in use), then select your type of amplifier in the amplifier list.

The amplifier configuration table shows the default primary and secondary input channels, voltage and current command output channels and gain and mode telegraph input channels (if these are required by the amplifier).

The basic (at minimum gain) scaling factors and units for the current and voltage outputs and the current and voltage command inputs are also displayed.

If required, the **Gain** and **Voltage/current clamp mode** telegraph inputs can be changed to another analog input channel. However, if this is done, the physical connections between the amplifier and the laboratory interface must be adjusted to match. Telegraphs can also be disabled by selecting **Off** as the input channel. When telegraphs are disabled, gain and/or voltage/current clamp mode can be entered manually by the user.

If necessary, primary and secondary channel scaling factors, units, voltage- and current-clamp command scaling factors can be altered by the user. This is most likely to be case for the command scaling factors where where a range of division factors are available on most common patch clamp amplifiers. The default settings are set to the most commonly

used stimulus scalings (0.02 or 0.1) for the amplifier. In the case of amplifiers where the primary/secondary channels switch output signals in current- or voltage-clamp mode, two different primary and secondary channel scaling factors can be entered, by selecting the ICLAMP or VCLAMP options.

Note that, in the case of amplifiers

## Manual amplifier configuration

If the amplifier in use is not specifically supported by WinEDR, current and voltage scaling information can still be entered manually by the user. To enter up a manual amplifier configuration, select

### Manual Gain Entry

as the amplifier type and enter the following information.

#### Primary Channel (Im)

The amplifier membrane current output should be connected to this input channel (AI Ch.0 for Amplifier#1). Enter the measurement units of the current (pA, nA, uA, mA, A) into the **Units** box and the amplifier current scaling factor of the amplifier at minimum gain in V/units into the **Scale factor** box. The scaling factor can typically be obtained by reading the minimum gain setting of the rotatable current gain switch, found on most patch-clamp amplifiers (e.g. a minimum gain settings of 1mV/pA is equivalent to a scaling factor of 0.001V/pA.)

#### Secondary Channel (Vm)

The amplifier membrane potential output should be connected to this input channel (AI Ch.1 for Amplifier#1). Enter the measurement units of the current (mV) in the **Units** box and the amplifier voltage scaling factor, in V/Units, into the **Scale factor** box. Most amplifiers have a fixed scaling factor of 0.01 V/mV.

#### Voltage-clamp command channel

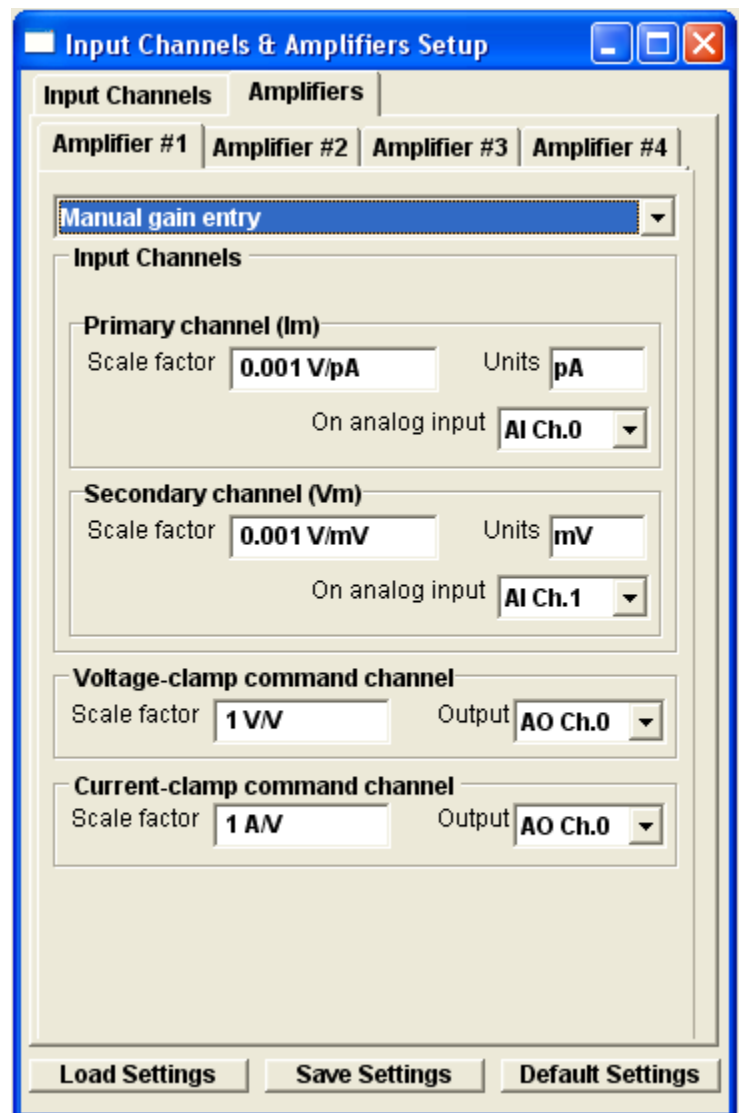
Analog output (AO Ch.0 for Amplifier#1) should be connected to the amplifier command voltage input. Enter the voltage-clamp command potential scaling factor (membrane potential/command voltage) into the **Scale factor** box. (A label specifying the command voltage scaling factor can usually be found beside the command voltage input socket on the amplifier. Typical values are 0.1 V/V or 0.02 V/V)

#### Current-clamp command channel

Enter the current-clamp command potential scaling factor (membrane current/command voltage) into the **Scale factor** box. The current clamp command scaling factor can usually be found in the amplifier user manual. Typical values are between  $10^{-9}$  A/V and  $10^{-10}$  A/V. (Usually the current command signal is applied to the same input on the amplifier as the voltage command (AO Ch.0 for Amplifier#1), however on some there may be a separate current command input.)

### Saving/Loading Settings

Amplifier and input channel settings can be saved to a settings file by clicking the **Save Settings** button and saving the settings to an XML settings file. Settings can be reloaded from a settings file by clicking **Load Settings** and selecting



the file. The default scaling and channel settings for an amplifier can be reset by clicking the **Restore Default Settings** button. Default settings are also loaded whenever the amplifier type is changed.

[Next](#)



## Getting Started > Amplifiers > Channel Calibration Table

WinEDR displays the signals stored in each signal channel in the units appropriate to each channel. The names, units and scaling information for each channel are entered into the channel calibration table which can be displayed by selecting the **Input Channels** tab in the Input Channels & Amplifiers dialog box.

The channel calibration table settings for the patch clamp current and voltage channels are automatically configured with appropriate names, units and V/Units scaling when an amplifier is selected from the **Amplifiers** list. However, when no amplifier is selected for use (i.e. Amplifier=None) the calibration information for a channel must be entered directly into the table.

There are 5 entries in the table for each channel.

**Name:** A 1-4 letter name used to identify the source of the channel (e.g. Vm, Im).

**V/Units:** The scaling factor relating the voltage level at the inputs of the A/D converter (in V) to the actual signal levels in each channel (in the units defined in the **Units** column).

**Units:** The measurement units of the signal (e.g. mV, pA etc.).

**ADC Ch.:** The analog/digital converter input channel from which the signal for this channel is being acquired. (Note. Signal channel to analog input mapping is currently only available with National Instruments interface cards.)

**Amplifier:** Indicates whether an amplifier has been defined for this channel.

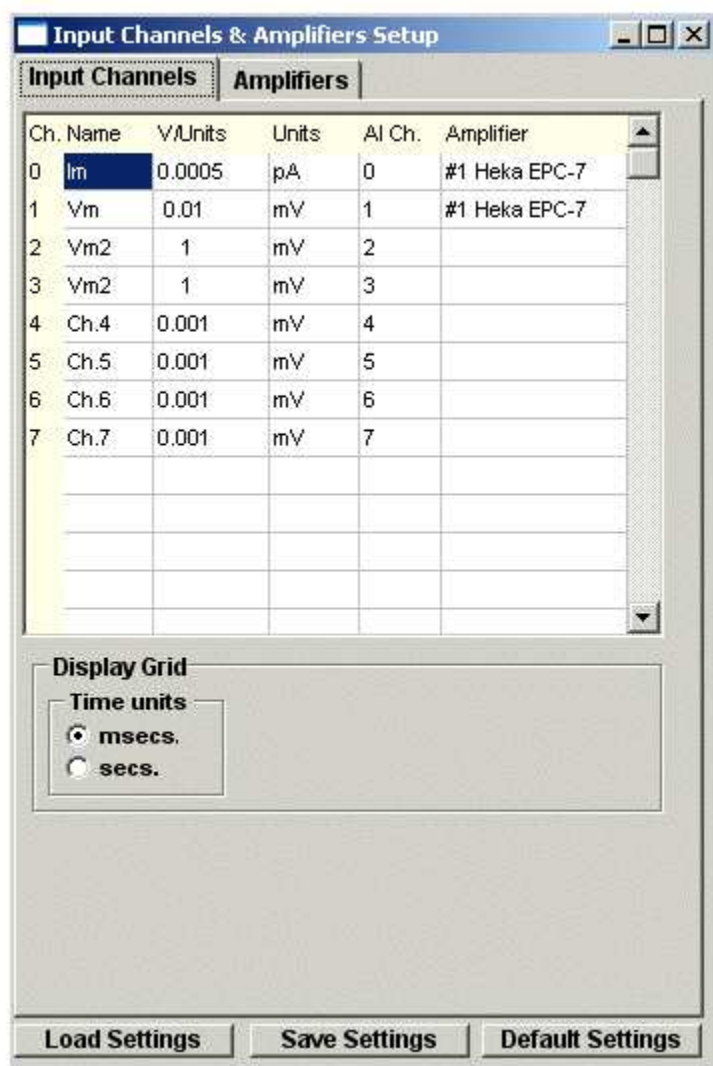
For example, if the membrane voltage output of your amplifier supplies a signal which is 10X the measured membrane potential of the cell, and the units have been defined as mV, then the appropriate V/Units setting is 0.01 (since the amplifier voltage output is 0.01 Volts per mV).

Amplifier

### Display Grids and Time Units

The **Time Units** options determine the units (secs or msecs) used to display time intervals in signal display windows.

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## Getting Started > Amplifiers > CED 1902 Amplifier

Cambridge Electronic Design Ltd ([www.ced.co.uk](http://www.ced.co.uk))

The Cambridge Electronic Design 1902 is a computer-controlled amplifier with built-in isolation circuits for recording ECG and EMG signals from humans, and a bridge circuit for recording from tension or pressure transducers. It can also be used to record extracellular electrical activity from nerve and muscle. It has two inputs.

- The **Electrodes** input is an electrically isolated differential amplifier input used to record ECG, EMG and similar signals. Isolation makes it safe to attach recording leads to human subjects.
- The **Transducer** input is a differential amplifier input used to record from transducers such as force and pressure transducers. It is not isolated.

### Connections

In order to control the amplifier, the CED 1902 serial communications cable must be connect to a serial port (COM1 or COM2). The analog output of the CED 1902 should also be connected to analog input Ch.0 of the laboratory interface. The amplifier gain setting is taken into account by WinEDR in scaling the signal level on this channel.

### Control Panel

Select

#### Setup

#### CED 1902 Amplifier

to open CED 1902 control panel



The following amplifier settings can be configured using the panel:

**COM Port:** Selects the serial port used to communicate with the 1902.

**Input:** Selects the amplifier input; **Grounded** to connect the input to ground (i.e. there is no signal.), **Single Ended** for the transducer input in singled ended (i.e. non-differential mode), **Normal Diff+** for the Transducer input in differential amplifier mode, **Inverted Diff-** for the transducer input in differential mode with the signal inverted, or **Isolated EEG** for the electrically isolated differential input.

Note. **Normal Diff+** mode is used when recording from a force or pressure transducer and **Isolated EEG** used when making EMG, ECG recordings.

**Amplifier Gain:** Sets the gain of the CED 1902 amplifier. The range of gain settings depends on which input is in use. When using the Transducer input there are 11 gain settings (**X1, X3, X10, X30, X100, X300, X1000, X3000, X10000, X30000, X100000**). When using the Electrodes input (**X100, X300, X1000, X3000, X10000, X30000, X100000, X300000, X1000000, X3000000, X10000000**). Note. When **CED 1902** is selected as the **Amplifier** in the Recording setup dialog box, the gain setting is automatically included in the scaling of input channel 0

**Low Pass Filter:** Sets the cut-off frequency of the CED 1902's built-in low pass filter. The filter can be set to **None**

(out of use), **1000Hz, 500Hz, 100Hz**. The low pass filter removes signal frequencies signal higher than the cut-off, smoothing the signal.

**High Pass Filter:** Sets the cut-off frequency of the CED 1902's built-in high pass filter. The filter can be set to **None** (out of use), **50Hz, 100Hz, 200Hz**. The high pass filter removes signal frequencies signal lower than the cut-off, removing steady and slowly changed components of the signal.

**AC Coupled:** Check this box to make the CED 1902 input AC (alternating current) coupled. In this mode only variations in the signal are allowed through to the amplifier, constant (DC) levels are blocked. (AC coupling should be used with EMG and ECG recordings but not with force or pressure transducer recordings.)

**50 Hz Filter:** Enable/disables a notch filter which selectively removes frequencies around 50Hz. (Used to remove 50Hz interference from mains power lines).

**DC Offset:** The DC Offset facility adds or subtracts a DC voltage level from the input. The offset range depends upon the input mode (Normal Diff+ and Inverted Diff- = +/-0.5mV, Single Ended = +/-500mV, Electrodes = +/-0.1mV). The offset facility is often used to cancel out the standing DC voltage signal from tension transducers.

[Next](#)

## Getting Started > Amplifiers > Tecella Patch Clamp Amplifier Control Panel

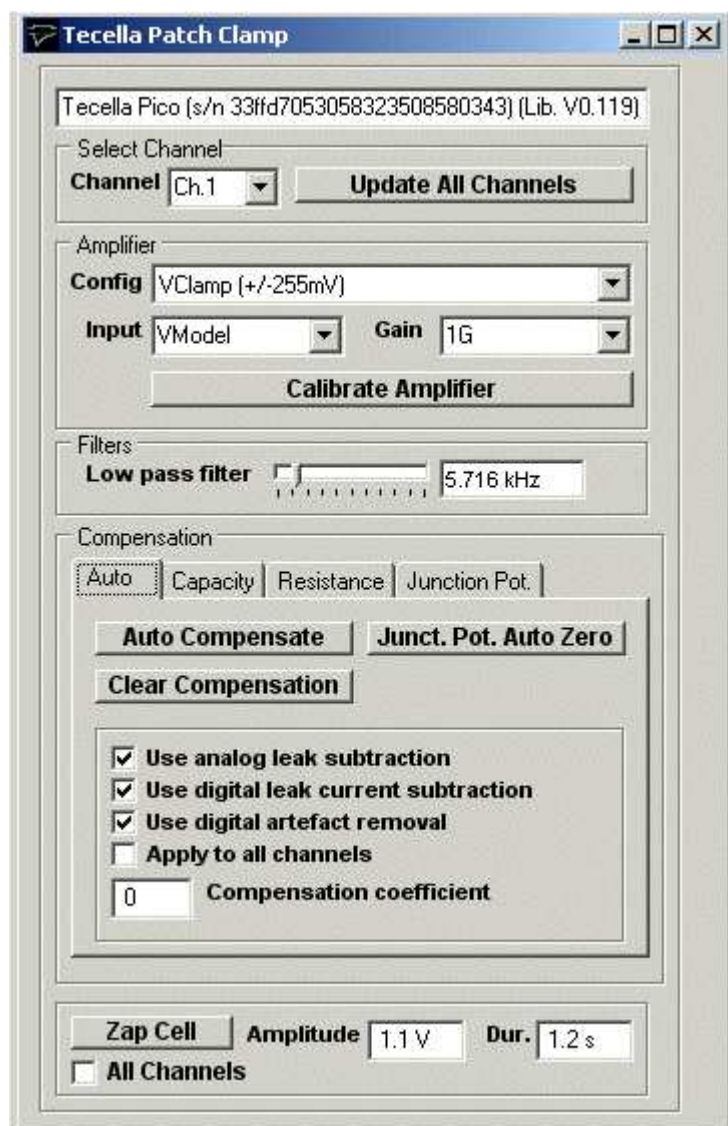
The amplifier gain, compensation and current/voltage clamp mode of Tecella patch clamp amplifiers can be set from this control panel.

Select

### Setup

#### Tecella Patch Clamp

to display the Tecella Patch Clamp control panel.



**Config:** Selects the voltage/current clamp mode and stimulus current/voltage limits.

**Input:** Selects the amplifier input (**None** = No input, **Head** = headstage input, **VModel** = 100 MOhm model cell, **IModel** = 1 MOhm model cell).

**Gain:** In voltage-clamp mode, selects the headstage feedback resistor. In current-clamp mode, selects amplifier gain.

**Low pass filter:** Slider selects cutoff frequency of Bessel low pass filter.

### Compensation

**Auto page:** Automatic capacity/leak current compensation.

Click **Auto Compensate** to automatically compensate for pipette/cell capacity and cell leak conduction.

Click **Junct. Pot. Auto Zero** to compensate for the electrode junction potentials, setting the input current to zero.

Click **Clear Compensation** to set all compensation to zero.

### Options

Tick the **Use analog leak subtraction** option to use the amplifier analog leak current subtraction circuits in the automatic leak current compensation.

Tick the **Use digital leak current subtraction** option to use the digital leak current in the automatic leak current compensation.

Tick the **Use digital artefact removal** option to use the digital artefact function in the automatic leak current compensation.

Tick **Apply to all channels** to apply automatic compensation to all amplifier channels (only applies to multi-channel amplifiers).

**Compensation coefficient:** Sets the compensation coefficient factor (0=optimal compensation, >0=under-compensation, <0 = over-compensation).

**Capacity page:** Capacity compensation settings in use. Can be adjusted by the user or set automatically using **Auto Compensate**.

**Resistance page:** Cell leak and pipette series resistance compensation settings in use. Can be adjusted by the user or set automatically using **Auto Compensate**.

**Junction Pot. page:** Electrode junction potential compensation settings in use. Can be adjusted by the user or set automatically using **Junct. Pot. Auto Zero**.

**Zap Cell:** Click **Zap Cell** to apply a voltage pulse of amplitude set by **Amplitude** and duration set by **Dur**.

## Recording Experimental Signals > Monitoring Input Signals & Patch Pipette Seal Test

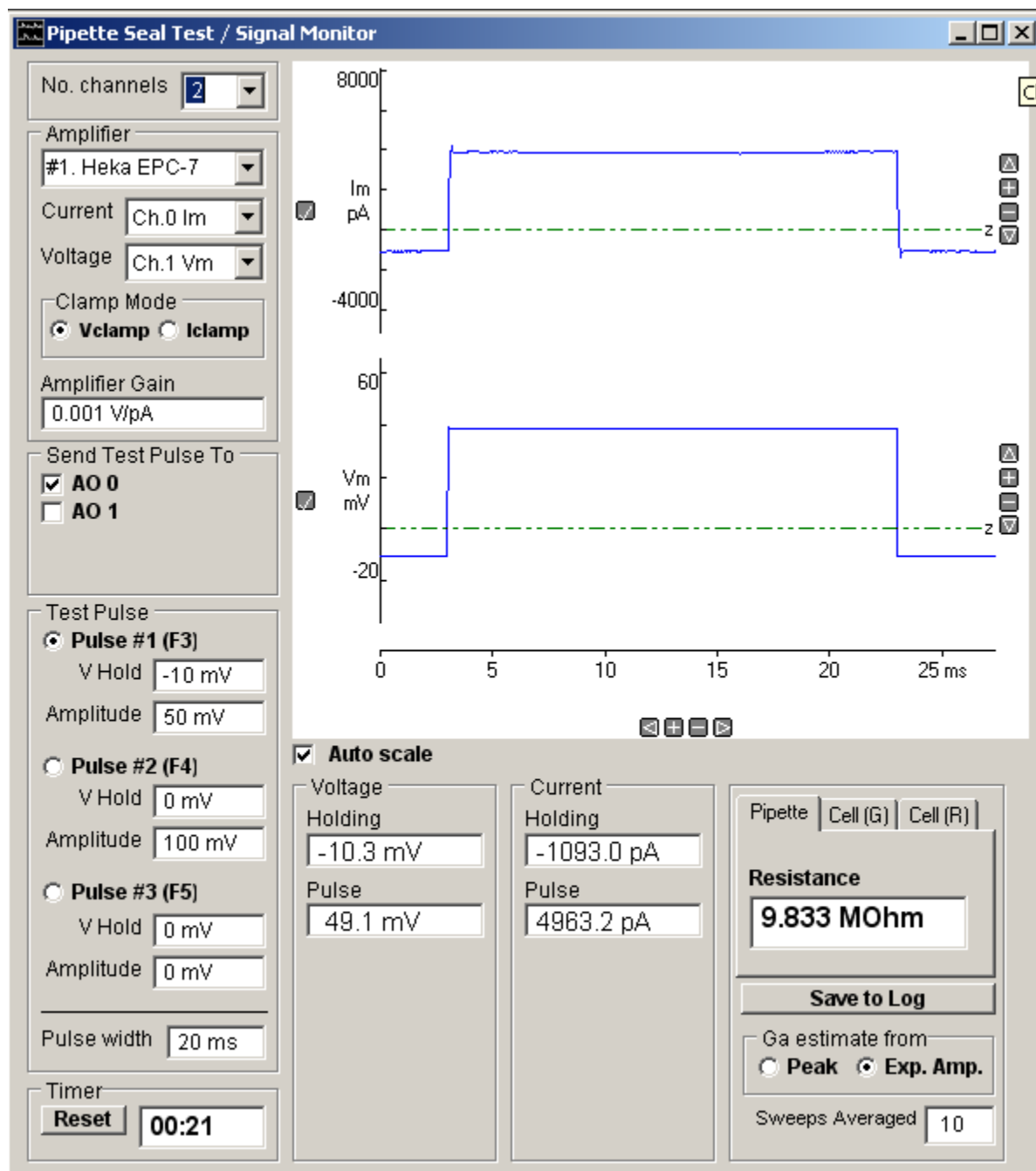
You can monitor the signals appearing on each channel using the signal monitor/pipette seal test module which provides a real-time oscilloscope display and digital readout of the signal levels on the cell membrane current and voltage channels. A test pulse can also be generated for monitoring pipette resistance in patch clamp experiments.

To open the monitor/seal test module, select from the menu

### Record

#### Pipette Seal Test / Signal Monitor

An oscilloscope trace showing the current signal on each input channel is displayed.



### 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.

## Amplifiers

The Amplifier selection box indicates which amplifier is currently selected for seal test and the current and voltage input channels being monitored. If two or more amplifiers are in use the seal test can be switched between amplifiers by selecting **Amplifier #1, #2** etc. ([See Amplifiers](#))

The amplifier voltage/current- clamp mode is indicated by the **Clamp Mode** options. (When mode telegraphs are operational for the amplifier these indicate the actual state of the amplifier. When telegraph information is not available the **Vclamp** and **Iclamp** buttons must be set by the user to the amplifier clamp mode.)

The amplifier gain (current channel gain in voltage-clamp mode, voltage channel gain in current-clamp mode) is indicated in the **Amplifier Gain** box. When gain telegraphs are operational these indicate the actual state of the amplifier. *When telegraph information is not available the current gain setting is entered here by the user.*

The analog output channel(s) to which the test pulse is applied is indicated in the **Send Pulse To** list of check boxes. Selecting an amplifier in the Amplifier selection box, automatically selects the output channel connected to the amplifier stimulus command input. The test pulse output can be routed to a different output channel (or to additional channels) by ticking the required channels.

## Cell 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 type is set by entering an appropriate value for holding voltage and pulse amplitude into the **Holding voltage** or **Amplitude** box for each pulse.

The width of the pulse 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 #2 = F4, Pulse #3 = F5).

## Current and voltage readouts

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

Clicking the **Save to Log** button saves the current Pipette or Cell readings to the log file.

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

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

where  $V_{\text{pulse}}$  and  $I_{\text{pulse}}$  are the steady-state voltage and current pulse amplitudes. The **Cell (G)** page displays the cell membrane conductance,  $G_m$ , capacity,  $C_m$ , and access conductance,  $G_a$ , and the **Cell (R)** page displays the cell membrane resistance,  $R_m$ , (1/ $G_m$ ) capacity,  $C_m$ , and access resistance,  $R_a$ , (1/ $G_a$ ).  $G_m$ ,  $C_m$ , and  $G_a$  are computed from

$$G_a = \frac{I_0}{V_{\text{pulse}}}$$
$$G_m = \frac{I_{\text{pulse}}}{\left( V_{\text{pulse}} - \frac{I_{\text{pulse}}}{G_a} \right)}$$

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

where  $I_0$  is the initial current at the start of the capacity transient and  $\tau$  is the exponential time constant of decay of the capacitance current (See Gillis, 1995, for details).  $I_0$  can be estimated either The **Ga estimate from** option determines how  $I_0$  is estimated (**Peak:**  $I_0$  estimated from the peak current of the capacity transient. **Exp. Amp.:**  $I_0$  estimated from amplitude of the fitted exponential at the start of the voltage step).

**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.

### **Sweeps Averaged**

The **Sweeps Averaged** setting determines the number of test pulse sweeps averaged to calculate the displayed pipette and cell parameters ( $R_{\text{pipette}}$ ,  $R_m$ ,  $G_m$ ,  $C_m$ ,  $R_a$ ,  $G_a$ ). Values can range from 1 (no averaging) to 10 (averaging of the 10 most recent test pulses).



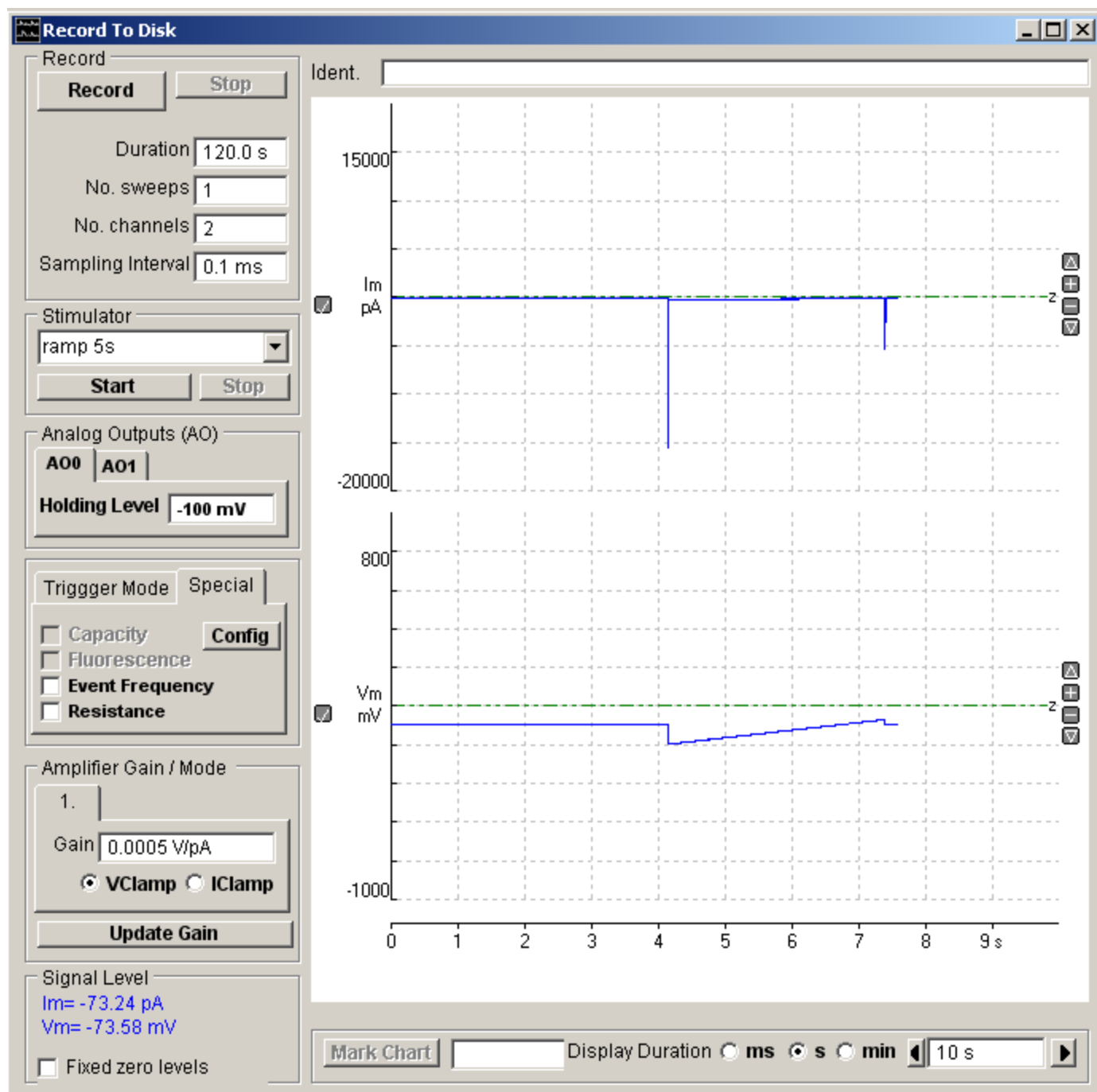
## Recording Experimental Signals > Recording Signals

To make a recording of signals from your experiment, select

**Record**

**Record to disk**

to open the recording window.



The display area of the screen acts like a digital oscilloscope, displaying live traces of the incoming analogue signals.

The **Signal Level** box provides a readout of the signal level on each input channel. The duration of the live display window can be adjusted using the **Display** slider bar at the bottom right of the screen or by entering a duration in the **Display** box.

The vertical magnification for each channel 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.

Individual channels can be added/removed from the display by clicking the / button at the left edge of each channel. The vertical area of the display devoted to each channel can be adjusted by dragging the top/left edge of each channel Y axis

up or down.

## Recording Experimental Signals > Recording Modes > Trigger Modes

There are two trigger modes **Free Run** and **External Trigger**.



When the trigger mode is set to **Free Run**, recording starts immediately after the **Record** button is pressed and continues until the required recording duration has been acquired. Choose the free run mode for signals, such as single-channel currents, spontaneous synaptic currents or any signal where synchronisation with an external event is not possible or required.

When the trigger mode is set to **External Trigger**, recording only starts after a trigger pulse is received on the External Trigger input of the laboratory interface. External Trigger mode is used when it is necessary to synchronise recording with an external event such as the pulse from a stimulator (ideally so that the sweeps starts shortly before the cell is stimulated).

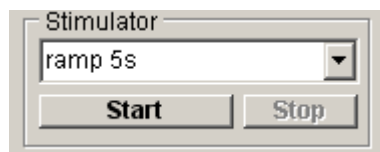
If the **5V Trigger Level** option is selected, recording will be triggered by a 0V-to-5V transition on the Ext. Trigger input. If the **0V Trigger Level** option is selected, recording will be triggered by a 5V-to-0V transition. (NOTE. Some laboratory interfaces support only one or other of the two trigger polarities.)

The **No. Sweeps** box determines the number of externally triggered sweeps to be collected during a recording session.

## Recording Experimental Signals > Recording Modes > Stimulator

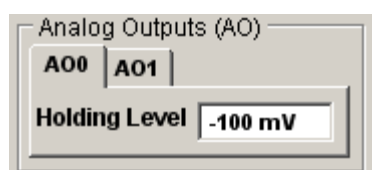
A wide variety of stimulus voltage or current waveforms can be generated on analog output channels 0 & 1 and 5V TTL digital waveforms on up to 8 TTL digital output channels.

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 analog outputs to the holding levels.

The holding voltages applied to analog outputs can be changed by entering a new voltage then pressing the **Enter** key. (Note. Stimulus voltages waveforms generated during a voltage protocol are added to the existing holding voltages.)



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 (\*.xml files).

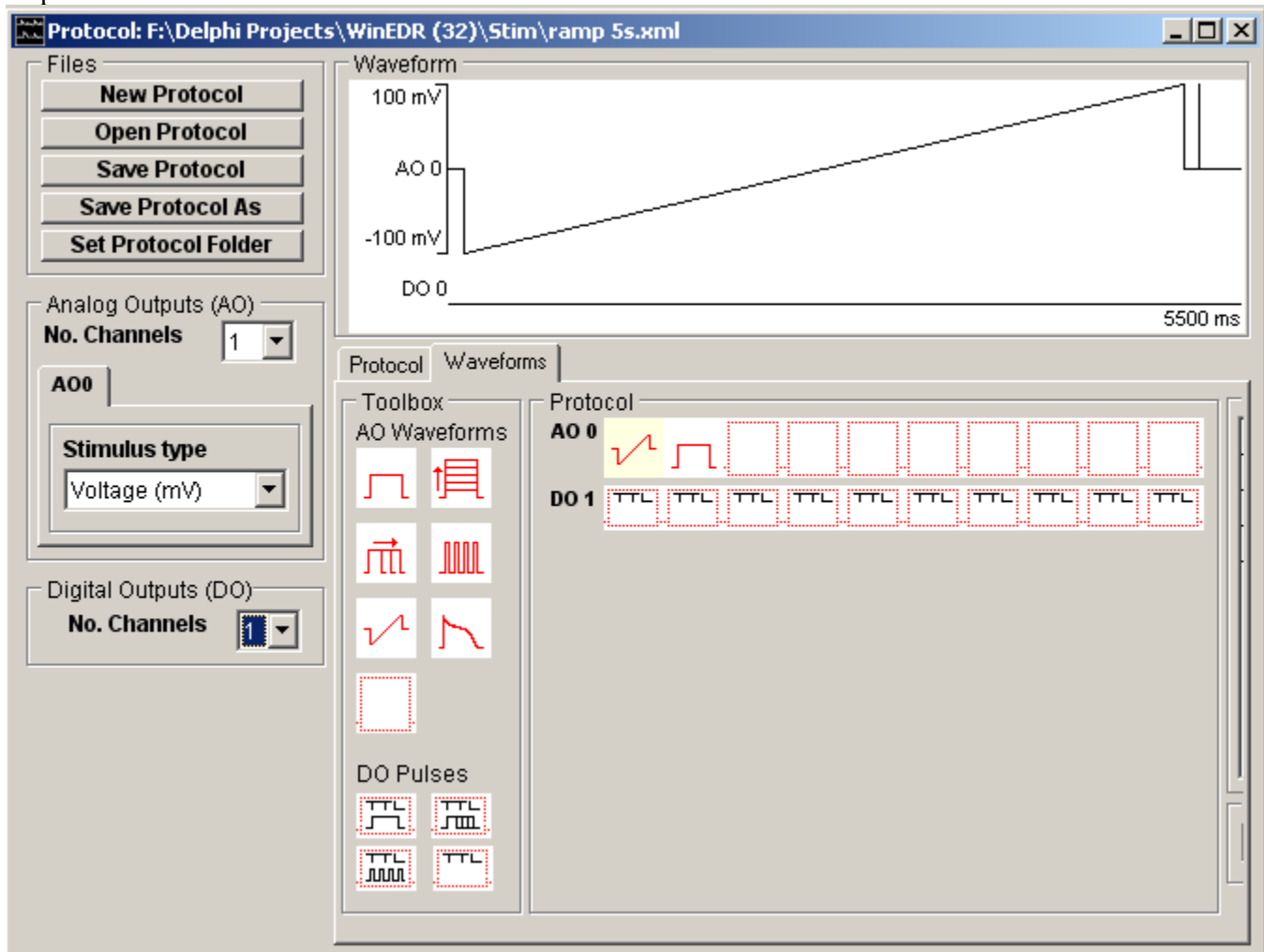
(Note. Stimulus voltage outputs are only supported with National Instruments and Cambridge Electronic Design laboratory interface units.)

## Recording Experimental Signals > Creating Stimulus Protocols > Opening the Stimulus/Recording Protocol Editor

To create a stimulus protocol, select

### Setup Stimulus/Recording Protocol Editor

to open the stimulus editor window.



Up to 4 voltage waveform output channels (AO0-AO3) are available and 8 TTL digital pulse channels (DO0-DO7). A diagram of the output waveforms appears in the **Waveform** display box.

To create a stimulus protocol, click the

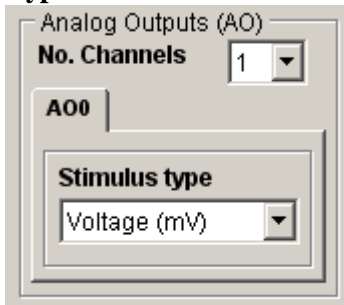
### New Protocol

button to create a blank protocol.

[Next](#)

## Recording Experimental Signals > Creating Stimulus Protocols > Analog & Digital Output Channels

**Analog Outputs (AO):** Select the number of analog output channels to be used in the analog outputs **No. Channels** list. For each AO channel defined, select the type of stimulus (Voltage or Current) and the stimulus units in the **Stimulus Type** list.



**Digital Outputs (DO):** Select the number of digital output channels to be used in the digital outputs **No. Channels** list.



(Note, during execution of a stimulus protocol, the analog and digital holding levels override the default holding levels set in the Seal Test or Default Settings windows).

[Next](#)

## Recording Experimental Signals > Creating Stimulus Protocols > Protocol Settings

Select the **Protocol** tab page to set the number of steps and repeat period of the protocol.

The screenshot shows the 'Protocol' tab of a settings window. At the top are two tabs: 'Protocol' (selected) and 'Waveforms'. Below the tabs is a table with three rows: 'Stimulus repeat period' with a value of '5500.00 ms', 'No. stimulus increments' with a value of '1', and 'No. repeats per increment' with a value of '1'. Below the table is a large empty text area. At the bottom, there are two radio buttons: 'Repeat Protocol' (unselected) and 'Link to next protocol' (selected). Below the radio buttons is a dropdown menu that is currently empty.

Stimulus repeat period	5500.00 ms
No. stimulus increments	1
No. repeats per increment	1

☐ Repeat Protocol  
☒ Link to next protocol

\_\_\_\_\_

**Stimulus repeat period:** the time interval between successive steps within a protocol.

**No. stimulus increments:** *For incremented step waveforms*, the number of times a stimulus is incremented within the protocol.

**No. repeats per increment:** *For incremented step waveforms*, the number of times a stimulus is to be repeated without incrementing the step size.

**Repeat Protocol:** Select the **Repeat Protocol** option to continuously repeat the protocol on completion.

**Link to next protocol:** Select the **Link to next protocol** option to load the specified protocol on completion of the current one.

This is a close-up of the 'Link to next protocol' section. It shows the 'Link to next protocol' radio button selected. Below it, a dropdown menu is open, displaying the text '100 - 100mV ramp'.

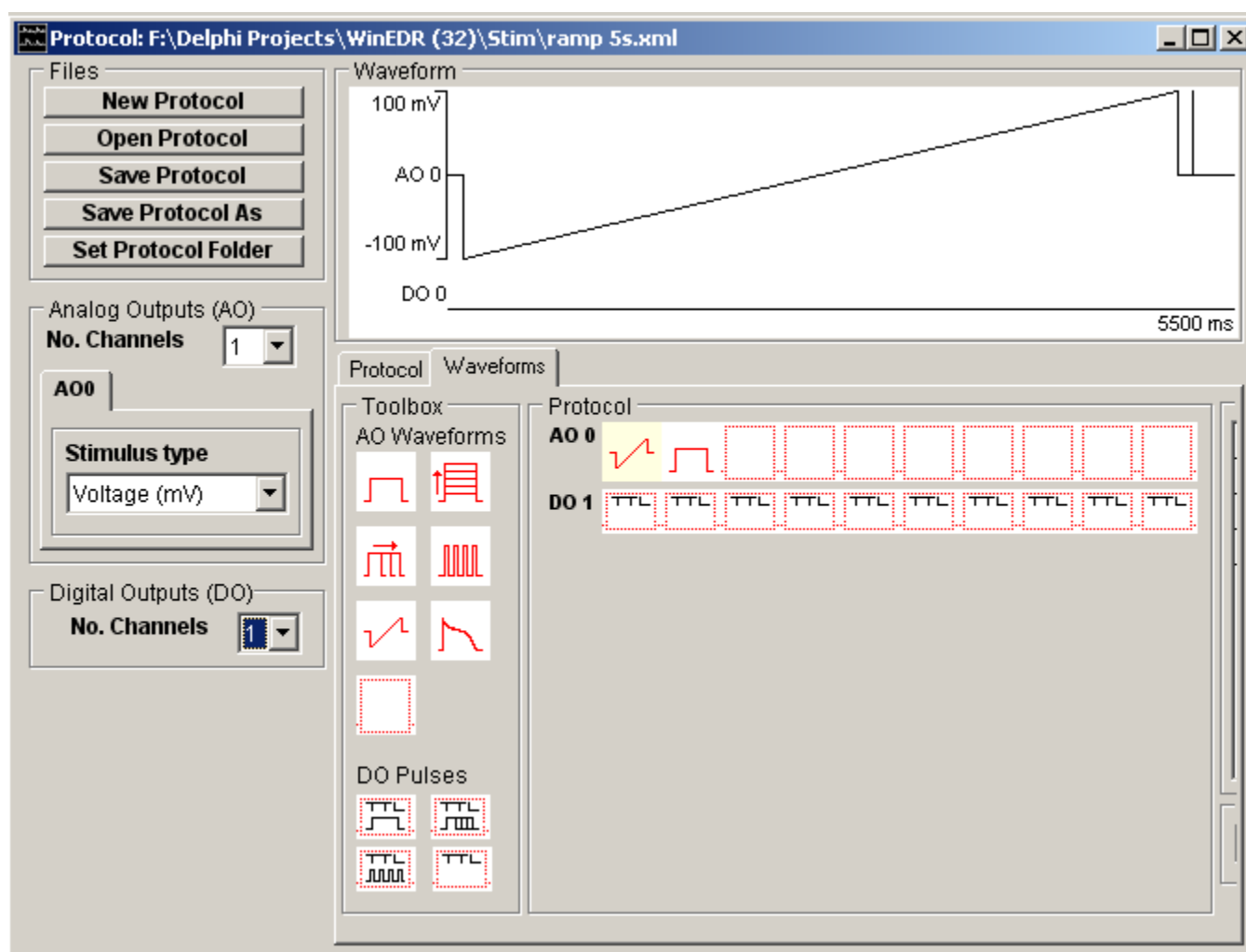
☒ Link to next protocol

100 - 100mV ramp

[Next](#)

## Recording Experimental Signals > Creating Stimulus Protocols > Adding Stimulus Waveforms to the Protocol

Select the **Stimulus** tab page to add stimulus waveforms to the analog or digital output channels.



Waveforms are constructed by dragging waveform step and ramp elements from the **Toolbox** and dropping them into the selected voltage channel (**AO 0 - AO 4**) or digital (**DO 0 – DO 7**) output list. A plot of the resulting stimulus protocol for each output channel is shown in the protocol display panel.

A stimulus waveform on each output channel can consist of up to 10 separate elements. The amplitude and duration for each element is defined in its parameters table which can be made to appear by clicking on the element.

Eight analog and 4 digital waveform elements are available in the toolbox, as detailed below.

### ***Rectangular voltage pulse of fixed size***



A simple pulse, which does not vary in amplitude and duration between records. 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.

	Parameters
<b>Initial Delay</b>	Delay (at the holding level) before the pulse begins.
<b>Amplitude</b>	Pulse amplitude.
<b>Duration</b>	Pulse duration.



### *Family of rectangular pulses varying in amplitude*



A rectangular voltage pulse whose amplitude is automatically incremented between recording sweeps. 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.

	Parameters
<b>Initial Delay</b>	Delay (at the holding level) before the pulse begins.
<b>Amplitude</b>	Amplitude of the first pulse in the series.
<b>Amplitude (increment)</b>	Increment to be added to amplitude between records.
<b>Duration</b>	Pulse duration.

### *Family of rectangular voltage pulses varying in duration*



A rectangular voltage pulse whose duration is automatically incremented between recording sweeps. 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.

	Parameters
<b>Initial Delay</b>	Delay (at the holding level) before the pulse begins.
<b>Amplitude</b>	Pulse amplitude.
<b>Duration</b>	Pulse duration.
<b>Duration (increment)</b>	Increment to be added to duration between records.

### *Series of rectangular voltage pulses*



A train of rectangular voltage pulses of fixed size. 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.

	Parameters
<b>Initial Delay</b>	Delay (at the holding level) before the pulse begins.
<b>Amplitude</b>	Pulse amplitude.
<b>Duration</b>	Pulse duration.
<b>Repeat period</b>	Interval between pulse in train.
<b>No. repeats</b>	Number of pulses in train.

## Voltage ramp



A linear voltage ramp between two voltage levels. 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.)

	Parameters
<b>Initial Delay</b>	Delay (at the holding level) before the pulse begins.
<b>Amplitude</b>	Amplitude at start of ramp.
<b>End Amplitude</b>	Amplitude at end of ramp.
<b>Duration</b>	Ramp duration.

## Digitised analog waveform



A digitised analog waveform loaded from an external data file.

	Parameters
<b>Initial Delay</b>	Delay (at the holding level) before the pulse begins.
<b>File Name</b>	Name of text file containing digitised waveform.
<b>D/A update interval</b>	Time interval between digitised waveform points.
<b>No. Points</b>	No. of digitised waveform data points to be used in the stimulus.
<b>Starting point (increment)</b>	Increment to be added between records to the first data point of the digitised waveform to be used in the stimulus.

Digitised waveforms are loaded into the stimulus protocol from text files containing the digitised data points. The waveform data can be formatted either as a single column of amplitude data or a pair of columns of time (in seconds) and amplitude (in the stimulus units of the output channel to contain the waveform) data points separated by <tab> characters, i.e.

$T_0$        $V_0$   
 $T_1$        $V_1$   
...etc

To load a digitised waveform from a text file, click the  button next to the **File Name** table entry and select the file containing the digitised waveform.

After loading the data, the **No. Points** entry in the parameter tables indicates the number of points loaded from the file. For two column data files which contain time data the **D/A update interval** is set to the time difference between the first and second rows of data. For single column data files, **D/A update interval** must be entered by the user.

## Empty analog element



Empty analog waveform element. Dragging this element on to an analog output list, erases the element it is placed on.

### ***Digital pulse (fixed duration)***



A fixed duration digital pulse. This 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.

	Parameters
<b>Initial Delay</b>	Delay (at the holding level) before the pulse begins.
<b>State (0=0V,1=5V)</b>	Digital output state during pulse: 0=0V, 1=5V.
<b>Duration</b>	Pulse duration.

### ***Family of digital pulse (varying in duration)***



A digital pulse whose duration is automatically incremented between recording sweeps.

	Parameters
<b>Initial Delay</b>	Delay (at the holding level) before the pulse begins.
<b>State (0=0V,1=5V)</b>	Digital output state during pulse: 0=0V, 1=5V.
<b>Duration</b>	Pulse duration.
<b>Duration (increment)</b>	Increment in duration between records.

### ***Train of digital pulses***



A train of digital pulses of fixed intervals and of fixed duration. This element can be used to apply a rapid train of stimuli to a cell.

	Parameters
<b>Initial Delay</b>	Delay (at the holding level) before the pulse begins.
<b>State (0=0V,1=5V)</b>	Digital output state during pulse: 0=0V, 1=5V.
<b>Duration</b>	Pulse duration.
<b>Repeat period</b>	Interval between pulse in train.
<b>No. repeats</b>	Number of pulses in train.

### ***Empty digital element***



Empty digital waveform element. Dragging this element on to a digital output list, erases the element it is placed on.

## D/A Update Interval

The D/A Update Interval box displays the D/A converter update interval to be used to produce the analog stimulus waveforms within the protocol.

A screenshot of a software dialog box titled 'D/A Update Interval'. It contains a text input field with the value '0.2734 ms' and a checkbox labeled 'Fix Interval' which is currently unchecked.

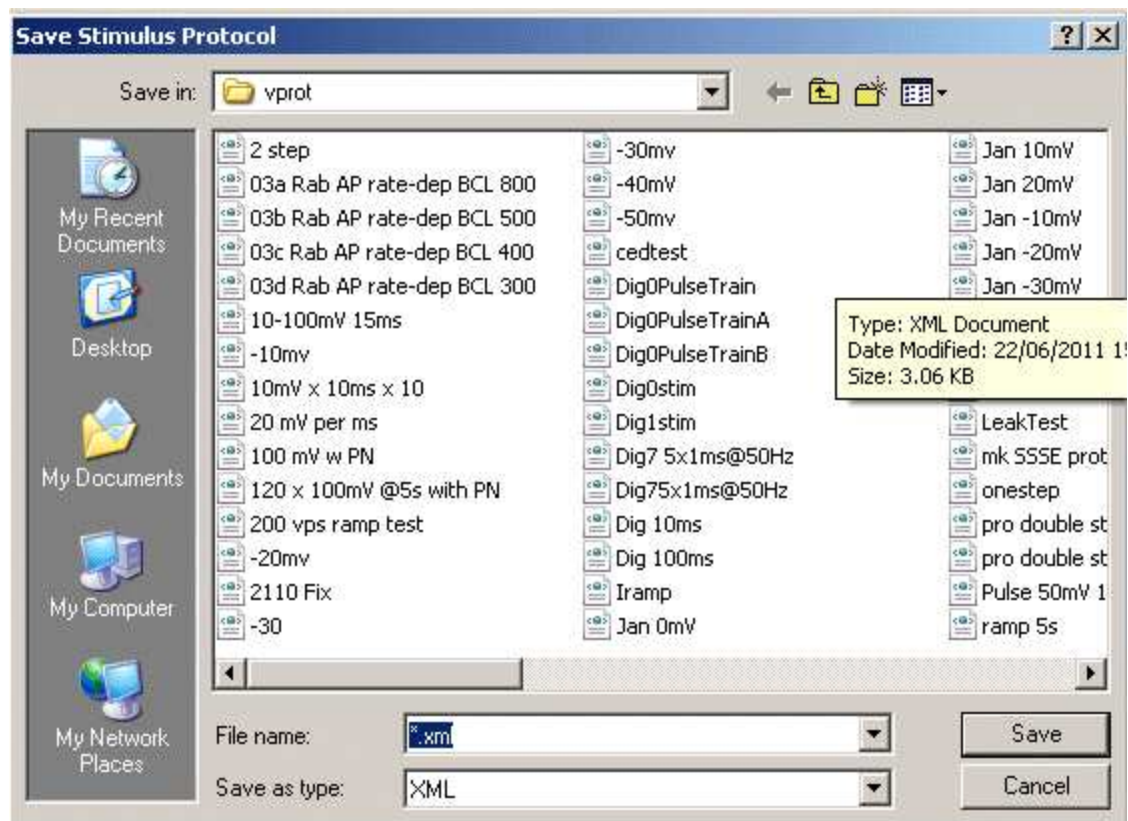
The interval is normally set to **Recording Duration/No. Samples**, ([see Recording Settings](#)) but may be greater than this if the laboratory interface cannot support this D/A update rate, or less if short duration pulses exist within the protocol. If digitised analog waveform elements exist within the protocol, the update interval is set to the D/A update interval for these waveforms.

To keep the D/A update interval fixed at a specified value, enter the update interval into the **D/A Update Interval** box, and tick the **Fix Interval** option. (Note. If the laboratory interface cannot support this D/A update rate it will be adjusted to the minimum possible update interval for the interface.)

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## Recording Experimental Signals > Creating Stimulus Protocols > Saving and Loading Stimulus Protocols

When you have created a stimulus protocol, you can save it to a protocol file by clicking the **Save Protocol As** button to open the **Save Stimulus Protocol** dialog box.



Stimulus protocols are stored as files with .XML file extensions, in the directory, **C:\WinEDR\vprot\**

Protocol files can be re-loaded for editing, by clicking the **Open Protocol** button, and selecting a protocol file from the list presented in the **Load Stimulus Protocol** dialog box.

The folder used to store protocols can be changed by clicking the **Set Protocol Folder** button to open the protocol folder selection dialog box, clicking on a folder, then clicking the Open button.

[Next](#)

## Recording Experimental Signals > Creating Stimulus Protocols > Example Protocols

A number of example protocols are installed in the vprot folder with WinEDR.

VSteps 10-100mV 100ms.xml	A family of 10 depolarising, 100 msec duration, voltage steps, ranging from 10 mV to 100 mV.
ISteps 10-100pA 100ms.xml	A family of 10 depolarising, 100 msec duration, current steps, ranging from 10 pA to 100 pA.
tail current.xml	A two step pulse protocol for recording tail currents. A 500 msec pre-pulse, followed by a 60 mV, 50 msec duration test pulse. The pre-pulse steps from 10mV to 120 mV.
Ramp -100..100mV 1s.xml	A voltage ramp, slewing from –100 mV to +100 mV over a period of 1 sec.
sinewave.xml	A digitised 1572 sample point sine waveform .
Digpulse.xml	A digital stimulus program controlling digital outputs 0 and 1. Dig.0 is OFF initially and pulses ON for a period of 50 msec, after a delay of 100 msec. Dig. 0 is ON initially and pulses OFF for 50 msec.

[Next](#)

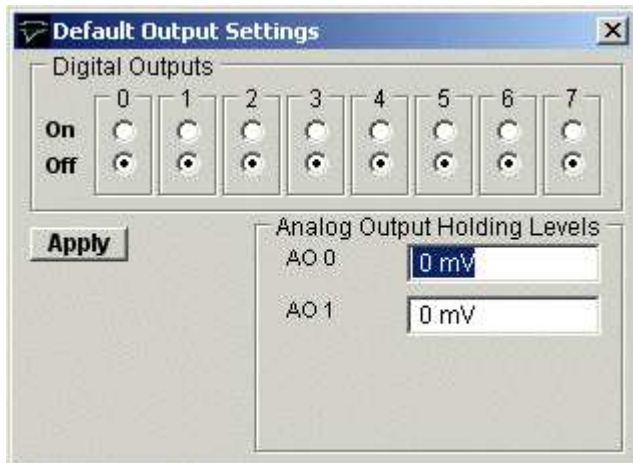
## Recording Experimental Signals > Creating Stimulus Protocols > Default Output Settings

The default output settings panel sets the default analog and digital output holding levels when a stimulus protocol is NOT in progress.

Select

### Setup Default Output Settings

To open the Default Output Settings control panel



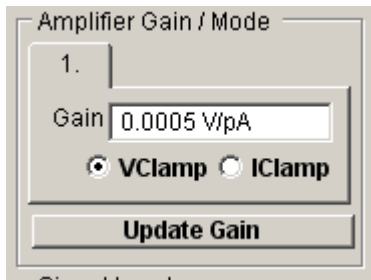
**Analog Output Holding Levels:** Sets the default holding level for the analog outputs. (Note. When an amplifier is defined, the default holding level for the command output of that amplifier can also be set by changing the holding level in the [Pipette Seal Test](#) window.)

**Digital Outputs:** Selecting the appropriate On/Off switch, sets the default voltage levels (ON=5V, OFF=0V) to be output on the digital output lines.

Click the **Apply** button to apply the settings to the outputs.

## Recording Experimental Signals > Amplifier Gain Setting

The amplifier gain (current channel gain in voltage-clamp mode, voltage channel gain in current-clamp mode) is indicated in the **Amplifier Gain/Mode** box. When gain telegraphs are operational these indicate the actual state of the amplifier. *When telegraph information is not available the current gain setting is entered here by the user.* ([See Amplifiers](#))



The screenshot shows a software dialog box titled "Amplifier Gain / Mode". It contains a small box with the number "1." and a "Gain" input field displaying "0.0005 V/pA". Below the input field are two radio buttons: "VClamp" (which is selected) and "IClamp". At the bottom of the dialog is an "Update Gain" button.



## Recording Experimental Signals > Experiment Identification/Comments

A line of text identifying the purpose of the recording can be entered into the **Ident** box at the top of the recording window.

A screenshot of a software interface showing a text input field labeled 'Ident.' with a cursor inside, ready for text entry. The field is part of a larger window with a blue header bar.

This line is stored in the data file. When the Return key is pressed the contents of the line is written to the log file.

Short tags (up to 8 characters) can be associated with each record by entering text into the **Marker** box and clicking the **Add** button.

A screenshot of a software interface showing a text input field labeled 'Marker' with the letter 'A' inside, and a button labeled 'Add' below it.

## Log File

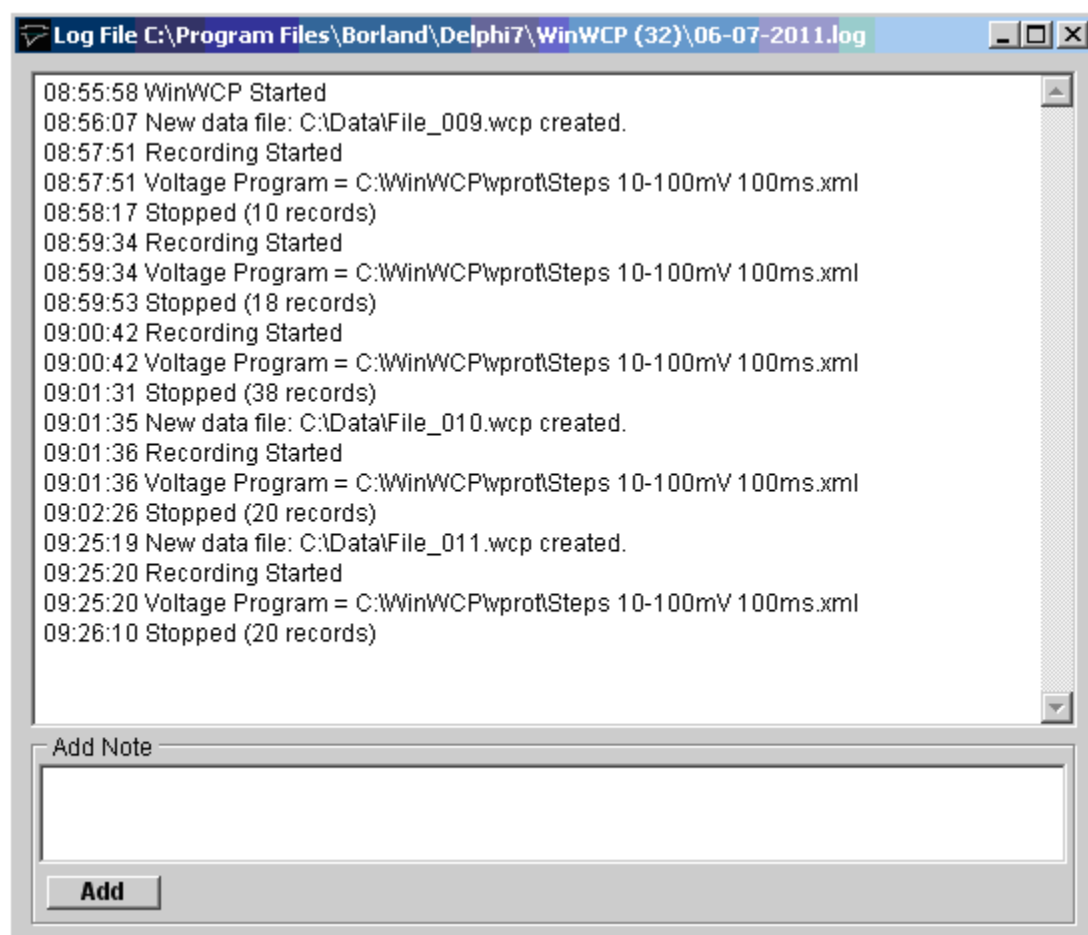
A log file of the operations initiated by the user is updated during the course of recording or analysing an experiment. The names of data files created or loaded, comments entered, stimulus programs used, and other events are stored along with the time that the event occurred. The log file can be used like an experimenter's notebook to keep a written record of the experiment. A new log file is opened on a daily basis with a name in the form **dd-mm-yy.log** and stored in the WinEDR program directory.

Select

**File**

**Inspect Log File**

to display the experimental log:



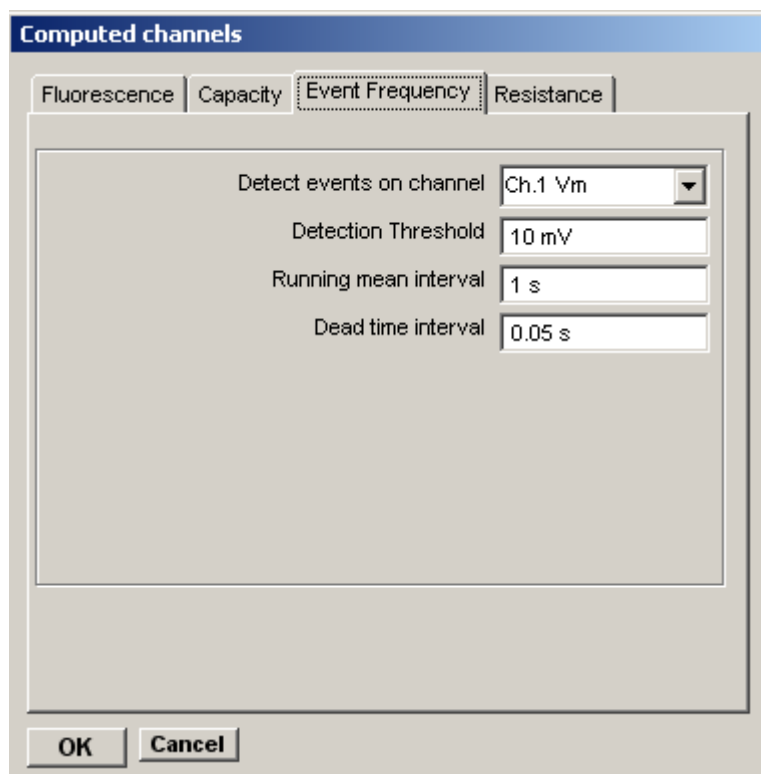
Additional notes can be added to the log file by entering text into the Add Note box and clicking the **Add** button.

## Recording Experimental Signals > Special Modes > Special Modes

WinEDR has a number of special features for processing of FURA-2 fluorescence, cell capacity and real-time event frequency. In these modes of operation certain input channels are computed on-line from the others. To define the input channels and computed results channels click the **Config** button to open Computed Channels configuration dialog box.

## Recording Experimental Signals > Special Modes > Event Frequency

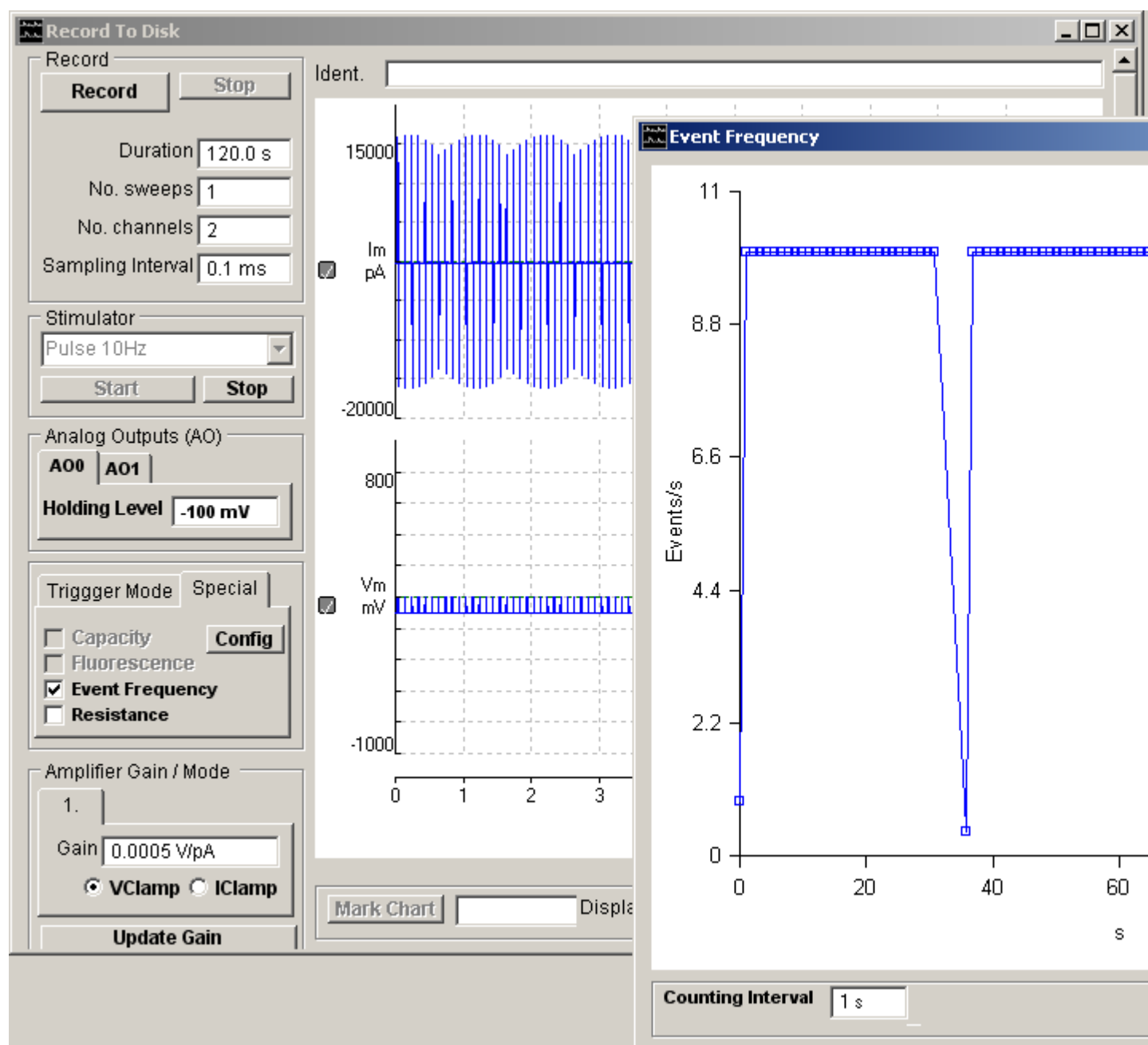
Selecting the **Event Frequency** special option opens a display window showing the frequency of occurrence of signals from a selected input channel. Signals are detected using a threshold-based event detector which is configured on the Event Frequency page of the Computed Channels dialog box.



To configure event detection:

1. Select the input channel on which the signals are to be detected from the **Detect events on channel** list.
2. Set the threshold which the signal must exceed to be detected in the **Detection threshold** box
3. Set the averaging interval for the threshold baseline level in the **Running mean baseline** box.
4. Set the time after detection of an event within which detection is disabled in the **Dead time interval** box to exceed the duration of a signals to be detected.

The period over which events are counted to produce the event frequency is set by the Count Interval box in the Event Frequency display window.



## Recording Experimental Signals > Special Modes > Cell Capacity Mode

In cell capacity mode, activated by ticking the **Capacity** option, real and imaginary admittance signals from a patch clamp with a phase detector can be decoded to produce a continuous measure of cell capacity, membrane and access conductance.

The screenshot shows the 'Computed channels' dialog box with the 'Capacity' tab selected. The 'Input channels' section on the left includes dropdowns for  $I_m$  (Ch.0  $I_m$ ),  $V_m$  (Ch.1  $V_m$ ),  $G_{real}$  (Ch.2  $G_{real}$ ), and  $G_{imag}$  (Ch.3  $G_{imag}$ ). Below these are checkboxes for 'Invert  $G_{real}$  signal', 'Invert  $G_{imag}$  signal', and 'Use gain telegraph'. The 'Excitation freq.' is set to 1000 Hz and 'Reversal Pot.' is 0 mV. The 'Results channels' section on the right includes dropdowns for  $G_m$  (Ch.4  $G_m$ ),  $G_s$  (Ch.5  $G_s$ ), and  $C_m$  (Ch.6  $C_m$ ), each with a checked checkbox. Below these are 'Display ranges' for  $G_m$  (10 nS),  $G_s$  (500 nS), and  $C_m$  (200 pF). The 'Capacity Compensation' section at the bottom has an unchecked 'Use compensation' checkbox, and fields for 'Series Resistance' (0 MOhm) and 'Cell Capacity' (0 pF). 'OK' and 'Cancel' buttons are at the bottom left.

A total of 8 channels are required, four input channels, real admittance ( $G_{real}$ ), imaginary admittance ( $G_{imag}$ ), membrane current ( $I_m$ ), membrane potential ( $V_m$ ), 3 computed results channels, membrane conductance ( $G_m$ ), access conductance ( $G_a$ ) and membrane capacity ( $C_m$ ).

## Recording Experimental Signals > Special Modes > Cell Fluorescence

In cell fluorescence mode, activated by ticking the **Fluorescence** option, intracellular ion concentration can be computed from the ratio of two input channels. Four channels are required, two fluorescent input channels, the computed ratio of these two channels, and computed ion concentration.

The screenshot shows a dialog box titled "Computed channels" with a blue header bar. Below the header are four tabs: "Fluorescence" (selected), "Capacity", "Event Frequency", and "Resistance". The "Fluorescence" tab contains the following controls:

- Input channels:** A section labeled "R =" with two dropdown menus. The first dropdown is set to "Ch.0 Im" and the second to "Ch.1 Vm". Below these is a "Threshold" input field with the value "0".
- Equation:** A section with three input fields: "R.Max" (value 1), "R.Min" (value 0), and "K.Eff" (value 1).
- Results channels:** A section with two dropdown menus: "Ratio" and "Conc.". Each dropdown has a small square checkbox to its right.
- Display Ranges:** A section with two input fields: "Ratio" (value 10) and "Conc." (value 1).

At the bottom of the dialog are "OK" and "Cancel" buttons.

## Recording Experimental Signals > Special Modes > Resistance

Selecting the **Resistance** special option opens a display window showing cell resistance or conductance obtained from a voltage test pulse applied to the cell.

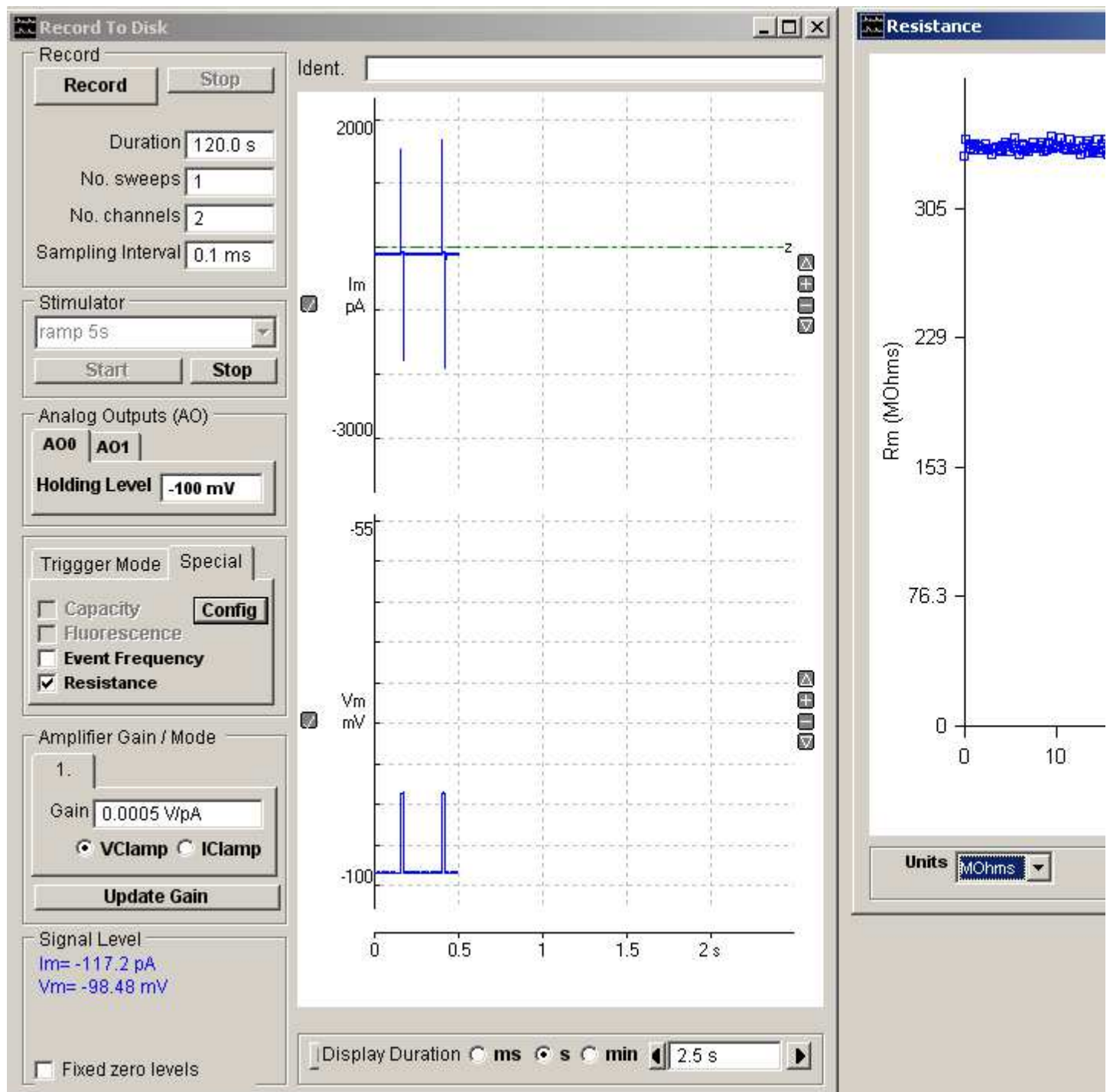
The test pulse properties and the selected current and voltage channels are configured on the Resistance page of the Computed Channels dialog box.

The screenshot shows the 'Computed channels' dialog box with the 'Resistance' tab selected. The dialog has a title bar 'Computed channels' and four tabs: 'Fluorescence', 'Capacity', 'Event Frequency', and 'Resistance'. The 'Resistance' tab is active. Inside the tab, there are three main sections: 'Channels', 'Plot', and 'Test Pulse'. The 'Channels' section has two dropdown menus: 'Current' set to 'Ch.0 Im' and 'Voltage' set to 'Ch.1 Vm'. The 'Plot' section has a dropdown menu set to 'Resistance'. The 'Test Pulse' section has three input fields: 'Amplitude' set to '10 mV', 'Duration' set to '20 ms', and 'Interval' set to '0.25 s'. At the bottom of the dialog are 'OK' and 'Cancel' buttons.

To configure the resistance plot:

1. Select the input channel on which the current signals are to be detected from the **Current** channel list.
2. Select the input channel on which the voltage signals are to be detected from the **Voltage** channel list.
3. Set the amplitude of the test pulse in the **Amplitude** box.
4. Set the duration of the test pulse in the **Duration** box.
5. Set the interval between pulses in the **Interval** box.
6. Select the type of plot to be produced from the **Plot** list. Plots of cell resistance, conductance, steady state pulse current and voltage can be produced.





The

The data displayed on the selected plot is stored in text file in the data folder.

## Displaying Records Stored on File > Compressed View > Displaying records (Compressed View)

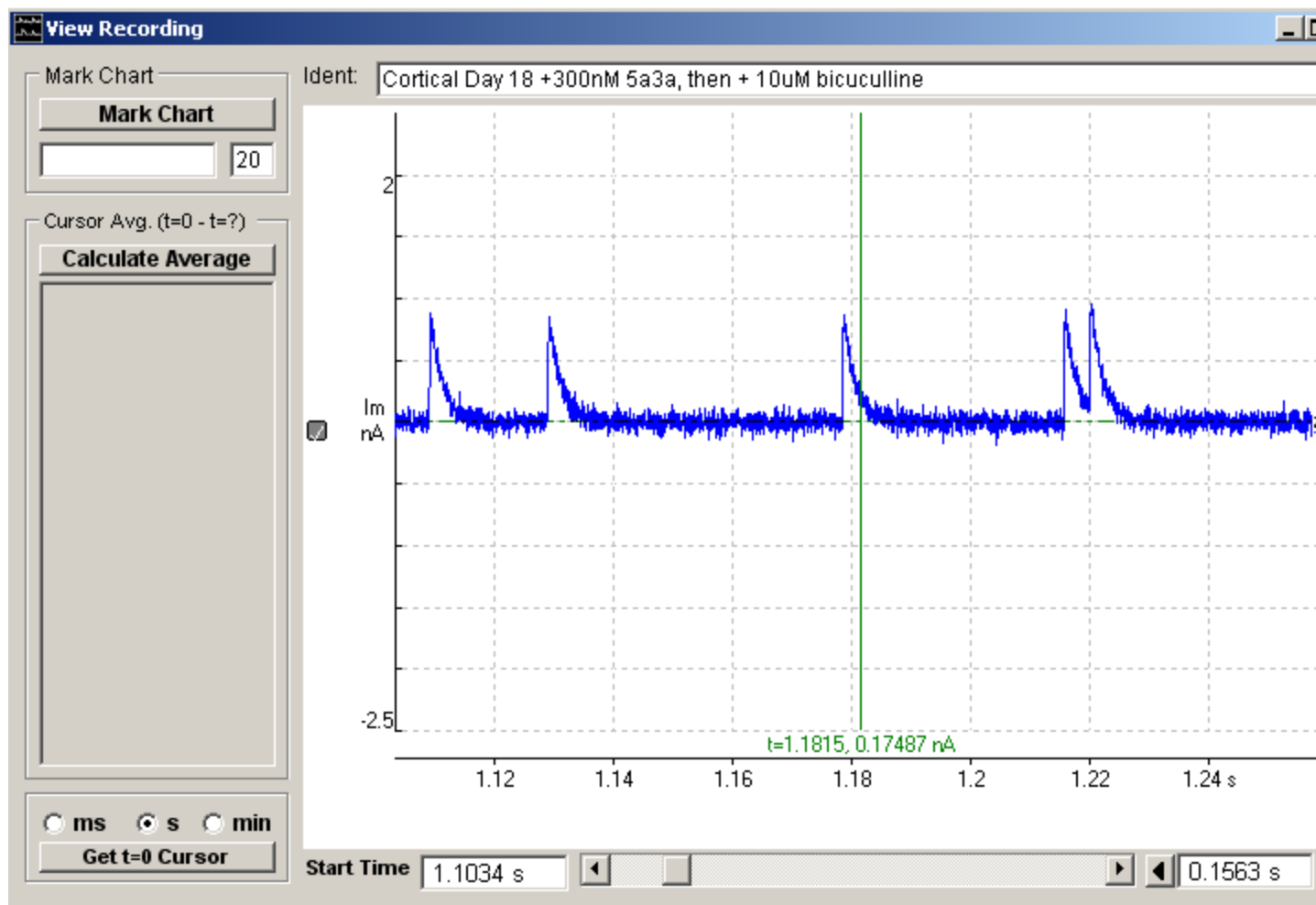
To review a recording after it has been acquired and stored in a data file, select one of the signal viewing options from the **View** menu. The **Compressed View** option displays the complete recording in compressed into a single window, with the facility to zoom into selected regions. The **Page View** option displays the un-compressed `recording within a single channel as a sequence of lines on page.

To display the recorded signal in the compressed view, select

### View

#### Compressed View

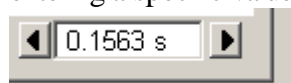
to open the **Compressed View** window.



The display window shows a segment of the recorded signals. The displayed segment can be scrolled through the recorded signals using the display scroll bar or moved to a specific time point within the recorded file by entering a value into the **Start Time** box and pressing the return key.



The size of the displayed segment can be increased or decreased in duration by clicking the arrow buttons or by entering a specific value and pressing the return key.



### Vertical Magnification

The vertical magnification of each plot can be expanded 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.

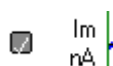


The vertical magnification and position of the displayed region within the recording can also be adjusted using the



buttons at the right edge of each plot.

Individual channels can be added/removed from the display by clicking the



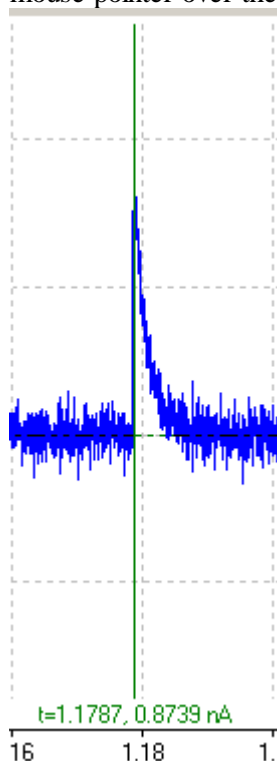
button at the left edge of each channel. The vertical area of the display devoted to each channel can be adjusted by dragging the top/left edge of each channel Y axis up or down.

All channels can be set back to minimum magnification by selecting

**View**  
**Zoom Out (All)**

## Displaying Records Stored on File > Compressed View > Measuring Signal Levels


To measure the signal at any point on the displayed record, use the mouse to drag the vertical readout cursor to the desired part of the trace. Fine positioning of the cursor can be achieved by pressing the left or right arrow keys with the mouse pointer over the selected cursor.



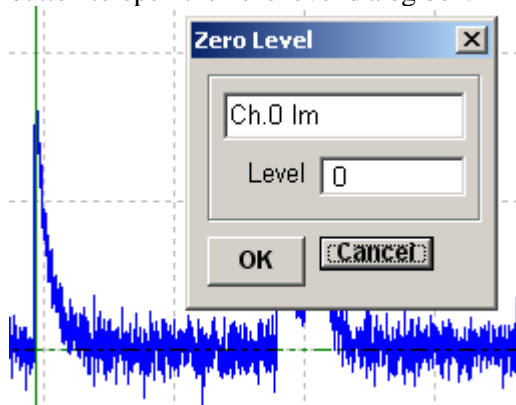
The signal level of the trace(s) at the cursor position is displayed at the bottom of each channel. Time measurements are made relative to the location of the  $t=0$  cursor. Signal levels are measured relative to each channel's horizontal zero level cursor.

### Zero levels

The signal zero level for each channel is indicated on the display by a green or red horizontal dotted line. It is initially set to the absolute zero voltage level of the analog input.

The zero level for a channel can be adjusted by moving the mouse over any point on the zero level line (the mouse cursor changes to ) , holding down the left mouse button and dragging the zero level up or down.

The zero level can also be entered numerically by moving the mouse over the zero level line clicking the right mouse button to open the zero level dialog box.



and entering a zero level value (in integer A/D converter units) into the **Level** box.



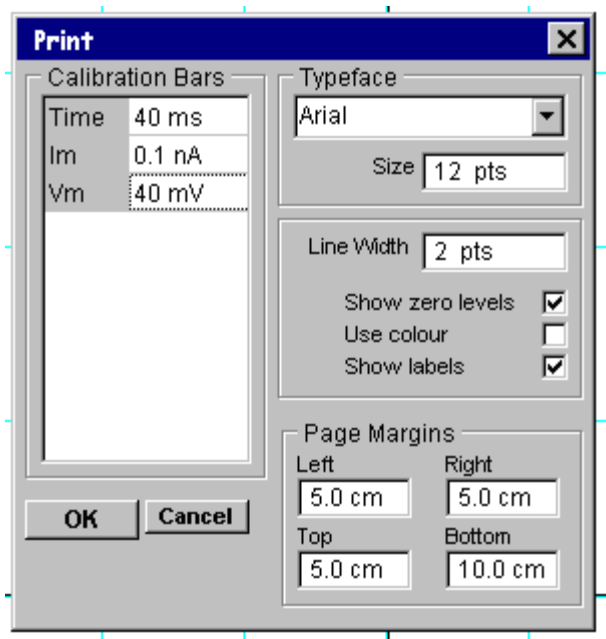
## Displaying Records Stored on File > Compressed View > Printing Records (Compressed View)

To print the records displayed on the screen, select

**File**

**Print**

To open the dialog box.



You can set the size of the plotted record on the printed page, by adjusting the size of the **page margins**.

The type face used to print text can be selected from the **font name** list and the type size entered into the **font size** box. The thickness of the lines used to draw the signal traces can be set using the **line thickness** box.

Vertical and horizontal calibration bars are added to the plot to indicate the units and scaling of the plotted signals. You can define the size of the bars by entering values into the **calibration bars** table.

The position of the zero level for each plotted trace is indicated by a horizontal dotted line. Zero levels can be disabled by un-checking **show zero levels**. Plot labelling can be disabled by un-checking the **show labels** check box. The use of colours within the plot can be disabled by un-checking **Use colour**.

When all plot parameters have been set, click the **OK** button to initiate printing.

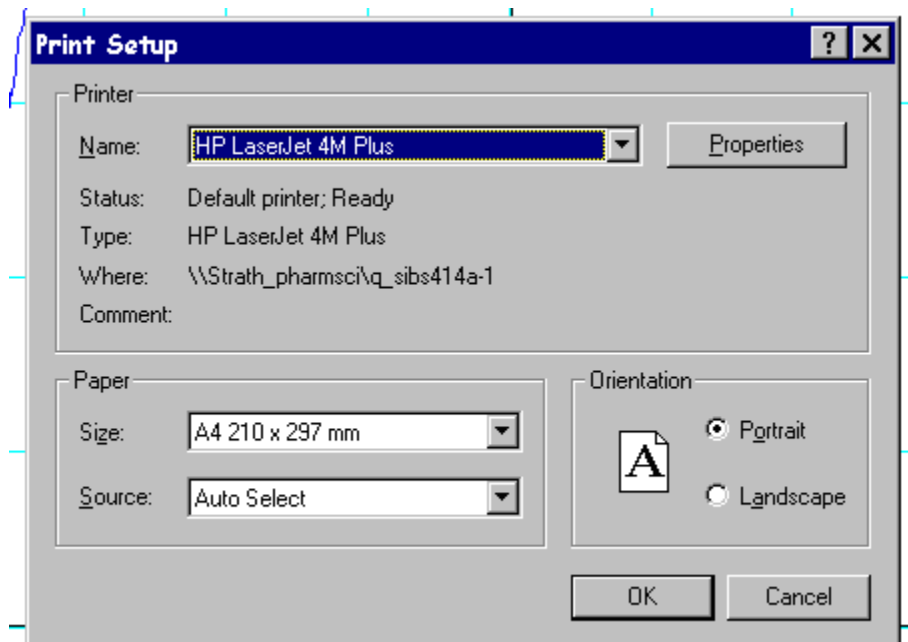
### Choosing a printer and output format.

To choose a printer and to select the paper format, select

**File**

**Print Setup**

to open the dialog box.



A printer can be selected from the list of currently installed printers. The orientation of the plot on the page can be selected as either **portrait** or **landscape**.

## Displaying Records Stored on File > Compressed View > Copying Records (Compressed View)

The displayed signal record(s) can be copied to the Windows clipboard in a variety of formats – a data table, an image, a WinEDR data record

### Copying data values

Each signal record consists of an array of digitised sample values. A table of data values for the active display record can be copied to the clipboard by selecting

**Edit**

**Copy Data**

The data is placed on the clipboard as a table, containing the scaled values for each sample in the record, in the measurement units defined for each channel. The table is stored in tab text format, allowing the data to be copied into programs such as spreadsheets and graph plotting packages, using an Edit/Paste command. (Note that due to limitations in the capacity of the Windows clipboard data points may be skipped to keep the size of the copied record within clipboard storage limits.)

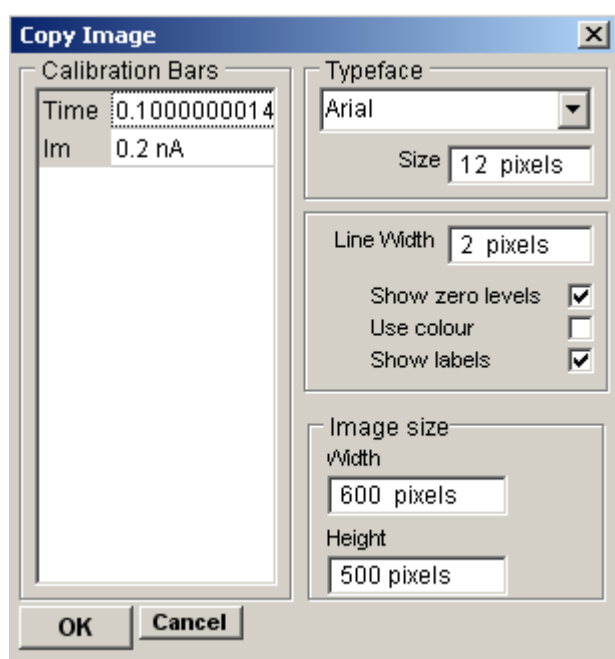
### Copying the displayed image

The signal record(s) on the display can be copied to the clipboard as a bit mapped image by selecting

**Edit**

**Copy Image**

to open the dialog box.



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.

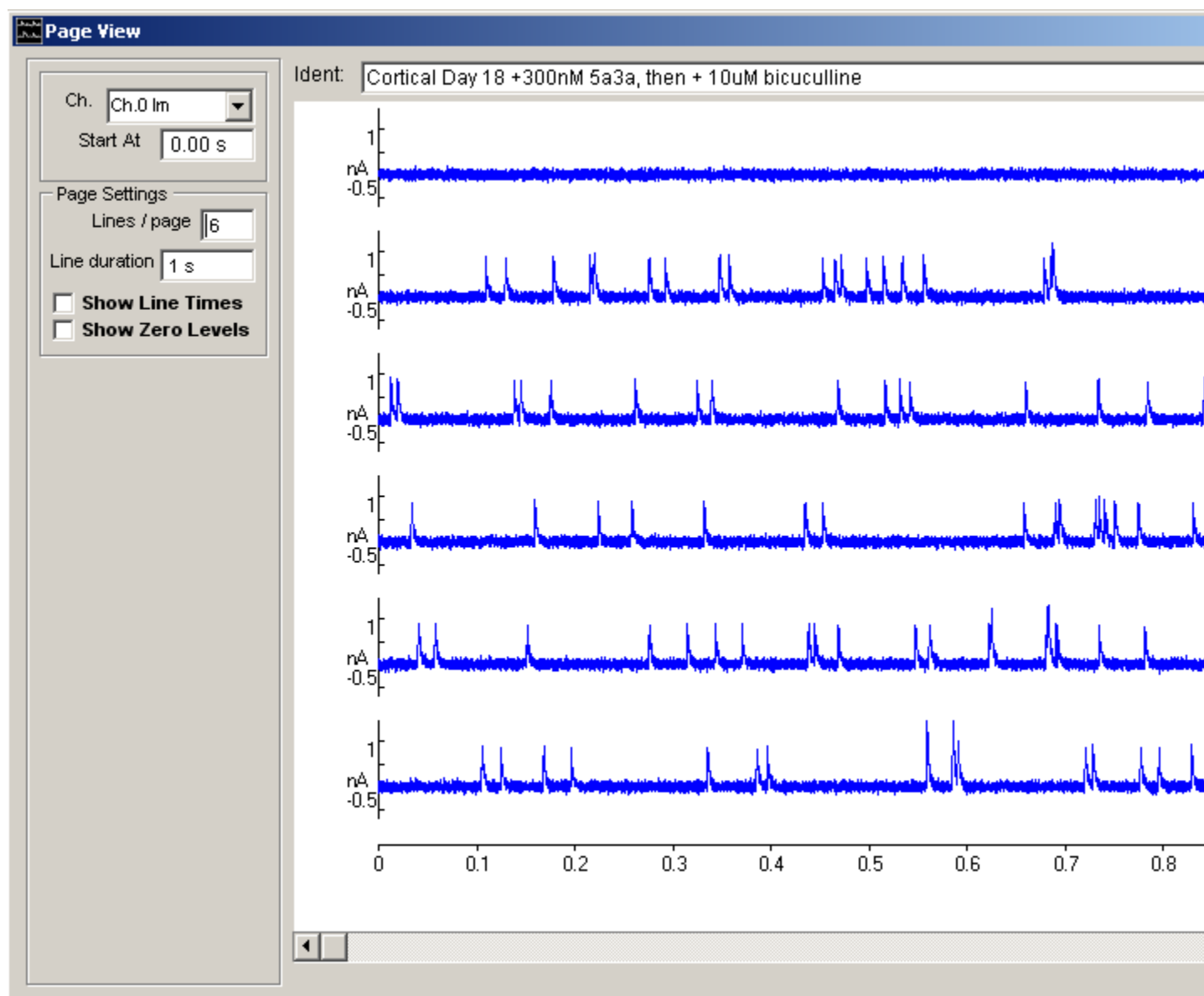
Calibration bars, zero levels and text font, size and line thickness can be set in the same way as for a printed image.

When the image parameters have been set, click the **OK** button to copy the image to the clipboard.



## Displaying Records Stored on File > Page View > Page View

To view a section of a selected channel within the digitised recording as a series of sequential records on a page, select **View**  
**Page View**  
to open the window.



One channel of the digitised recording is displayed (uncompressed) as a sequence of lines within the display window.

Dragging the scroll bar at the bottom of the display shifts the display forwards and backwards throughout the recording, one line at a time. The starting time of the displayed sequence of lines within the recording is indicated in the **Start At** box. (Note. Entering a value into **Start At** moves the sequence immediately to that time.)

The channel on display (if there is more than one in the recording) can be changed using the channel selection list

The number of lines (1-16) per page can be set using **Lines/Page** entry box and the number of sample points per line using the **Points/Line** box. (A maximum of 32768 samples can be displayed per page which limits the number of points per line to 32768/No. Lines).

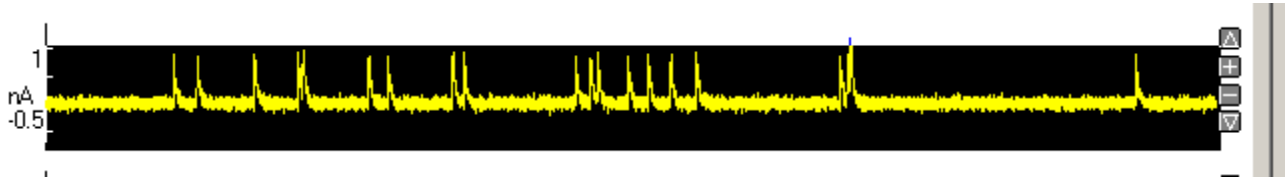
Tick the **Show Line Times** check box to display the time at which each line occurs within the recording at the beginning

of each line.

Tick the **Show Zero Levels** check box to display the signal zero level as a horizontal dotted line on each line.

### Vertical Magnification

The vertical magnification of each line can be expanded 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 and position of each line within the recording can also be adjusted using the



buttons at the right edge of each plot.

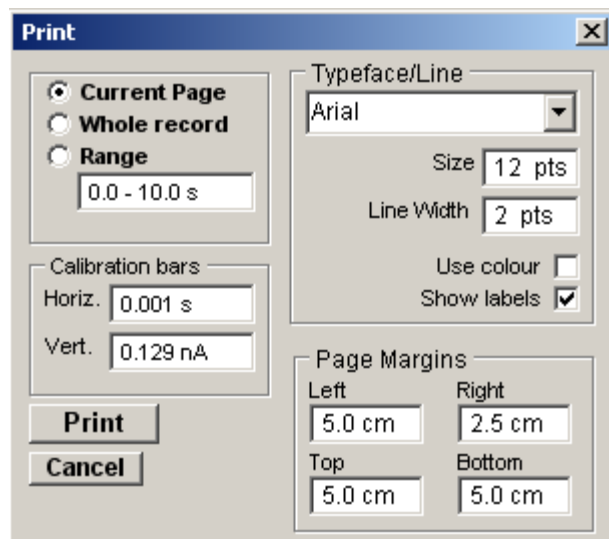
## Displaying Records Stored on File > Page View > Printing (Page View)

To print a section of the digitised recording as a series of lines, select

**File**

**Print**

to open the dialog box.



There are 3 printing options:

**Current Page:** Select the **Current Page** option to print out the current set of lines on display on a single printed page.

**Whole Record:** Select the **Whole record** option to print out the whole recording on to a series of printed pages. (Note that a large number of pages may be required for long data files acquired at high samplings rates.)

**Range:** Select the **Range** option to print out the recording over the specified range of times.

The type face used to print text can be selected from the **Typeface** list and the type size entered into the **Size** box. The thickness of the lines used to draw the signal traces can be set using the **Line Width** box.

Vertical and horizontal calibration bars are added to the plot to indicate the units and scaling of the plotted signals. You can define the size of the bars by entering values into the **calibration bars** table.

You can set the size of the plotted record on the printed page, by adjusting the size of the **page margins**.

To initiate printing, click the **Print** button.

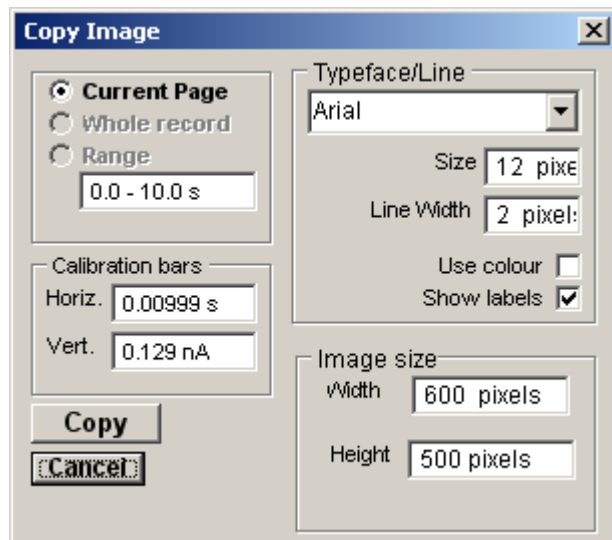
## Displaying Records Stored on File > Page View > Copying (Page View)

The currently displayed page can be copied to the clipboard as an image by selecting

**Edit**

**Copy Image**

to open the dialog box.



The dimensions of the image, can be set using the width and height image size boxes. Calibration bars, zero levels and text font, size and line thickness can be set in the same way as for a printed image.

To copy the image to the clipboard., click the **Copy** button

## Detecting & Analysing Events > Event Detection

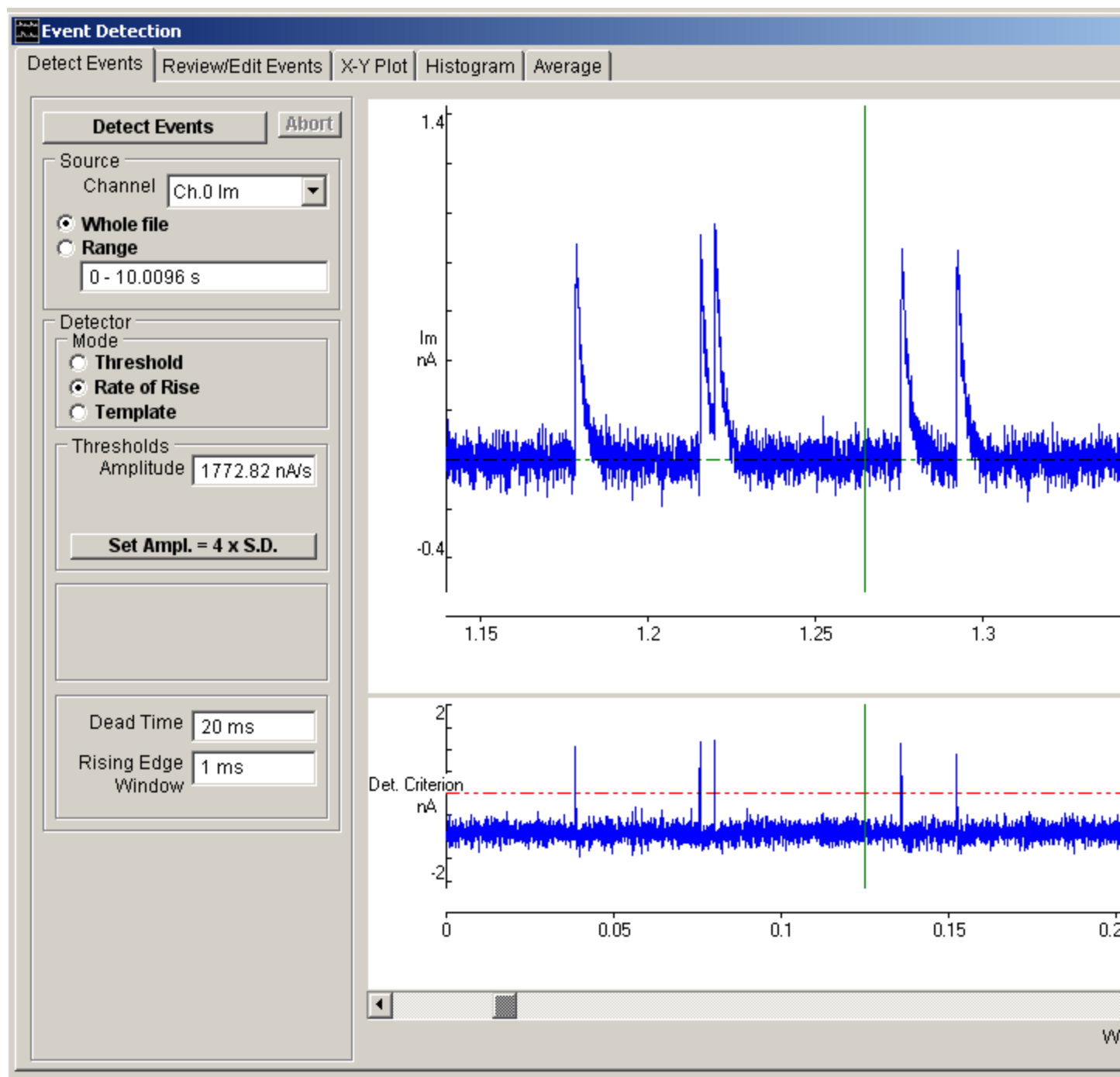
WinEDR's event detection module can be used to locate individual transient signals (e.g. miniature synaptic signals, actions potentials) occurring within a continuous recording. The frequency of occurrence and inter-event time intervals can be analysed within WinEDR. Detected signals can also be exported in a WCP format data file for more detailed analysis using WinWCP (a program in the Strathclyde Electrophysiology Software series designed for the analysis of transient signals).

To scan a recording for signals, select

### Analysis

#### Detect Events

to open the Event Detection module.



The module is split into 5 pages

- Detect Events
- Review/Edit Events

- X/Y Plot
- Histogram
- Average

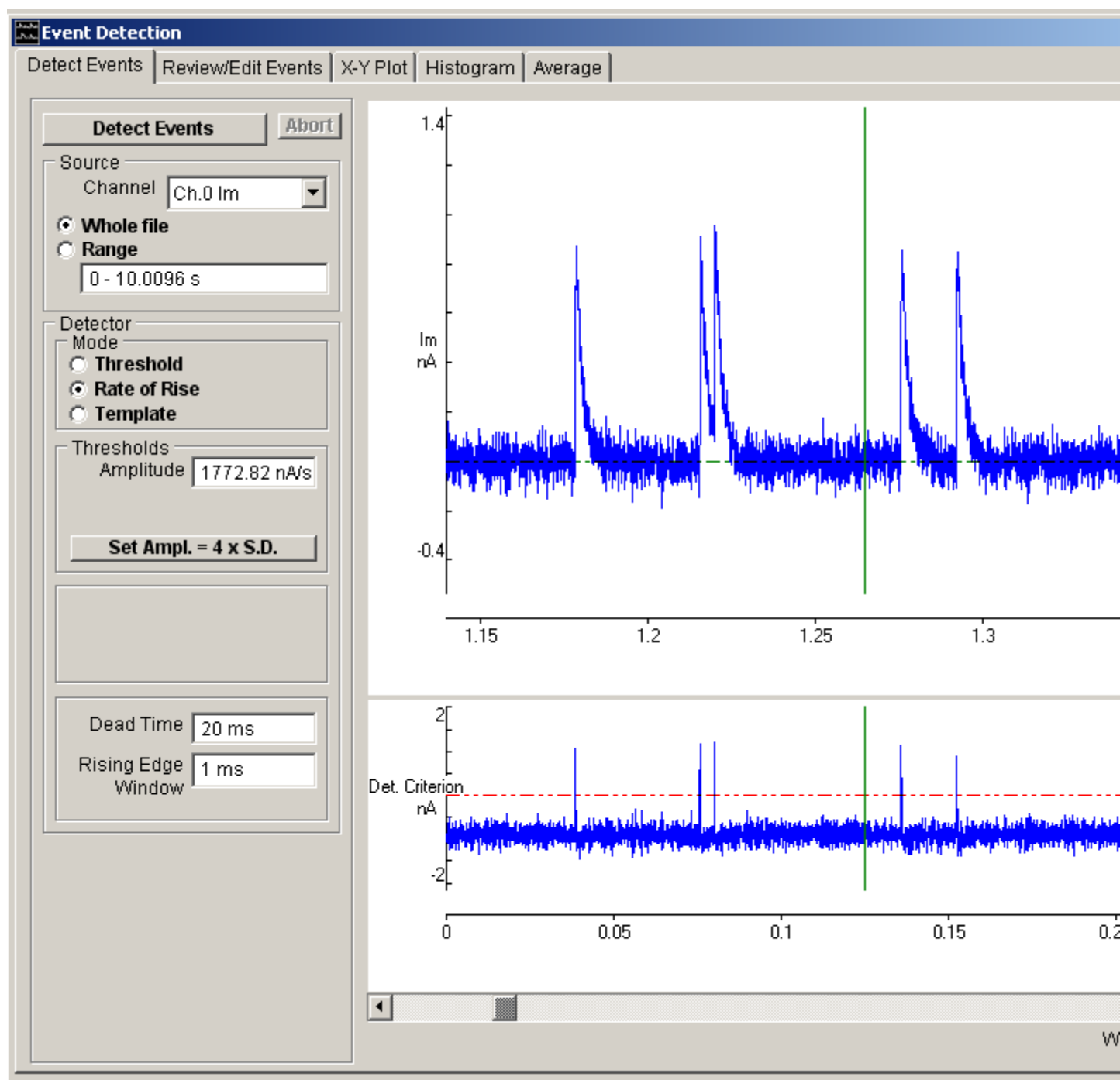
The **Detect Events** page provides the tools for setting up and running an event detection scan of the continuous recording.

The **Review/Edit Events** page allows the visual inspection of detected events and the manual insertion or deletion of events.

The **X/Y Plot** page allows event detection times, inter-event intervals and frequency to be plotted.

## Detecting & Analysing Events > Detecting Events

To detect events within a continuous record, select the **Detect Events** page. The recording to be scanned is displayed in the upper panel and the detection criterion derived from it is displayed in the lower.



Three different types of event detection method are supported:

- **Threshold:** The amplitude **threshold** method uses a baseline-tracking amplitude threshold algorithm, the detection criteria being that the signal exceeds the threshold level for more than a predetermined period of time. The signal baseline level is computed from a running average used to automatically adjust the threshold for slow changes in baseline level.
- **Rate of rise:** The **rate of rise** method uses a signal detection criterion based upon the signal rate of change computed using a 5-point Savitsky-Golay differentiation algorithm.
- **Template:** The **template matching** method is an implementation of the optimal template matching algorithm

developed by Clements & Bekkers (1997) An exponentially decaying waveform template defined by the equation

$$y = A \left( 1 - \exp \left( - \frac{t}{\tau_{rise}} \right) \right) \exp \left( - \frac{t}{\tau_{decay}} \right) + C$$

is slid point-by point along the digitised signal, its amplitude,  $A$ , and offset,  $C$ , being adjusted at each step using least squares methods to obtain the best fit. The ratio between best fit template amplitude and residual sum of squares is used as a detection criterion. See Clements & Bekkers (1997) for details of the method.

The rate of rise and amplitude threshold methods are applicable to any shape of signal while the template matching method is specifically designed for decaying exponential signals such as postsynaptic currents or potentials.

To scan a segment of the continuous digitised recording for event:

1. **Source:** Select the **Whole File** option to scan the complete file, or select **Range** and enter a time interval defining the section of the recording to be scanned.
2. **Source:** If there is more one channel within the recording, select the channel to be scanned for signals, from the **Channel** list.
3. **Mode:** Select the event detection method (**Amplitude**, **Rate of rise** or **Template**).
4. **Thresholds (Amplitude mode):** Set the level which the signal detection criterion has to cross for an event to be detected in the **Amplitude** box (or adjust the threshold cursor in the Det. Criterion display). Set the period of time which the signal has to remain above the amplitude threshold before detection is accepted in the **Time** box. Set the period of time over which the baseline tracking average is computed in the **Baseline Tracking Time** box. (The smaller the time period, the faster the tracking rate of the baseline.)

Detector Mode

☒ **Threshold**

☐ **Rate of Rise**

☐ **Template**

Thresholds

Amplitude 0 nA

Time 0 ms

**Set Ampl. = 4 x S.D.**

Baseline Tracking Time

1000 ms

**Thresholds (Rate of Rise mode):** Set the level which the signal detection criterion has to cross for an event to be detected in the **Amplitude** box (or adjust the threshold cursor in the Det. Criterion display).

Detector Mode

☐ **Threshold**

☒ **Rate of Rise**

☐ **Template**

Thresholds

Amplitude 1702.32 nA/s

**Set Ampl. = 4 x S.D.**

**Thresholds (Template mode):** Set the level which the signal detection criterion has to cross for an event to be detected in the **Amplitude** box (or adjust the threshold cursor in the Det. Criterion display). Enter the rising and decaying time constants which define the shape and duration of the template waveform into the **Tau(rise)** and



**Tau(decay)** boxes. (Suitable values can be obtained by detecting a typical signals using one of the other detection methods, exporting the record to WinWCP and fitting an MEPC curve to it.).

Detector Mode

☐ Threshold

☐ Rate of Rise

☒ Template

Thresholds

Amplitude 55.78

Set Ampl. = 4 x S.D.

Template

Tau(rise) 0.1 ms

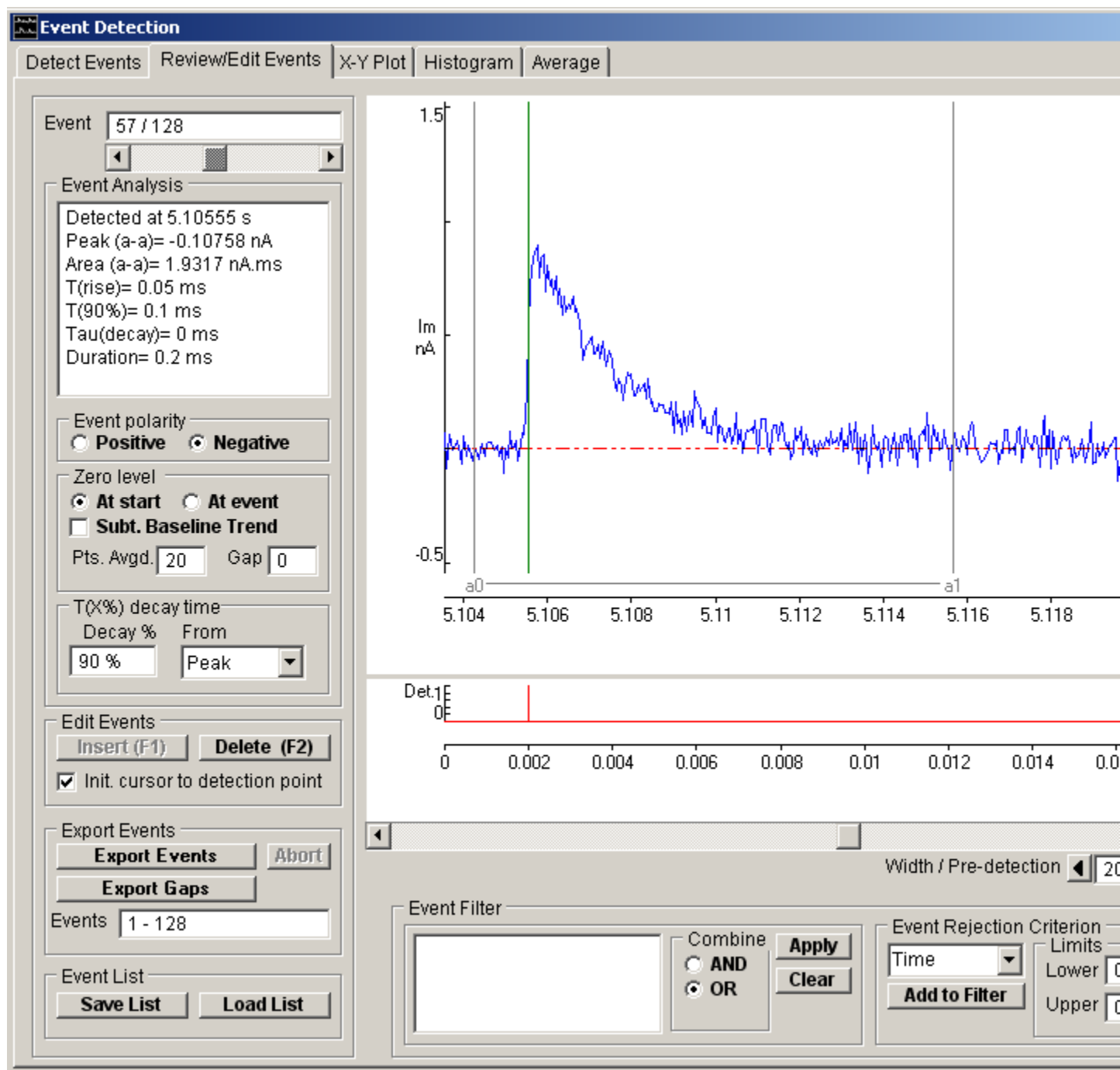
Tau(decay) 10 ms

(Note. The **Set Amp.=4xS.D.** button can be used to set the amplitude threshold to 4x the standard deviation computed from the displayed signal).

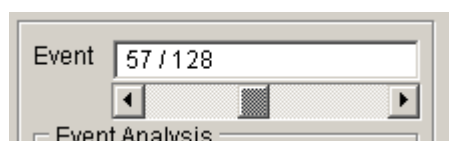
5. **Dead Time:** To avoid repeated detection of the same signal, set the time period before another signal can be detected in the **Dead Time** box. (Usually set equal to the expected duration of the longest signals being detected.)
6. Rising Edge Window:
7. Click **Detect Events** to begin the event detection process.

## Detecting & Analysing Events > Reviewing & Editing Events

After a list of detected events has been created, each event can be inspected on the **Review/Edit Events** page.

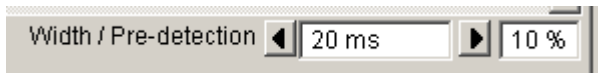


The detected event is displayed in the upper panel with the detection point displayed as a vertical red line in the lower. Each detected event can be selected for display using the event selection scroll bar or by entering the desired event number into the box.



Regions before and after the event can be inspected by shifting the display window using the scroll bar at the bottom of the display. The period of time displayed around the detected event can be set by entering a value into the display **Width** box. The position of the detected event within the display window can be adjusted by setting the **Pre-detection**

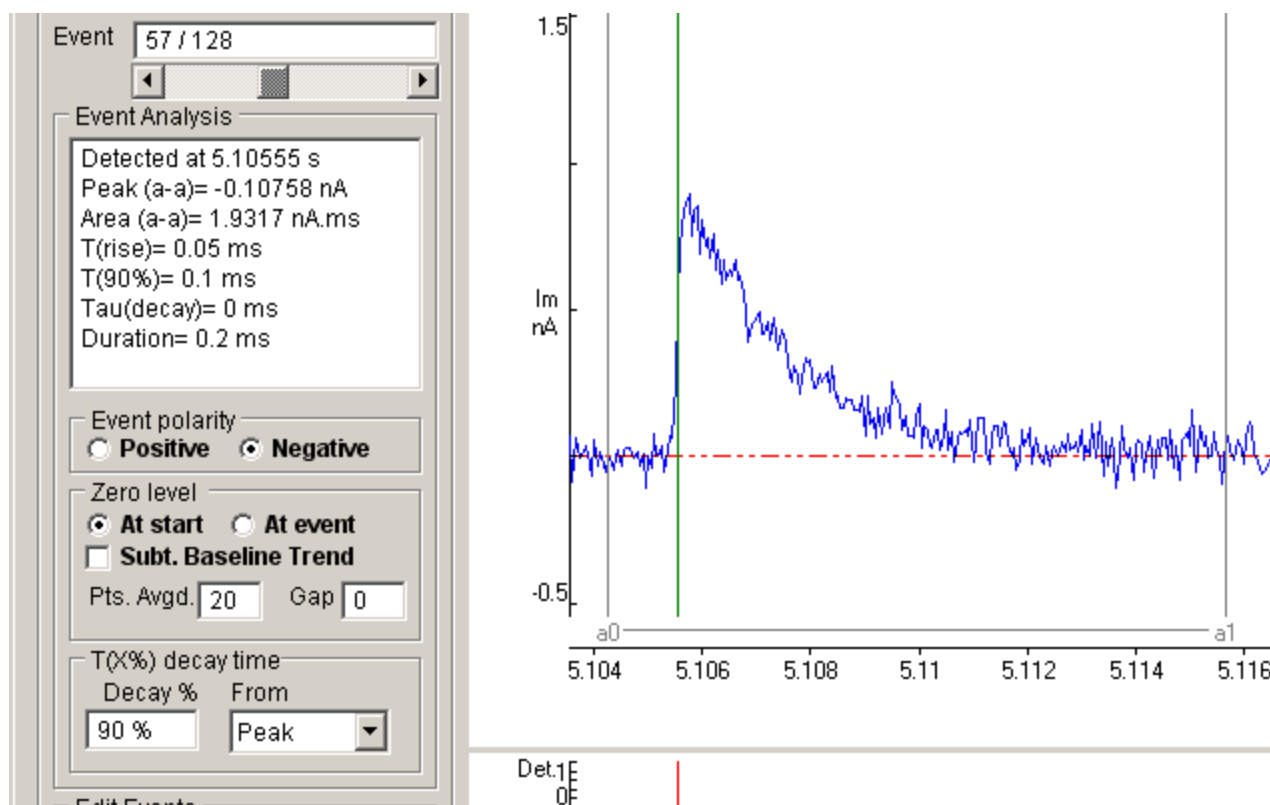
percentage.



**Note** . When measurements are being made, the display width should be adjusted to display one event only.

## Detecting & Analysing Events > Event Analysis

The **Event Analysis** box displays a set of waveform measurements, computed by analysing the signal waveform within the display window.



**Analysis Region:** The pair of cursors (**a0** and **a1**) define the region within the event display window containing the waveform to be analysed. This region can be adjusted to exclude stimulus artefacts from the waveform analysis.

Measurements include:

- The time at which the event was detected (**Detected at**).
- The peak amplitude (positive or negative depending upon the setting of the **Polarity** option) of the event (**peak**).
- The integral of the event waveform (**area**).
- The 10%-90% rise time of the event (**T(rise)**).
- The time to decay to a user set percentage (0-100%) after the peak value (**T(x%)**)
- The time constant of exponential decay (**Tau(decay)**).

The following options configure the waveform analysis:

**Event Polarity:** Set the **Polarity** option to determine whether the signals are to be treated as positive- or negative-going waveforms.

**Zero Level:** Select the **At start** option to use the average of a series of sample points at the start of the event detection window as the zero level for amplitude measurements or the **At event** option to use a block of points immediately preceding the event. The number of samples to be averaged is defined by the **Pnts Avgd.** box. To shift the points used to compute the zero level away from the start or event, enter a non-zero number of points in the **Gap** box. Select the **Subt. Baseline Trend** option to subtract any upward or downward trends caused by drifting of the baseline on which the detected signals are superimposed. (A cubic spline interpolation between signal baseline estimates obtained from the

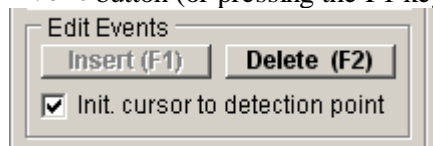
average of 10 samples immediately preceding each event is subtracted from the each event waveform.

**T(X%) Decay Time:** Enter the required decay time percentage for the signal decay time measurement in the **T(X%)** box. Select the **Peak** option to measure the decay from the peak of the signal, the **50% rise** option to measure from the mid-point of the signal rising phase, or the **a0 cursor** option to measure from the position of the a0 cursor.

## Detecting & Analysing Events > Adding/Removing Events

### Manual Event Editing

The currently displayed event can be removed from the event list by clicking the **Delete Event** button (or pressing the F2 key). An event can be added to by placing the vertical display cursor over the position of an event and clicking the **Insert Event** button (or pressing the F1 key).

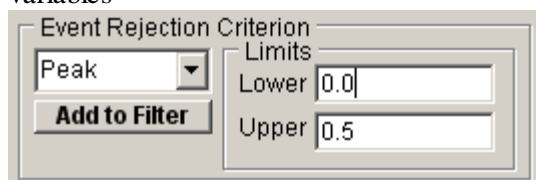


### Automatic Event Filtering

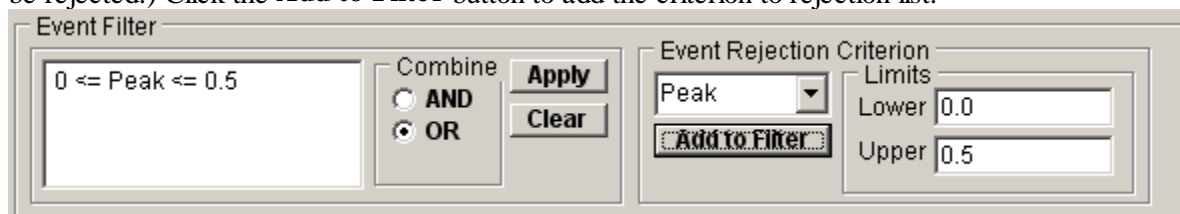
Events can be automatically deleted from the detected event list using a set of filter criteria based upon the event analysis measurements.

To delete events matching a defined set of criteria :

1. Select the waveform measurement to be used as the **Event Rejection Criterion** from the list of measurement variables



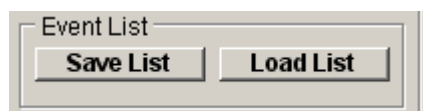
Enter the limits of the range of values which will result in the rejection of the event in the **Upper** and **Lower** limits boxes. (The example shows a criterion where events with a peak amplitude  $\geq 0.0$  pA and  $\leq 0.5$  pA will be rejected.) Click the **Add to Filter** button to add the criterion to rejection list.



2. If additional criteria are required, repeat step 1., selecting another variable and limits. Choose the way that the criteria are to be combined, selecting **AND** if a match to all criteria in the list is required for rejection or **OR** if a match to any criterion will suffice.
3. Click the **Apply** button to remove events matching the defined criteria.

### Saving/Loading detected event lists

**Note.** Events are permanently deleted from the event list by the filter. It is prudent to save the original detected event list before applying the filter, so it can be restored if necessary. The list of event detection times can be saved as an ASCII text file by clicking the **Save List** button and entering the name and location of a file into the Save Event List dialog box. A list of event times can be re-loaded (replacing the existing list) by clicking the **Load List** button and entering the name of a file in the Load Event List dialog box.





## Detecting & Analysing Events > Exporting Events

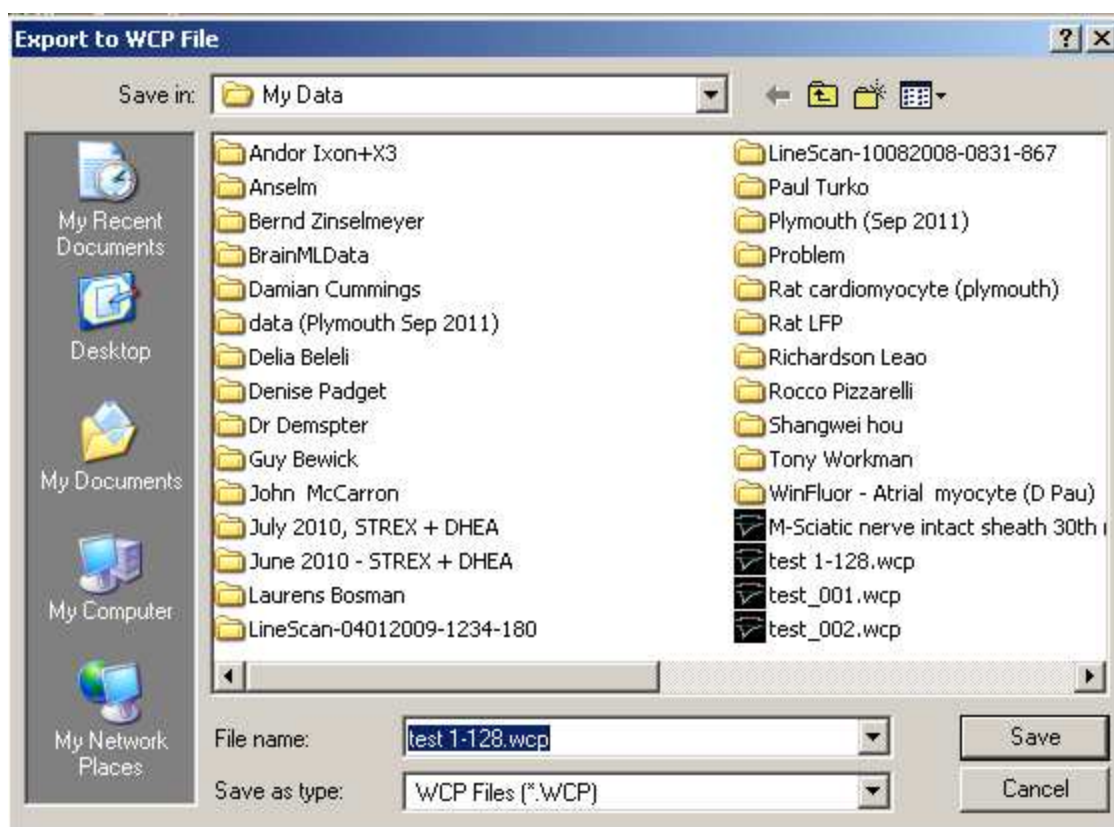
The digitised waveforms of the detected events can be extracted from the data file and exported as individual records for analysis by WinEDR's companion program WinWCP which has features for signal averaging, curve fitting and more detailed waveform analysis.



### Exporting Events

To export detected events :

1. Enter the range of events to be exported in the **Events** box.
2. Click the **Export Events** button and select the name of a WCP data file to hold the data.



The record size of the exported file is determined by the size of the Review/Edit Events display window.

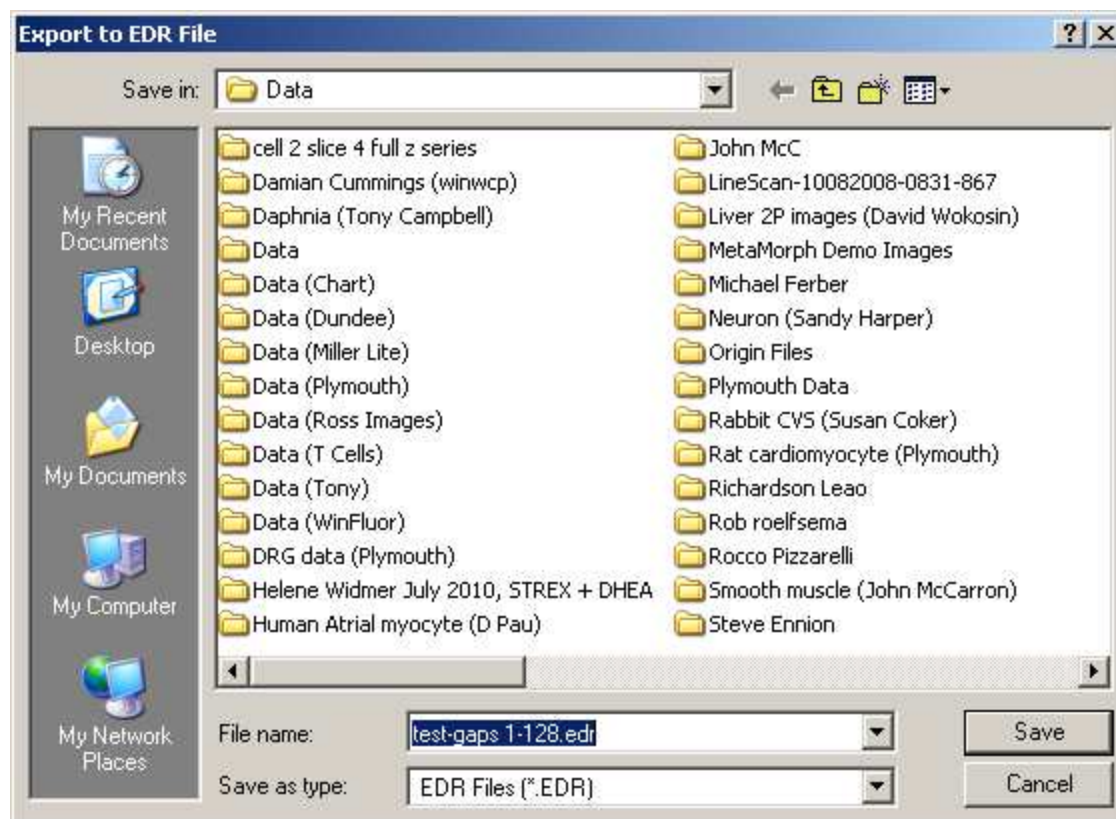
### Exporting the Gaps Between Events

The sections of the digitised recording which do not contain events can also be extracted and saved to another EDR format data file.

To extract and save the gaps between events:

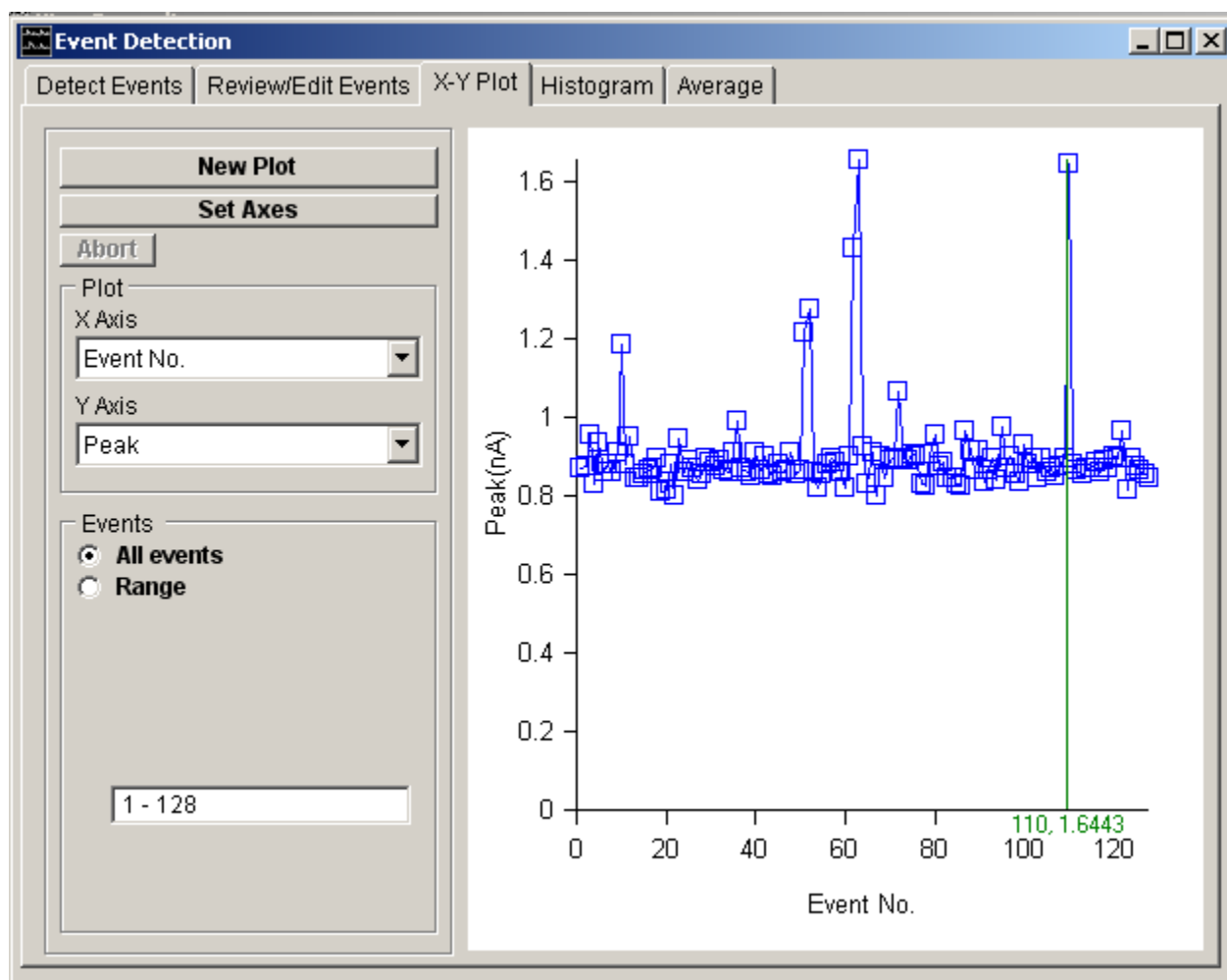
1. Enter the range of events to be exported in the **Events** box.
2. Click the **Export Gaps** button and select the name of a new EDR data file to hold the data.





## Detecting & Analysing Events > Plotting X-Y Graphs of Event Analysis Measurements

The **X-Y Plot** page allows the event analysis measurements for the series of detected events to be plotted against each other.



To create an X-Y plot :

1. Select the variable to be plotted on the horizontal axis from the **X Axis** variable list.
2. Select the variable to be plotted on the vertical axis from the **Y Axis** variable list. (If Rate has been selected enter the interval over which the event frequency is to be calculated.)
3. Select the **All Events** option to plot the results from all events in the list or select **Range** and enter a selected range of events.
4. Click the **New Plot** button to display the plot.
5. If you want to customise the axes ranges, click the **Set Axes** button.

The values of the x,y points in the plot can be read out using the blue readout cursor.

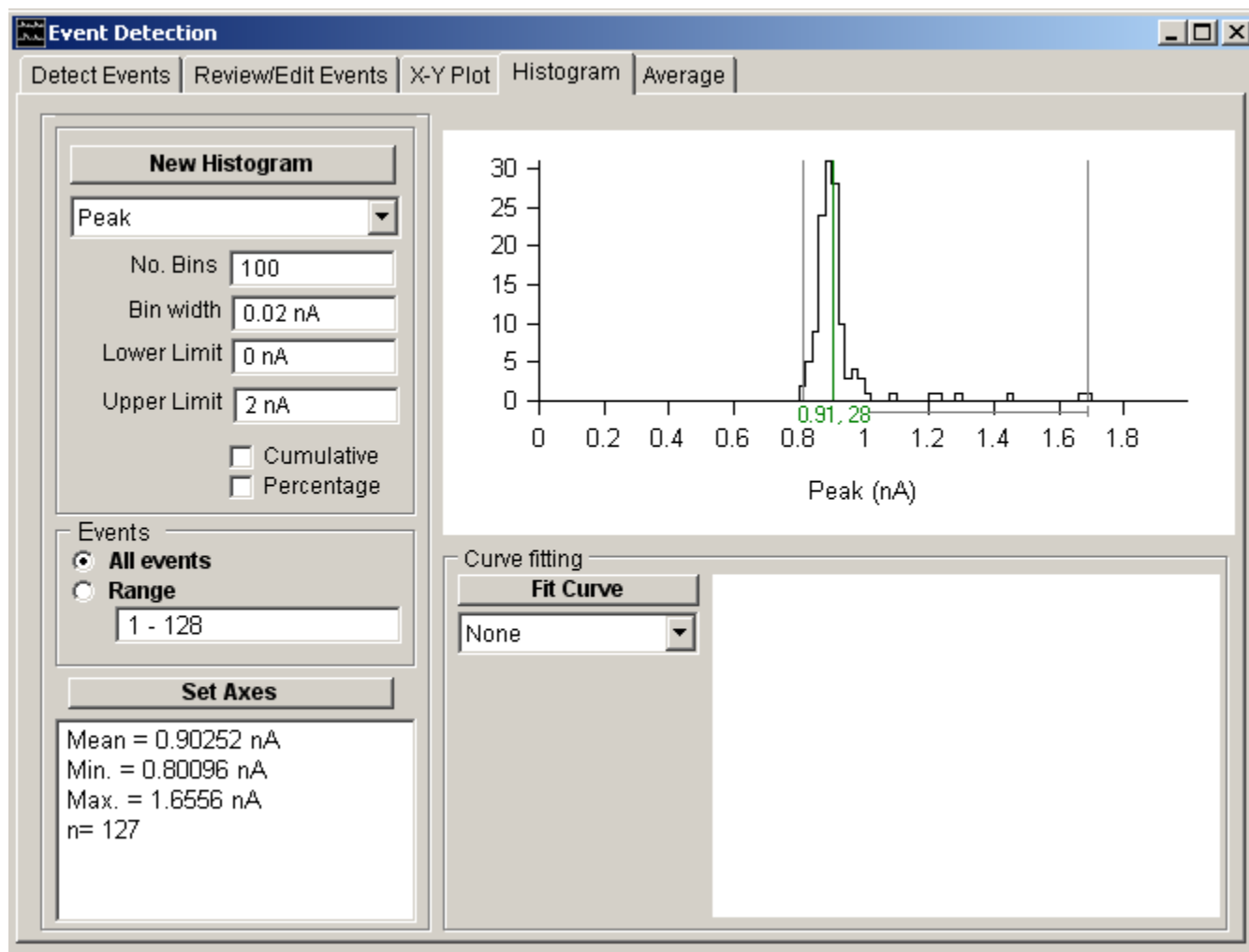
Variables that can be plotted include :

- The time at which the event was detected (**Detected at**).
- The time interval between successive events (**Interval**)
- The instantaneous event frequency (i.e. the reciprocal of Interval) (**Inst. Frequency**).

- The average event frequency computed over a defined period of time (**Rate**).
- The peak amplitude (positive or negative depending upon the setting of the **Polarity** option) of the event (**peak**).
- The integral of the event waveform (**area**).
- The 10%-90% rise time of the event (**T(rise)**).
- The X% decay time after peak (**T(X%)**)
- The time constant of exponential decay (**Tau(decay)**).
- Signal baseline level determined just before the onset of the signal (**Baseline**)

## Detecting & Analysing Events > Plotting Histograms of Event Analysis Measurements

The **Histogram** page allows histograms of the distribution of event analysis measurements to be plotted.



### Plotting a Histogram

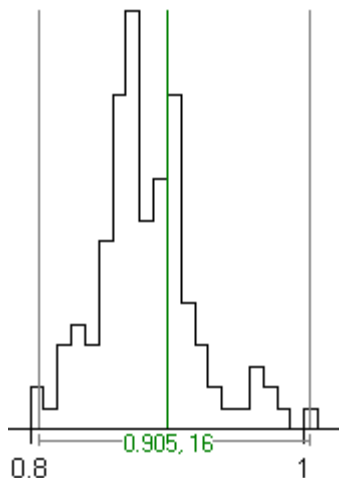
To plot a histogram :

1. Select the variable to be plotted from the **Histogram** variable list.
2. Select the **All Events** option to plot the results from all events in the list or select **Range** and enter a selected range of events.
3. Define the upper and lower limits of the range of variable values to be included in the histogram in the **Range** boxes. (Values outside this range are excluded from the histogram.)
4. Set the number of histogram bins into which the histogram range is divided, in the **No. Bins**. box.
5. Tick the **Percentage** check box to plot the histogram vertical axis as a percentage of the total number of events (rather than number of events in each bin).
6. Tick the **Cumulative** check box to plot a cumulative histogram.
7. Click the **New Histogram** button to display the plot.
8. If you want to customise the axes ranges, click the **Set Axes** button.

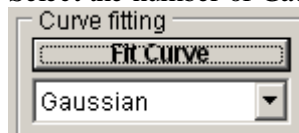
## Fitting Gaussian Curves to Histograms

To fit a gaussian curve to a histogram:

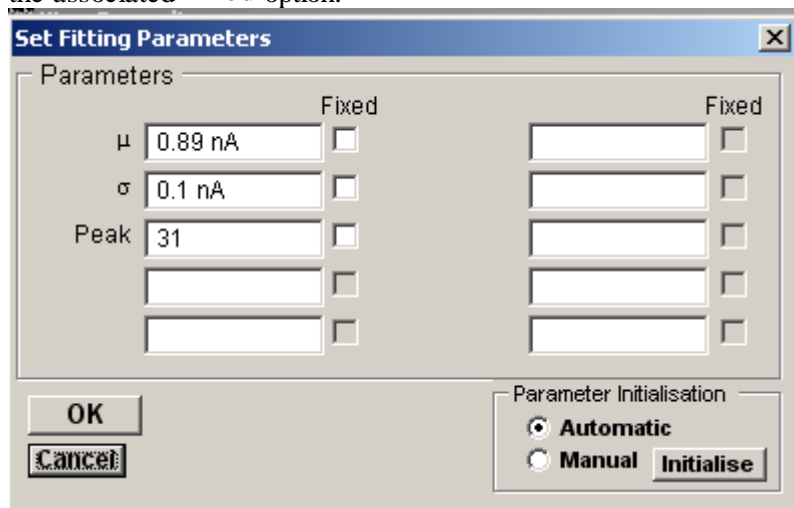
1. Define the range of amplitudes containing the peak(s) to be fitted using the pair of grey 'I' region of interest cursors.



2. Select the number of Gaussian functions to be fitted from the **Curve Fitting** list.

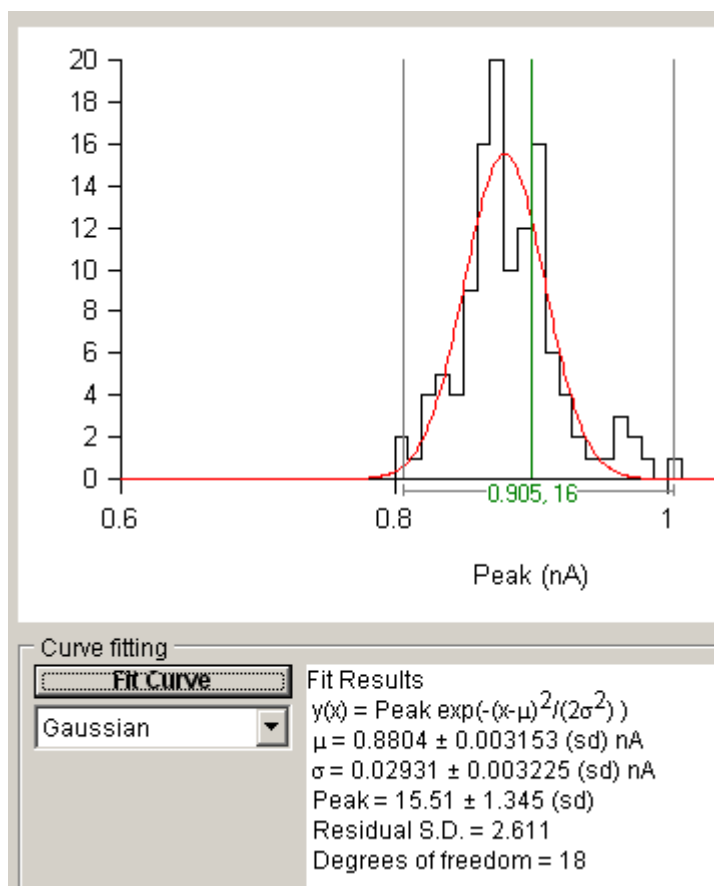


3. Click the **Fit Curve** button and enter an appropriate set of initial guesses for the function parameters. A set of initial guesses are computed automatically, but it is often necessary to adjust these to better match the location and size of the observed histogram peaks. Individual parameters can also be fixed at their initial values by ticking the associated **Fixed** option.



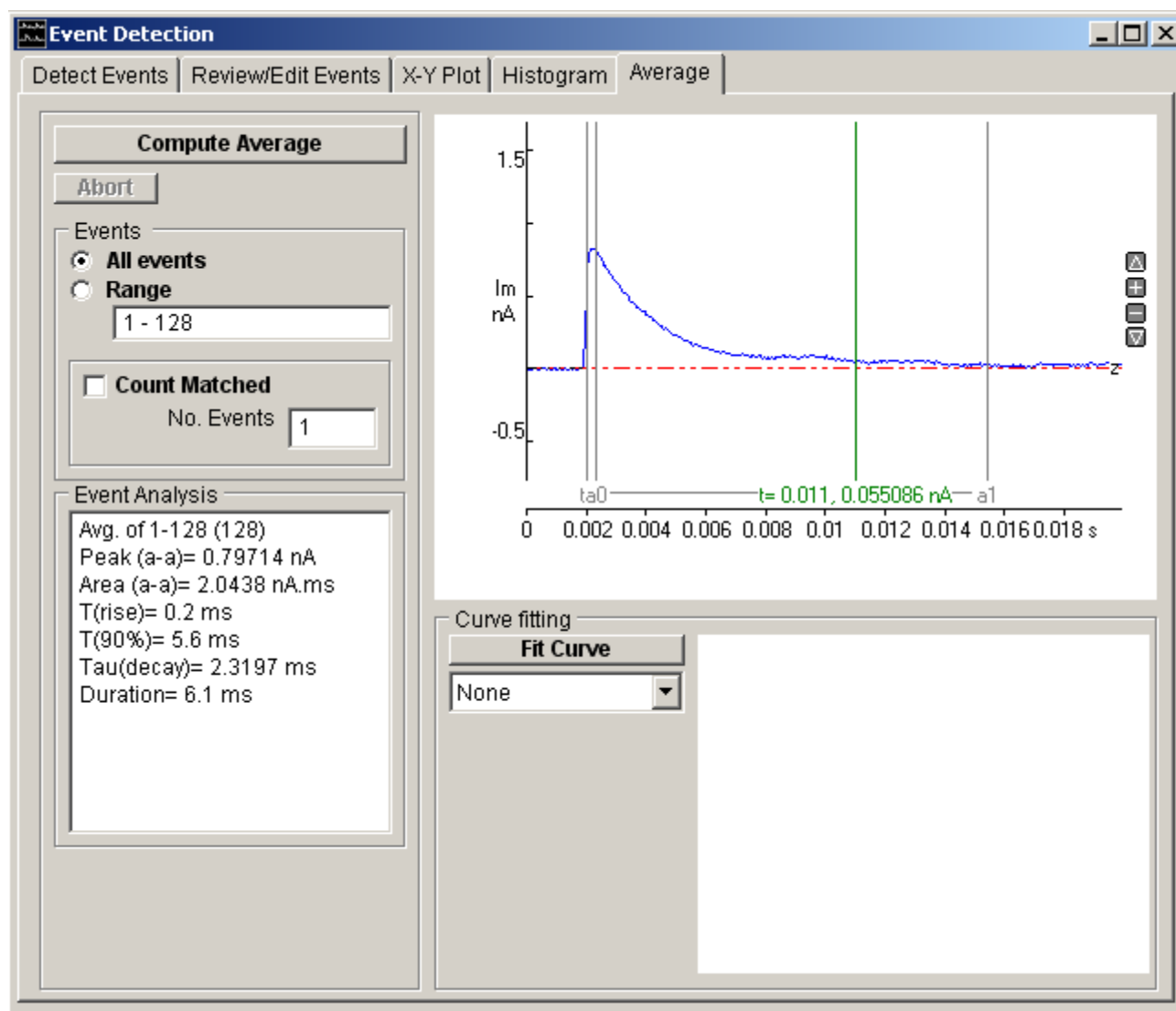
Click the **OK** button to begin fitting.

The best fitting gaussian function(s) are superimposed (in red) on the histogram. The values of the best fitting function parameters along with their estimated standard errors are displayed in curve fitting results area at the bottom of the display.



## Detecting & Analysing Events > Averaging Events

The averaged waveform of a series of events can be computed and displayed on the **Average** page. Averages can be computed from a specified range of events or using the count-matched averaging process to average the  $n$  largest events.



### Computing Averages

To compute the event average waveform:

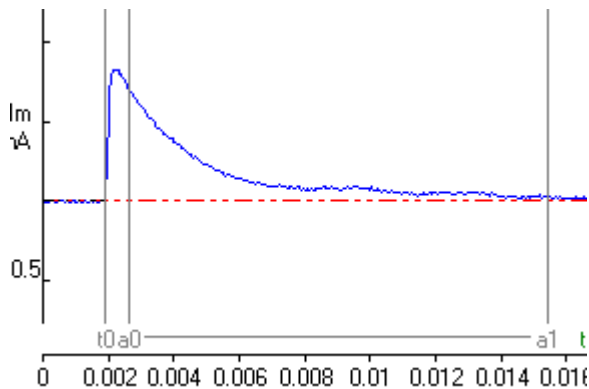
1. Select the **All Events** option to average all detected events or select **Range** and enter a selected range of events.
2. Select the **Count-Matched** option if you wish to compute a 'count matched' average and enter the number of events to be averaged in the **No. Events** box. (The count matched average is an average of a defined number of the largest amplitude events within the selected event range. It provides a method for comparing average event amplitude before and after an experimentally-induced reduction in amplitude which compensates for the failure to detect small events which have fallen below the detection threshold. See Stell & Mody, J. Neuroscience, 2002, Vol 22, RC223.)
3. Set the % of the averaged waveform, to be before the event detection point in the **Pre detection samples** box.
4. Set the Event Analysis Polarity of the average waveform measurements. Select **Positive** for positive-going waveforms and **Negative** for negative-going.
5. Define the peak detection region using the pair of "a" cursors. (This option can be used to exclude stimulus artefacts or other signals feature from the waveform analysis)

- Click the **Compute Average** button to calculate and display the average waveform.

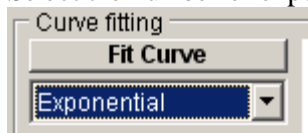
### Fitting curves to averages

To fit one or more exponential functions to the average:

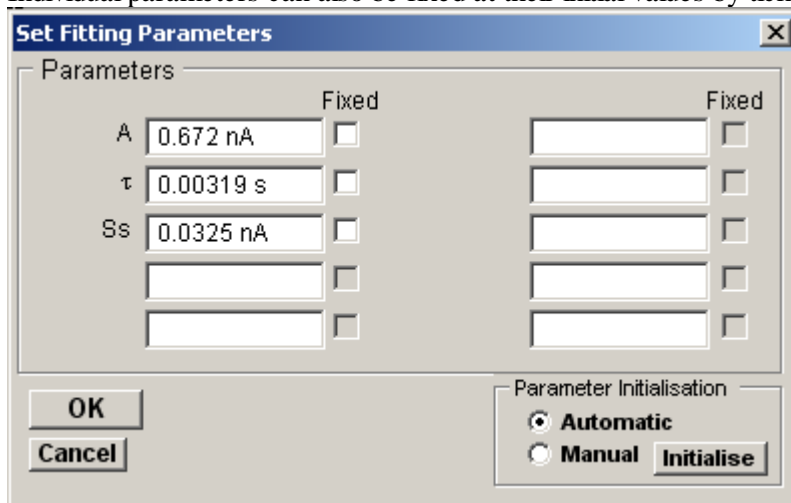
- Define the segment of the average waveform be fitted using the pair of 'a0-a1' analysis region cursors. Define the starting point (time = 0) of the exponential function to be fitted, using the t0 cursor.



- 
- 
- Select the number of exponential functions to be fitted from the **Curve Fitting** list.



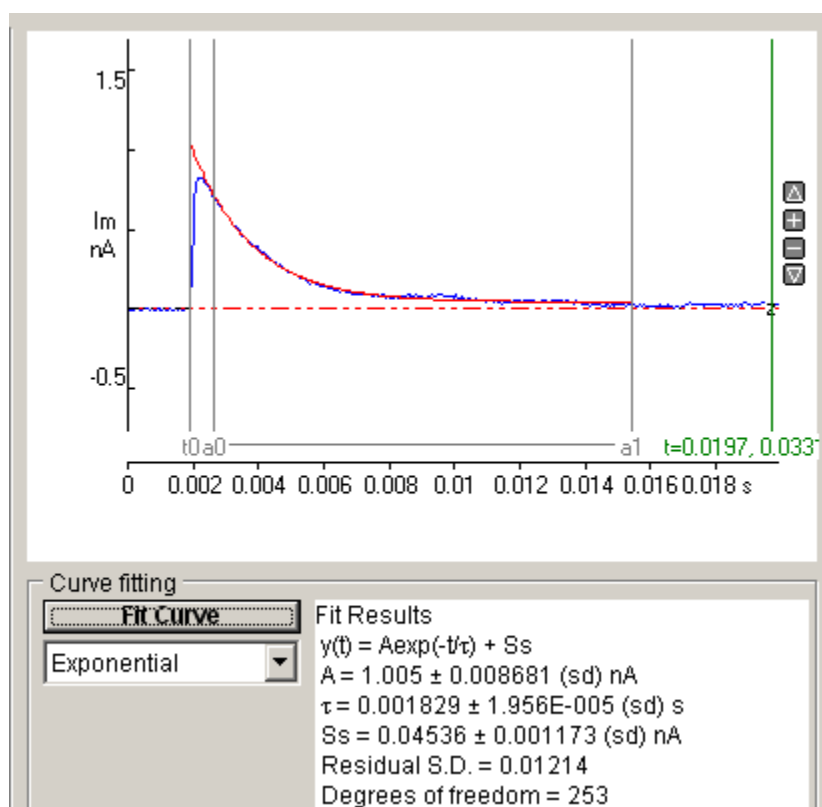
- Click the **Fit Curve** button and enter an appropriate set of initial guesses for the exponential function parameters. Individual parameters can also be fixed at their initial values by ticking its associated **Fixed** option.



Click the **OK** button to begin fitting.

The best fit curve is superimposed in red over the fitted region of the waveform. The parameters of the best fitting equation are displayed in the Curve Fitting results area.





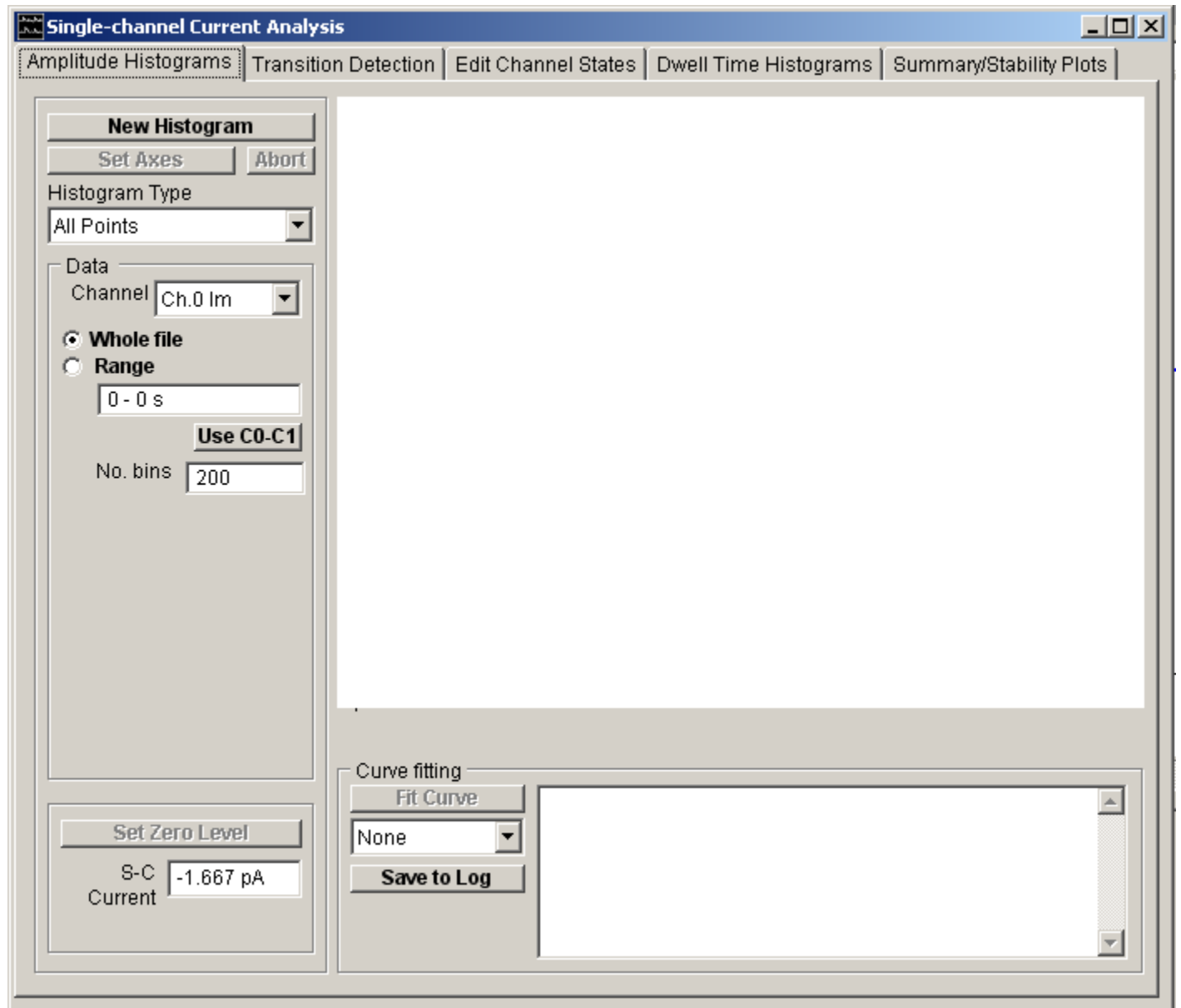
## Analysing Single-channel Currents > Single-channel Current Analysis

To analyse a single-channel current recordings, select

### Analysis

#### Single-channel Current Analysis

to open the window



The module is split into 5 pages each associated with a specific single-channel analysis operation.

- Amplitude Histograms: Provides tools for compiling and analysing the ion channel current amplitude distribution.
- Transition Detection: Provides tools for the detection of channel open/close transitions and the determination of the sequence of channel dwell times spent in each state.
- Edit Channel States: Provides an editor for visually inspecting each detected state, and rejecting artefacts from the analysis.
- Dwell Time Histograms: Provides tools for compiling and analysing histograms of the distribution of the channel open and closed times determined by the transition detection process.
- Stability Plots: Provides tools for determining whether the recording satisfies the necessary stationarity conditions

for a valid analysis.

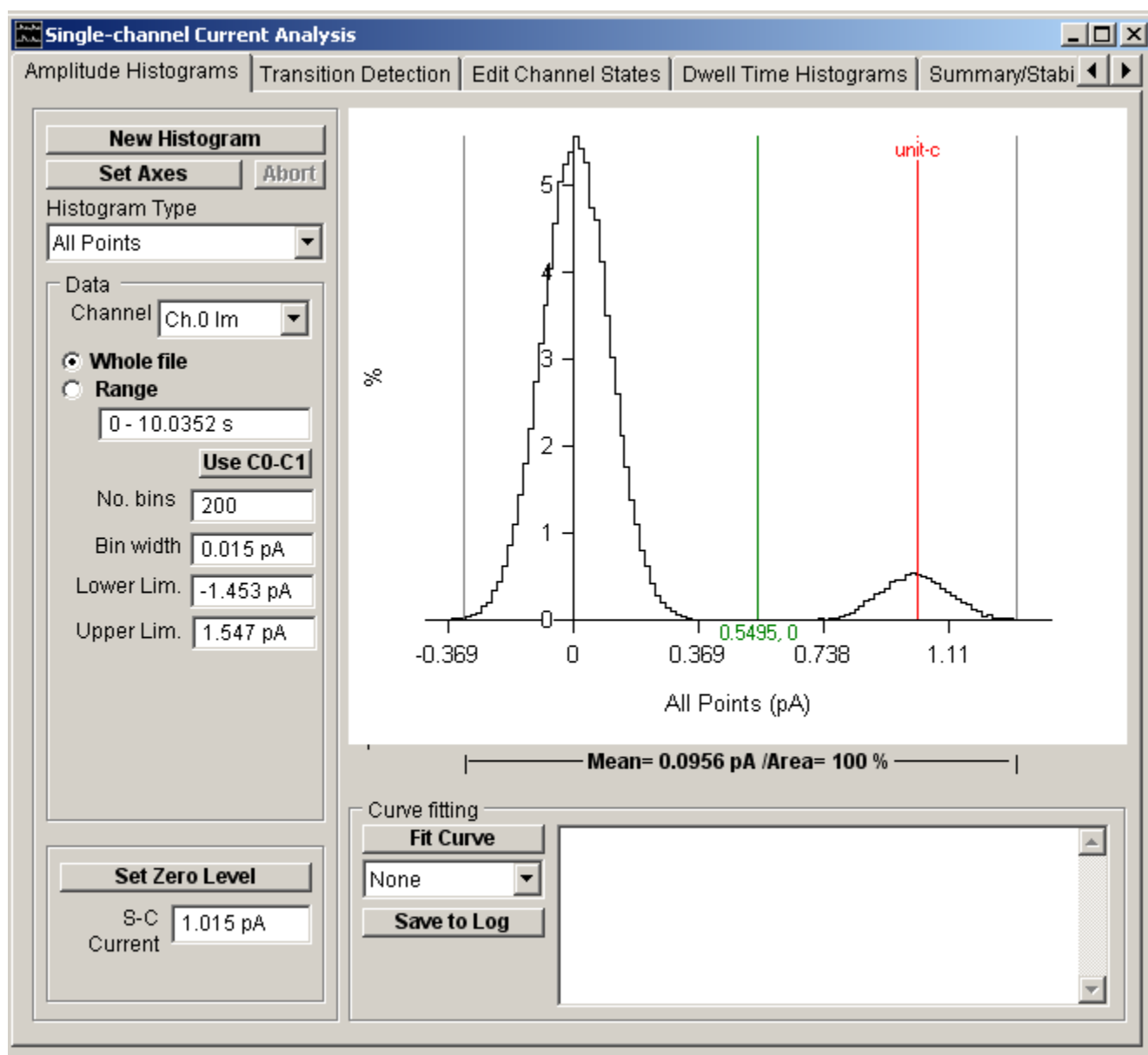
A typical single-channel analysis sequence is as follows:

1. Current amplitude histograms are computed to determine the single-channel current/conductance, number of channels in the patch, and the steady-state probability of a channel being open.
2. Once the single-channel current has been established, the transition detection procedure is used to determine the location of channel open/close transitions and the channel dwell times spent in each state.
3. Dwell time histograms are then be computed and exponential probability density functions fitted to determine the number and mean duration of channel open and closed states.

A detailed discussion of single-channel analysis techniques can be found in Sakmann & Neher (1995) or Ogden (1994).

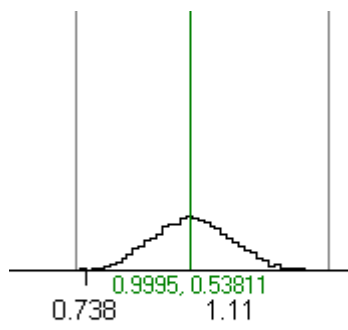
## Analysing Single-channel Currents > Current Amplitudes > Analysis of Current Amplitudes

Select the **Amplitude Histograms** page to compute and plot histograms of the distribution of current amplitudes within a single-channel recording.



Current amplitude histograms generally have at least two peaks, associated with the closed and open states of the ion channels. The horizontal distance between the peaks indicates the current passing through a single channel. The width of the peaks indicate the amount of background noise degrading the current signal. The area under each peak indicates the proportion of time spent in that particular channel state. A channel which is predominantly in the closed state will, for instance, have a large peak at the zero current level and a small one at the single-channel current level. Channels which have than one open conductance or patches which contain more than one channel will often result in histograms with more than two peaks.

The height of each histogram bin indicates the percentage of samples falling within the limits of the bin. The % within each bin and the current at its mid-point can be read out using the green readout cursor. The mean current and % of samples falling within a selected range of amplitudes can be computed by placing the pair of grey '|--|' cursors at the limits of the region of interest.



s (pA)

**Mean= 1.01 pA /Area= 8.73 %**

Six types of current amplitude histogram can be produced.

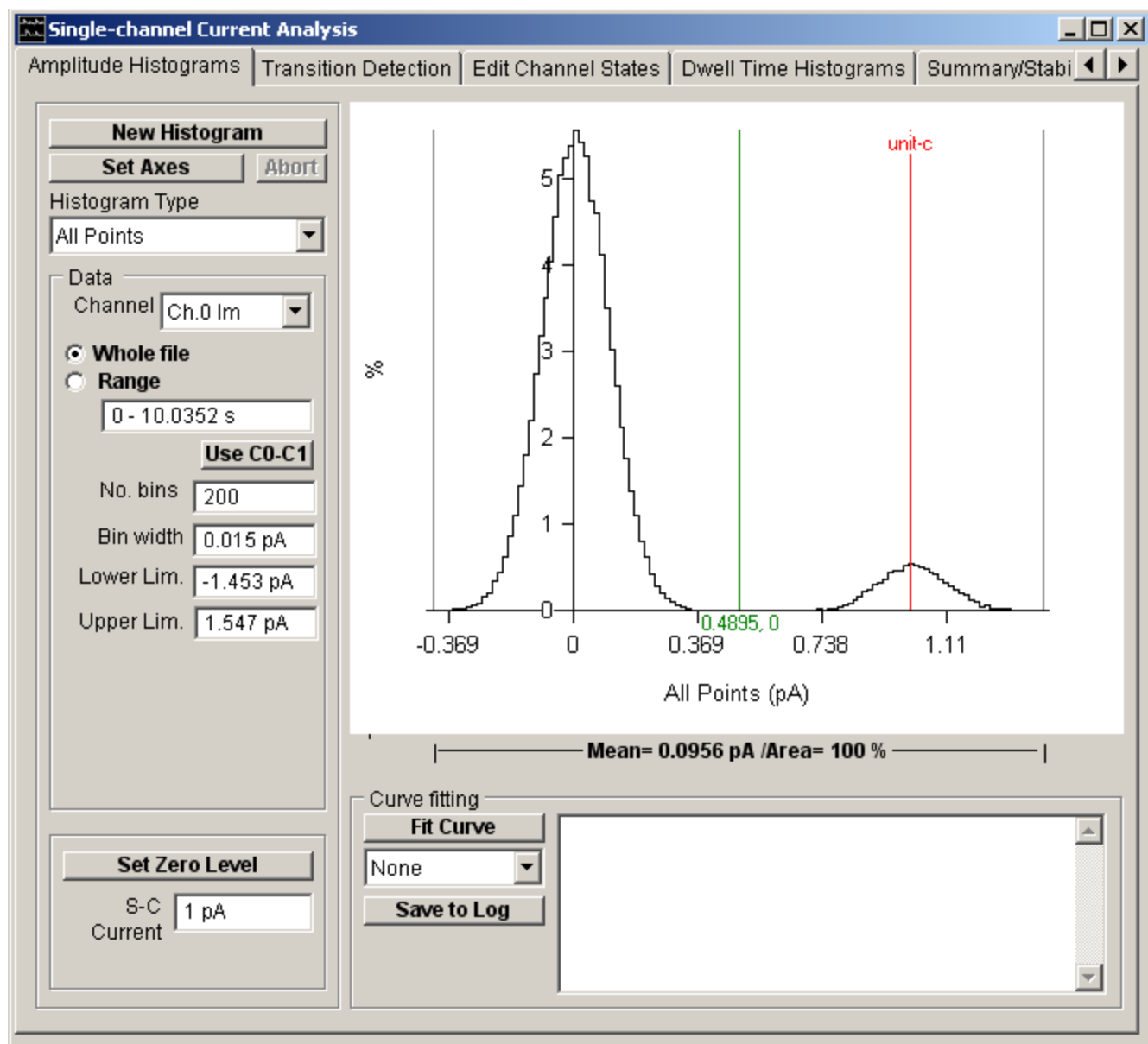
- All Points
- All Points in State
- Mean State Amplitude
- Patlak Average
- Cursor Measurements (avg)
- External file

## Analysing Single-channel Currents > Current Amplitudes > Amplitude Histograms > All Points Amplitude Histogram

The **All Points** histogram represents the distribution of current amplitude within the digitised record, irrespective of the open/close state of the channel. It is used for initial analysis of the current amplitude distribution, determination of single-channel current amplitude, number of channels in the patch, and the steady-state, open-channel probability, *p<sub>open</sub>*. The current amplitude range is divided into a series of equally spaced bins (up to 512). Each sample point within the digitised signal is allocated to a bin according to the formula

$$Bin.no = \frac{(I(i) - I_{lo})n_b}{I_{hi} - I_{lo}} + 1$$

where  $I_{hi}$  and  $I_{lo}$  are the upper and lower limits of the current range,  $n_b$  is the number of bins, and  $I(i)$  is the current for A/D sample  $i$ . The distribution is displayed as a histogram of the percentage of the total number of sample points contained within each histogram bin.



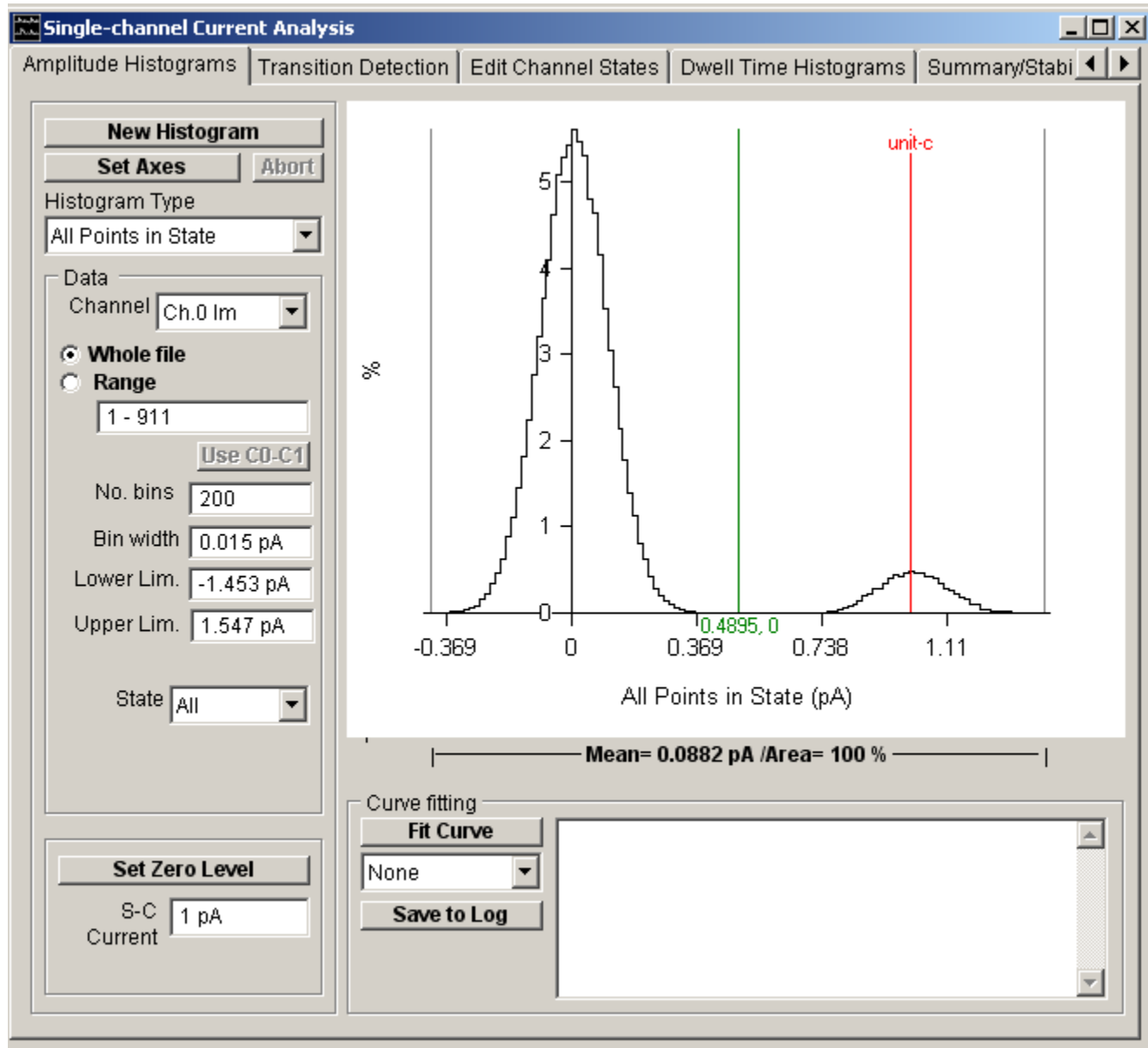
To compile an All Points histogram :

1. Select **All Points** from the **Histogram Type** list.
2. If more than one signal channel is available, select the channel to be used from the **Channel** list.
3. Select the **All** option to use all sample points contained in the recording or select **Range** and enter a time interval to use only samples within that region of recording.

4. Enter the width of the histogram bin in the **Bin Width** box.
5. Click the **New Histogram** button to compile and display the histogram.

## Analysing Single-channel Currents > Current Amplitudes > Amplitude Histograms > All Points in State Histogram

This histogram displays the current amplitude distribution of the sample points contained within a selected set of channels states (**closed**, **open**, and **all**). (**Note**. This option can only be used after transition detection has been completed.



To plot the histogram:

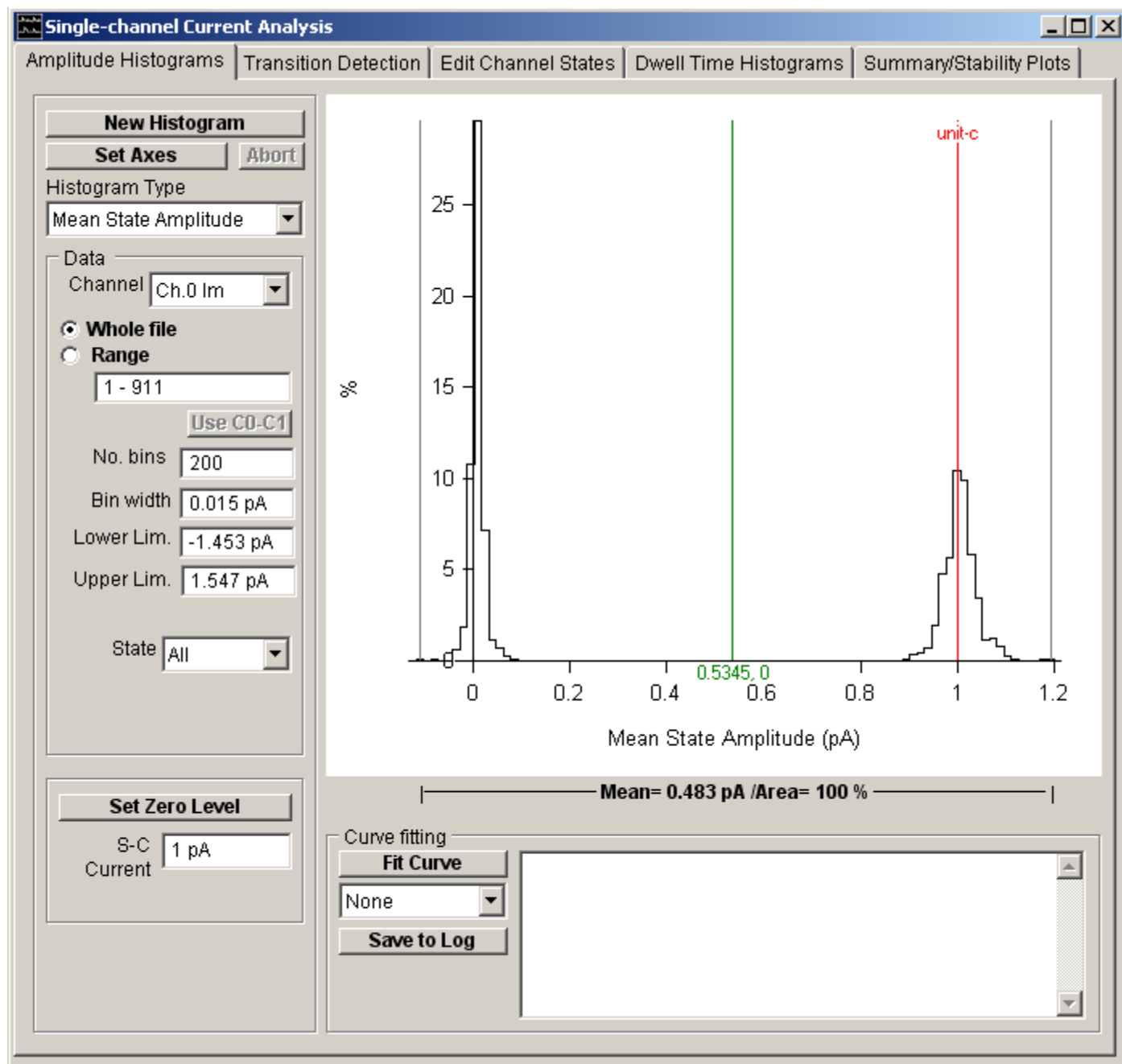
1. Select **All Points In State** from the **Histogram Type** list.
2. If more than one signal channel is available, select the channel to be used from the **Channel** list.
3. Select the **All** option to use all detected channel transition events in the recording or select **Range** and enter the sub-range of events to be included.
4. Select the channel state(s) (**Close**, **Open(X1)** or **All**) to be included in the histogram from the **State** list.
5. A number of (zero or more) samples at the beginning and end of each state can be excluded from the histogram to eliminate the parts of the signal associated with the transition between states. Enter the number samples to be excluded in the **Margin Points** box.
6. Enter the width of the histogram bin in the **Bin Width** box.
7. Click the **New Histogram** button to compile and display the histogram.





## Analysing Single-channel Currents > Current Amplitudes > Amplitude Histograms > Mean State Amplitude Histogram

This histogram displays the mean amplitude for each detected channel state, computed from the average of the sample points contained within the selected set of channels states (**closed**, **open**, and **all**). (**Note**. This option can only be used after transition detection has been completed.)



To compute the histogram :

- Select **Mean State Amplitude** from the **Histogram Type** list.
- If more than one signal channel is available, select the channel to be used from the **Channel** list.
- Select the **All** option to use all detected channel transition events in the recording or select **Range** and enter the sub-range of events to be included.
- Select the channel state(s) (**Close**, **Open(X1)** or **All**) to be included in the histogram from the **State** list.
- Enter the width of the histogram bin in the **Bin Width** box.
- Click the **New Histogram** button to compile and display the histogram.

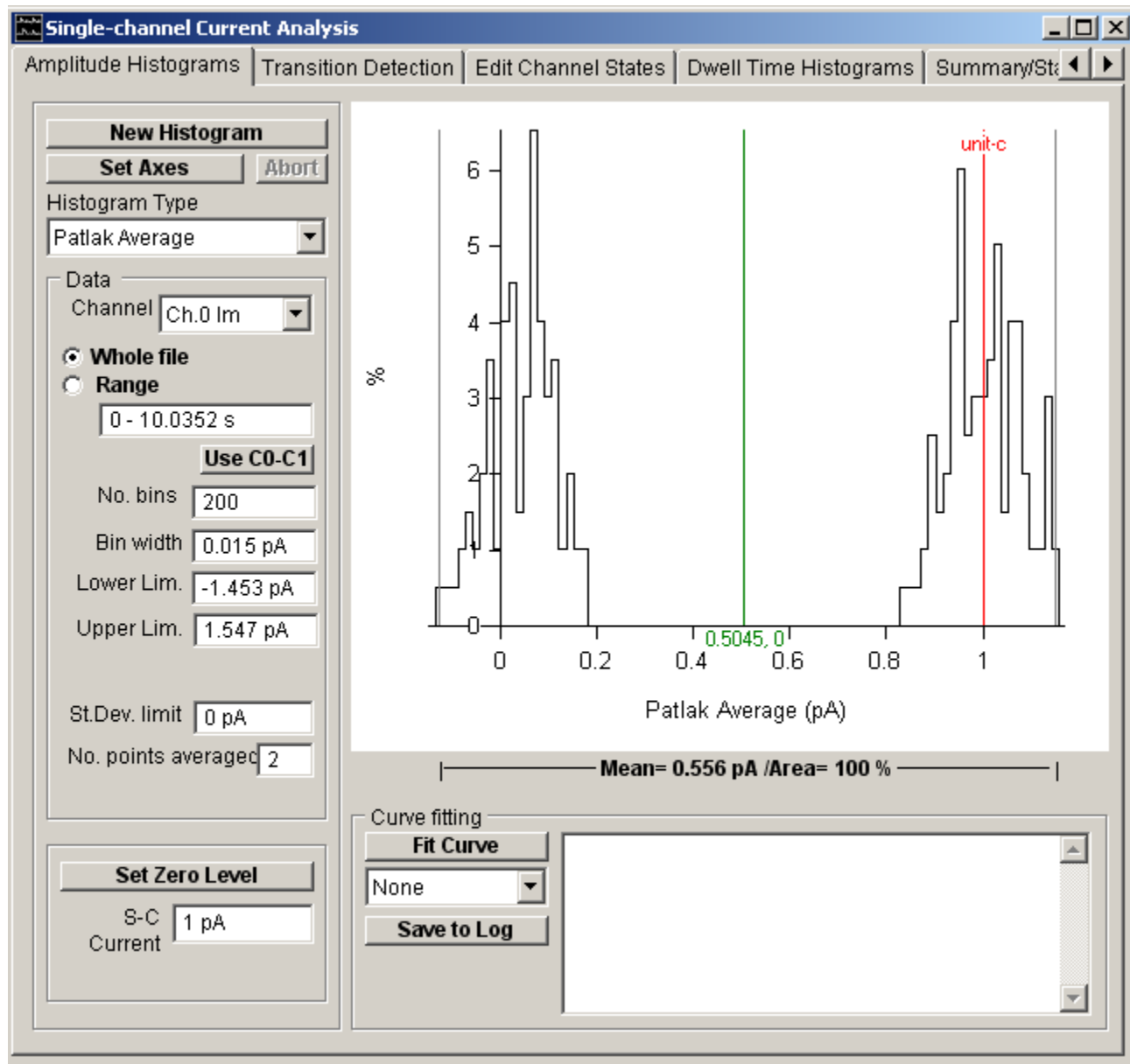
**Note.** The **Exclude Edges** entry on the **Edit Channel State** page determines the number of (zero or more) samples at

the beginning and end of each state which are excluded from the average to eliminate the parts of the signal which are in the process of transition between states. States with a duration shorter than twice the Exclude Edges settings are not included in the mean state amplitude histogram.

## Analysing Single-channel Currents > Current Amplitudes > Amplitude Histograms > Patlak Average Histogram

This is an implementation of a method developed by Joseph Patlak (Patlak, 1988) for improving the resolution of All Points histograms without resorting to transition detection procedures. The amplitude histogram is compiled from the running average of a series of  $n$  ( $2 \leq n \leq 256$ ) adjacent sample points. To avoid smearing of the distribution by including averages which contained transitions between states the standard deviation of the  $n$ -point average is also calculated and the measurement excluded if it exceeds a preset threshold.

A typical value of  $n$  would be 16. The variance threshold is normally set to be the variance of a stable open or closed state. In such circumstances, about 30% of all averages are excluded from the histogram.



To compute the histogram:

1. Select **Patlak Average** from the **Histogram Type** list.
2. If more than one signal channel is available, select the channel to be used from the **Channel** list.
3. Select the **All** option to use all sample points contained in the recording or select **Range** and enter a time interval to use only samples within that region of recording.
4. Enter the number of sample points in the running average in the **No points averaged** box.
5. Enter the exclusion limit for the running mean standard deviation in the **St. Dev. Limit** box. (**Note.** A suitable value is the standard deviation () of a gaussian function fitted to the all points current amplitude distribution around

the zero or single-channel currents levels.

6. Enter the width of the histogram bin in the **Bin Width** box.
7. Click the **New Histogram** button to compile and display the histogram.

The Patlak averaging method produces histograms with much narrower, better defined, peaks than All Points histograms. It is thus useful for resolving and viewing closely spaced sub-conductance levels. However, it should be borne in mind that the averaging process is biased in favour of longer lasting states and short-lasting events tend to be excluded from the histogram. It cannot be used to compute open channel probability values.

## **Analysing Single-channel Currents > Current Amplitudes > Amplitude Histograms > Cursor Measurements (avg) Histogram**

This option plots the histogram of the average amplitude measurements stored in the cursor measurement list (see Cursors Measurements)

## **Analysing Single-channel Currents > Current Amplitudes > Amplitude Histograms > External File Histogram**

This option plots the histogram of a list of amplitude measurements (ASCII text format, one amplitude per line) stored in a file.

## Analysing Single-channel Currents > Current Amplitudes > Fitting Gaussian Functions to Histograms

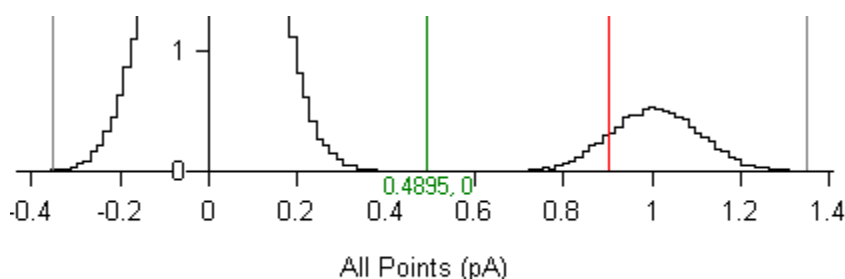
The peaks of the current amplitude histogram can often be fitted by a mixture of one or more gaussian probability density functions. The height  $y(i)$ , of bin  $i$  with its mid-point at current  $I(i)$ , is given by

$$y(i) = \sum_{i=1..n} \frac{A_i w}{\sqrt{2\pi\sigma_i^2}} \exp\left(-\frac{(I(i) - \mu_i)^2}{2\sigma_i^2}\right)$$

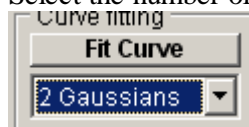
where  $w$  is the histogram bin width. Each gaussian component is defined by 3 parameters;  $A_i$  the percentage of the total area under the component,  $\mu_i$  the mean current, and  $\sigma_i$  the standard deviation about the mean value. WinEDR uses an iterative least squares curve fitting procedure to find the gaussian function which best fits the histogram data. Fitting a gaussian curve provides a convenient way of estimating the mean current for a channel state and the percentage of time spent in that state. In cases where the peaks are partially overlapping, mixtures of gaussians can be fitted simultaneously, allowing the relative proportions of each to be estimated.

To fit a gaussian curve to a histogram:

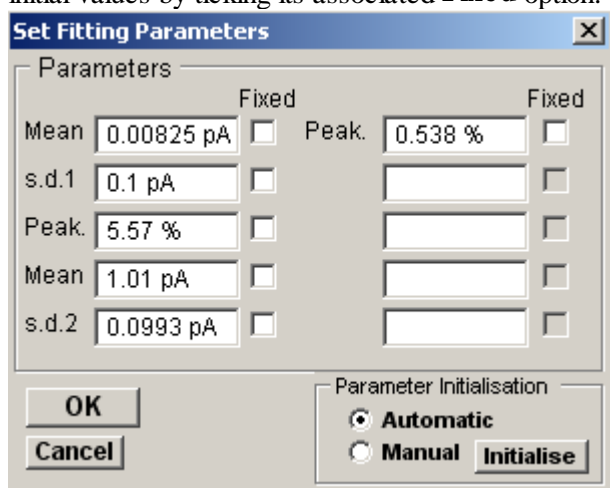
1. Define the range of amplitudes containing the peak(s) to be fitted using the pair of grey ']' region of interest cursors.



- 2.
- 3.
4. Select the number of Gaussian functions to be fitted from the **Curve Fitting** list.



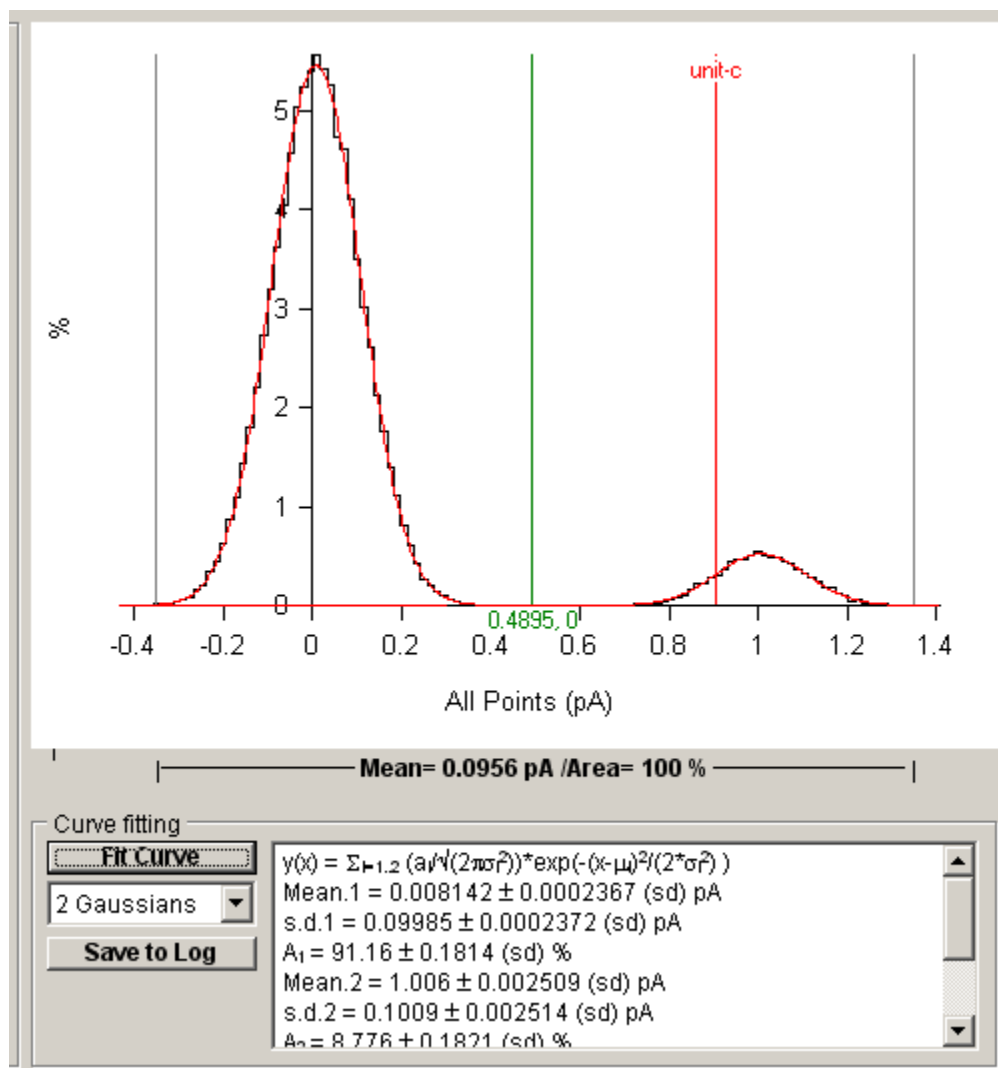
5. Click the **Fit Curve** button and enter an appropriate set of initial guesses for the gaussian function parameters ( $\mu_i$ ,  $\sigma_i$ ,  $Pk_i$ ) (Note. A set of initial guesses are computed automatically, but it is often necessary to adjust these to better match the location and size of the observed histogram peaks). Individual parameters can also be fixed at their initial values by ticking its associated **Fixed** option.



Click the **OK** button to begin fitting.



The best fitting gaussian function(s) are superimposed (in red) on the histogram. The values of the function parameters along with their estimated standard errors are displayed in curve fitting results area at the bottom of the display.

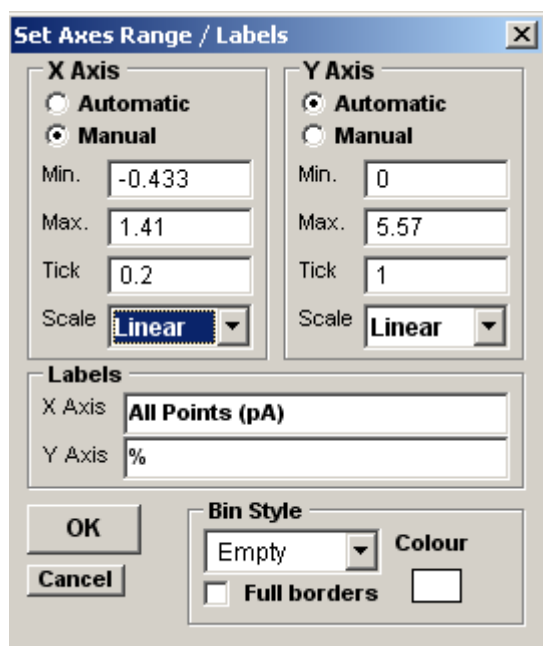


The residual standard deviation ( $SD_{res}$ ) between the histogram data and the fitted curve provides a measure of the quality of the fit, the smaller the  $SD_{res}$  the better the fit.

**Note.** The fitting procedure will generally work well when fitting gaussian curves to distributions with single peaks or two reasonably well separated peaks which do not differ by more than an order of magnitude in height. In other cases, such as overlapping 3-4 peak distributions the large number of free parameters can cause the procedure to fail to converge to a meaningful result. In such cases it is sometimes possible to obtain a better fit using a two stage procedure. First, a single gaussian is fitted to the largest and best defined peak. Then the resulting best fit parameters for that peak are used as **fixed** parameters in a 2 (or more) gaussian fit to the remaining peak.

## Analysing Single-channel Currents > Current Amplitudes > Customising Histogram Plots

If you want to alter the X or Y axis range, scaling or labels on a histogram, click the **Set Axes** button to open the dialog box.



The dialog box is titled "Set Axes Range / Labels" and contains several sections for configuring the histogram axes and appearance.

- X Axis:** Includes radio buttons for **Automatic** and **Manual** (selected). Below are input fields for **Min.** (-0.433), **Max.** (1.41), and **Tick** (0.2). A **Scale** dropdown menu is set to **Linear**.
- Y Axis:** Includes radio buttons for **Automatic** (selected) and **Manual**. Below are input fields for **Min.** (0), **Max.** (5.57), and **Tick** (1). A **Scale** dropdown menu is set to **Linear**.
- Labels:** Two text input fields. The **X Axis** label is "All Points (pA)" and the **Y Axis** label is "%".
- Buttons:** **OK** and **Cancel** buttons are located at the bottom left.
- Bin Style:** A section at the bottom right containing a **Bin Style** dropdown menu set to **Empty**, a **Colour** box, and a **Full borders** checkbox.

You can change the X or Y axis limits by selecting the **Manual** Axis option and entering new values into the **Min**, **Max**, and **Tick** (spacing) boxes. An axis can be made **Linear** or **Logarithmic** by selecting the option from its **Scale** list. Labels for the X and Y axes can be entered into the **Labels** boxes.

The style of rectangle used to plot the histogram bins can be changed using the **Bin Style** options. Select **No Fill** to display bins as rectangular outlines, **Solid Fill** to fill the bins in with a solid colour, and **Hatched Fill** for bins filled with a diagonal lines. You can define the colour used for the solid fill, by clicking the **Colour** box, and selecting a colour from the palette. The **Full Borders** check box determines whether the outline is drawn completely around each bar, or just where bars do not overlap.

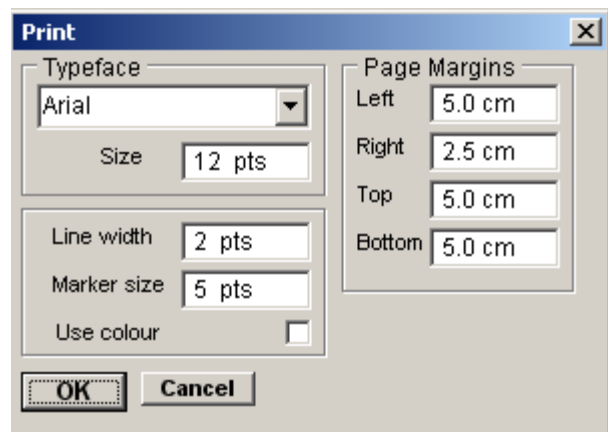
## Analysing Single-channel Currents > Current Amplitudes > Printing Histograms

To print the displayed histogram, select

**File**

**Print**

To open the dialog box.



Click the **OK** button to print the graph.

## Analysing Single-channel Currents > Current Amplitudes > Copying Histograms to the Clipboard

### Copying the histogram data points to the Windows clipboard

The numerical values of the X,Y data points which generate the histogram can be copied to the clipboard by selecting

**Edit**

**Copy Data**

The data is placed on the clipboard as a table of data values, in tab text format, defining the histogram. There are 4 values per row, and one row for every bin in the histogram. Each row has the format

```
<Bin Lower Limit> <tab> <Bin Mid-point> <Bin Upper Limit> <tab> <Bin Count> <cr> <lf>
```

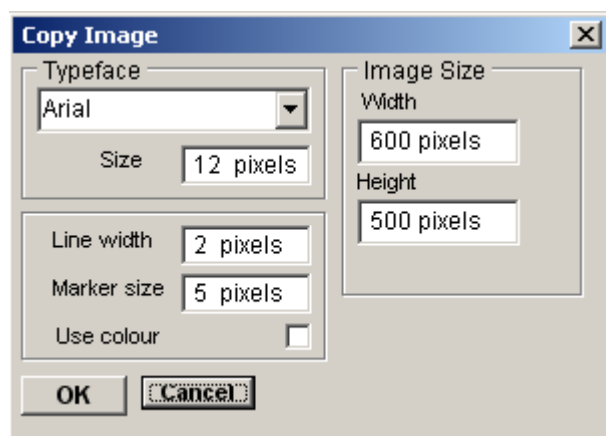
### Copying an image of the histogram to the Windows clipboard

An image of the histogram plot can be copied to the clipboard by selecting

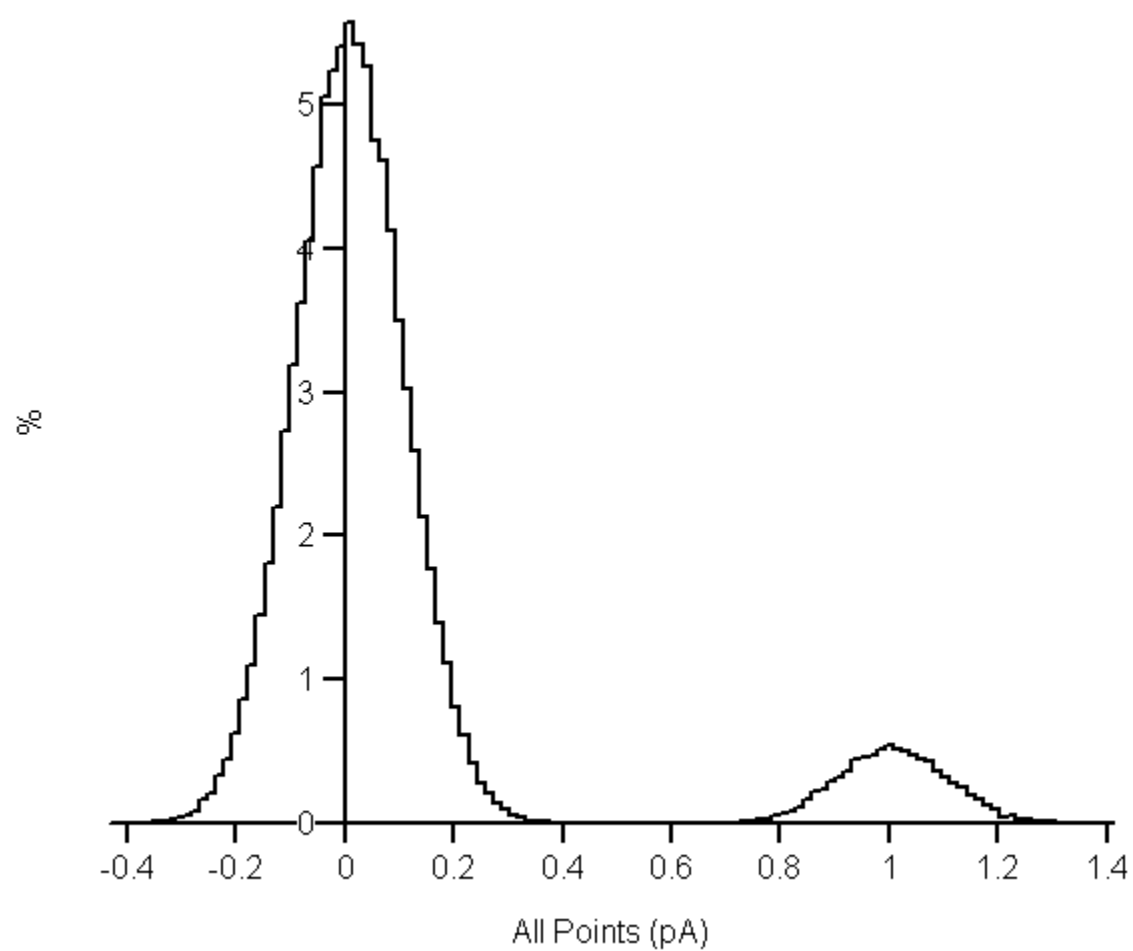
**Edit**

**Copy Image**

to open the dialog box.



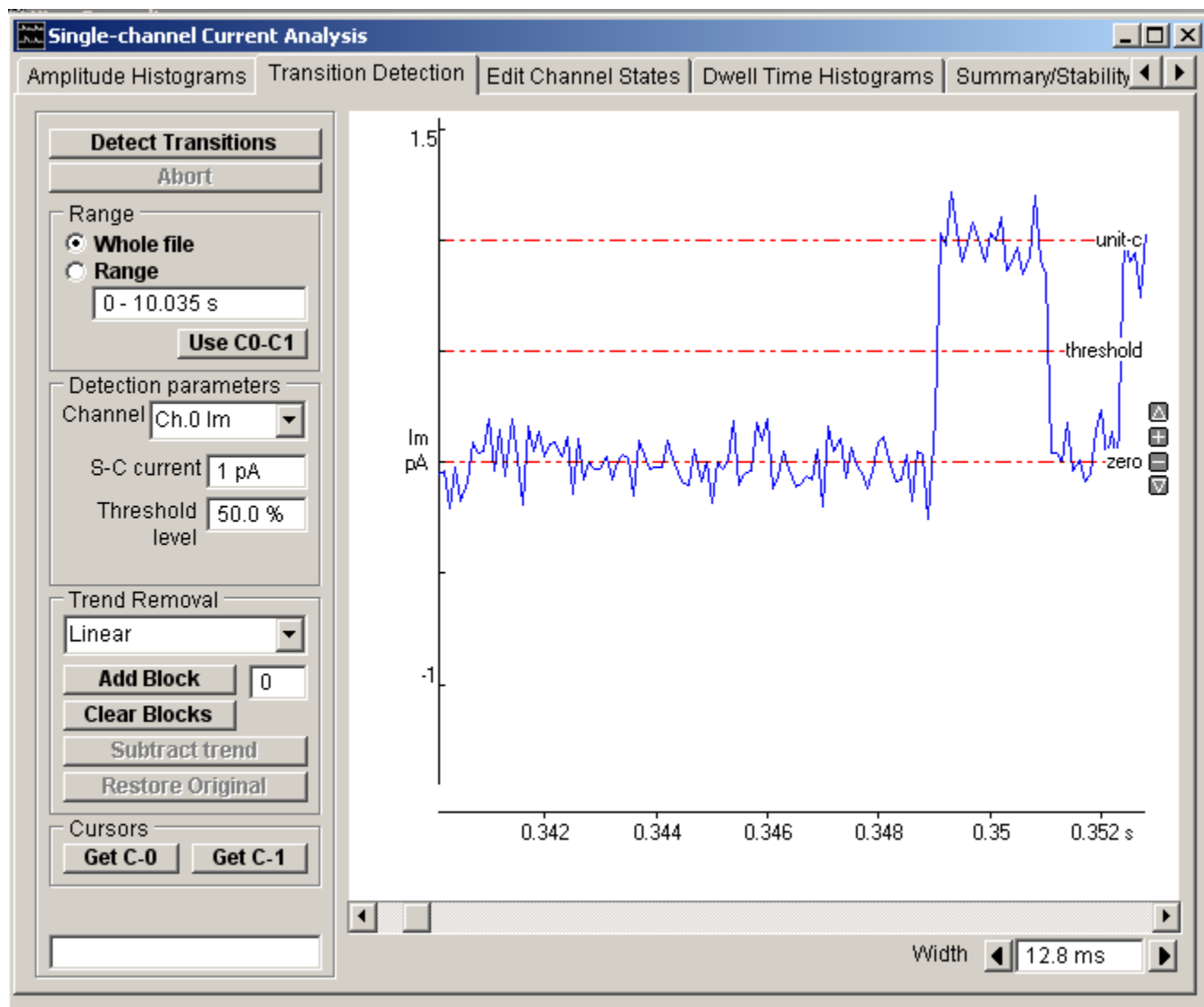
The dimensions (pixels) of the bit map, which will hold the image, can be set using the Width and Height image size boxes. The size and style of the typeface can be set using the Typeface and Size boxes. When the image parameters have been set, click the **OK** button to copy the image to the clipboard.



## Analysing Single-channel Currents > Detecting Channel Transitions > Transition Detection

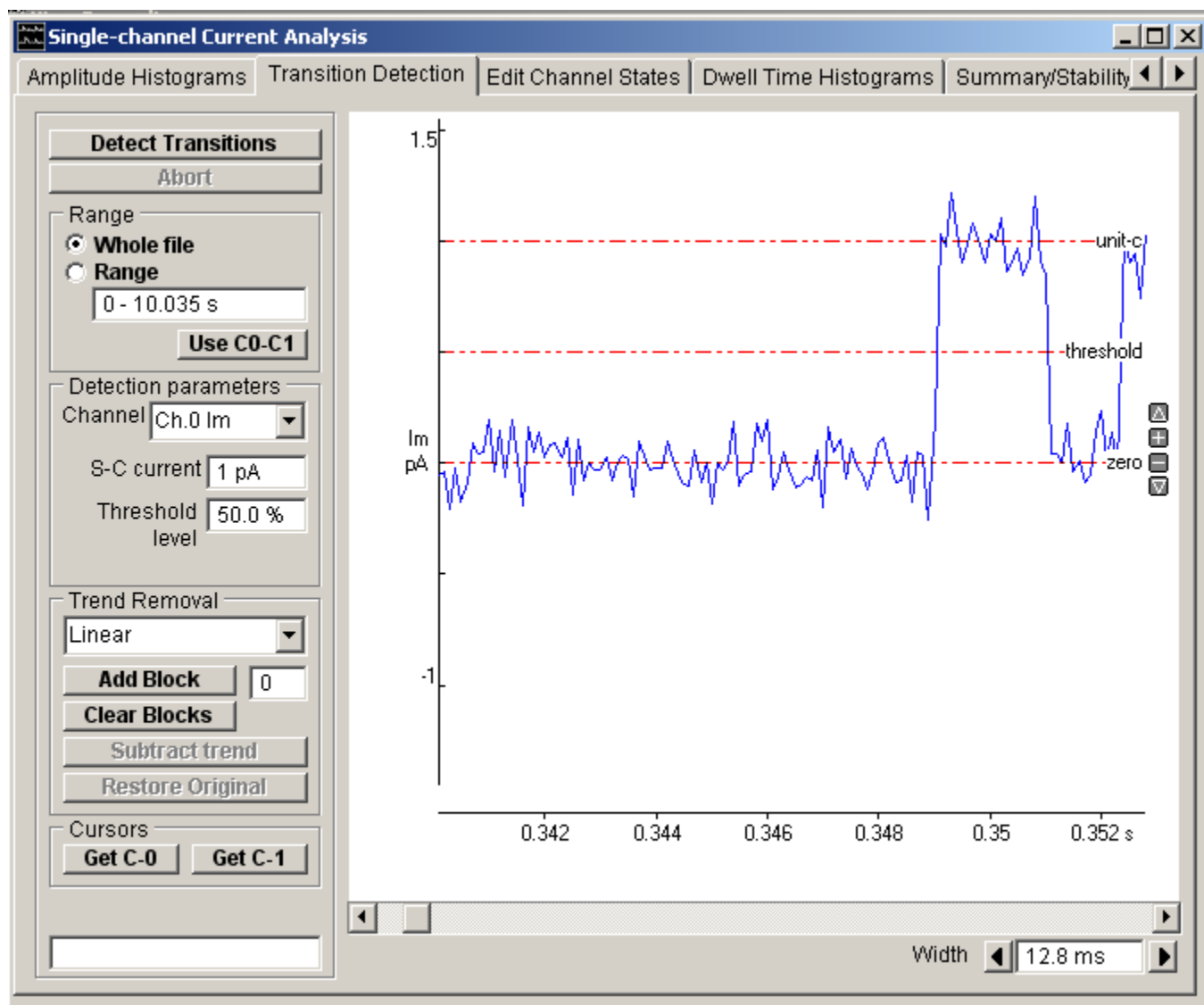
While analysis of current amplitude provides information concerning the number and size of channel conductance states it provides no information about channel kinetics. In order to do this it necessary to measure the time the channel spends in each state. Since ion channels fluctuate randomly between open and closed states the duration of a single opening or closure provides little information. It is necessary to measure the durations of hundreds or thousands of openings and closures and to analyse the distribution of dwell times spent in each state.

The **Detect Transitions** page provides tools for the detection of channel open/close transitions and the determination of the sequence of channel dwell times spent in each state.



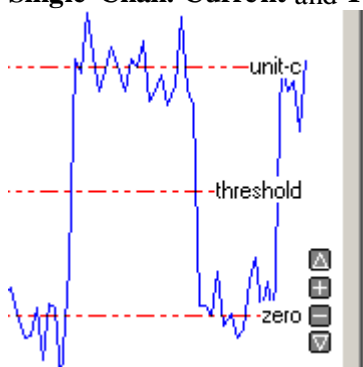
The simplest approach to the classification of the single channel current signal into open and closed states is to place a transition threshold at the mid-point between the zero and single-channel current levels. If the signal lies above the threshold the channel is deemed to be open, if it lies below the channel is deemed to be closed. An 'idealised' sequence of channel open and closed periods is thus obtained by scanning through the digitised recording point by point, counting the time between threshold crossings.

## Analysing Single-channel Currents > Detecting Channel Transitions > Detecting Channel Open-Close Transitions



To detect the channel open-close transitions in a single-channel current recording:

1. Define the section of the recording to scanned. Select the **Whole file** option to use all sample points contained in the recording or select **Range** and enter a time interval to scan only that region of recording.
2. Set the position of the zero current level (zero), single-channel current level (unit-c) and transition threshold (threshold), indicated by the horizontal cursors on the display. The levels can be adjusted by dragging the cursors up or down or (in the case of the single-channel level and threshold) by entering appropriate values in the **Single-Chan. Current** and **Threshold level** boxes



3. Click the **Detect Transitions** button to begin scanning through the recording.

During transition detection an idealised noise-free current signal, representing the detected sequence of open/closed channel states is superimposed (in red) on the recorded current signal. This process can take several minutes with large data files and/or slow computer, progress being indicated by the progress bar at the bottom-right of the window. (If necessary, transition detection can be aborted before completion by clicking the **Abort** button.)

**Note.** The half-amplitude threshold method works well when applied to signals with good signal-noise ratios, where the channel unitary current level is well separated from the zero level and the recording bandwidth is such that brief events are fully resolved. However, the method is prone to error when applied to channels with sub-conductance states close to the 50% threshold.

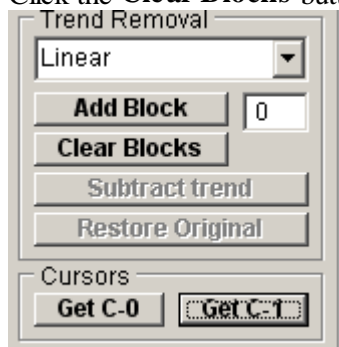


## Analysing Single-channel Currents > Detecting Channel Transitions > Removing Baseline Drift

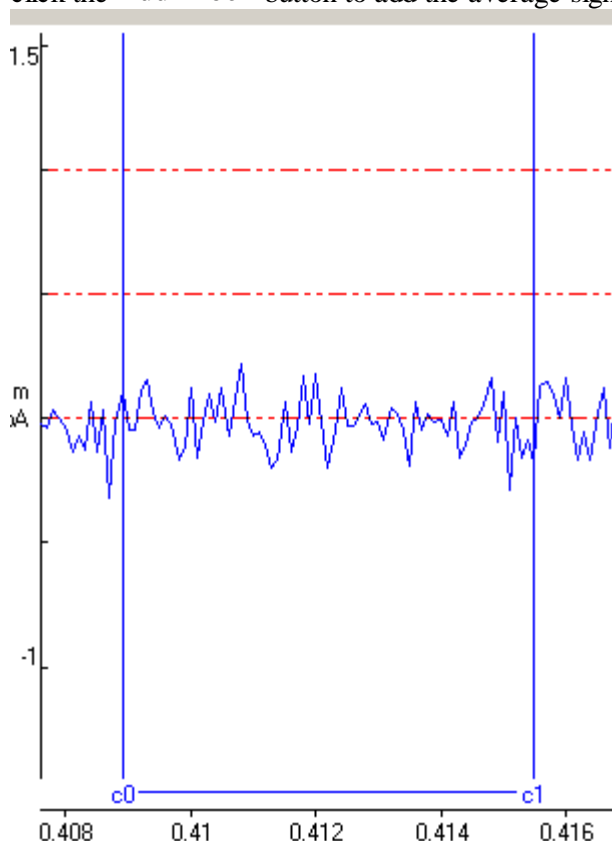
The accuracy of the transition detection process is crucially dependent upon the zero current baseline level remaining stable throughout the whole recording period. If any drift is apparent in the signal baseline level it must be removed before initiating the detection process. Trends can be removed by obtaining a series of estimates of the average signal baseline level throughout the recording period, fitting an equation to the resulting line, and subtracting this from the digitised signal.

To subtract a trend in the baseline level:

1. Click the **Clear Blocks** button to erase any existing baseline trend line.



2. Scroll through the displayed recording and locate a section where the channel is closed for an extended period. Select the closed interval for inclusion in the baseline current line, using the '|--|' region selection cursors, and click the **Add Block** button to add the average signal level during this period to the trend line.

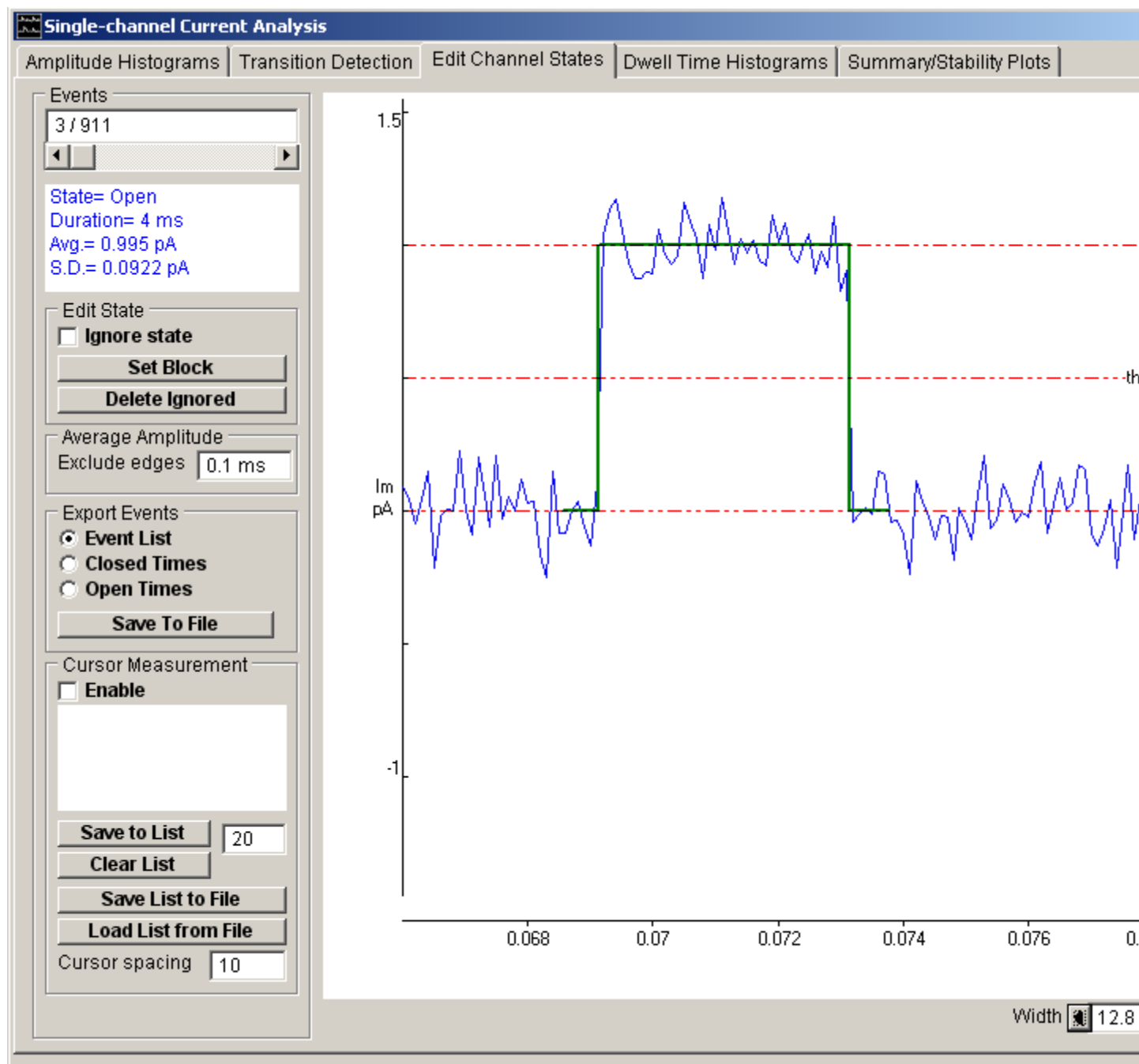


3. Repeat step (2), selecting baseline current periods throughout the recording until an adequate representation of the trend has been obtained.
4. Select the Trend Removal function (**Linear**, **Quadratic**, **Cubic**) to be fitted to the trend line. The Linear function represents the trend as a simple straight line. Quadratic and Cubic functions allow for curvature in the trend.
5. Click the **Subtract Trend** button to remove the baseline trend from the digitised signal. (If necessary, to. restore

the original signal click the **Restore Original** button.)

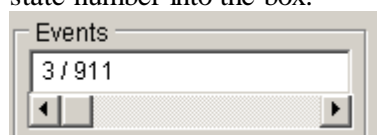
## Analysing Single-channel Currents > Detecting Channel Transitions > Viewing/Editing Channel Open/Close States

The individual channel open and closed states produced by the transition detection process are displayed on the **Edit Channel States** page.



The idealised channel open/closed state is superimposed (in green) on the digitised current signal. The time period on display can also be adjusted by increasing or decreasing the **Width** time value. The type of state (Closed, Open), its time duration, average current and signal standard deviation within the state are displayed in the status box.

Individual channel states within the state list can be selected for display using the state selection scroll bar or by entering a state number into the box.



The **Exclude Edges** box determines the number of (zero or more) samples at the beginning and end of each state which

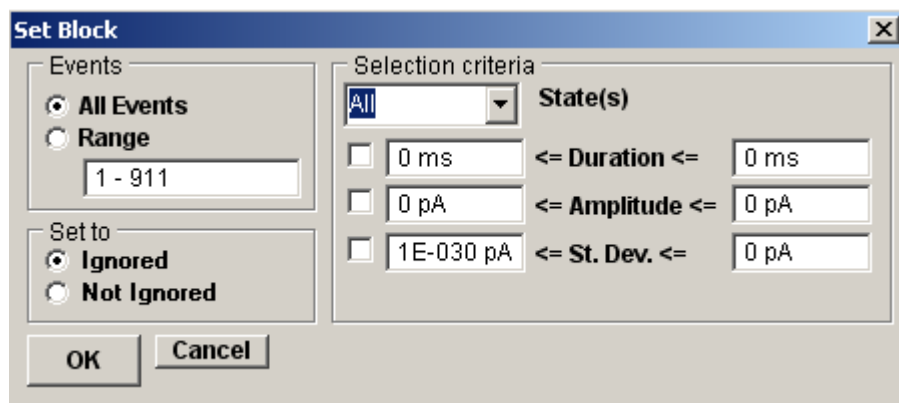
are excluded from the state average current to eliminate the parts of the signal which are in the process of transition between states.

## Analysing Single-channel Currents > Detecting Channel Transitions > Editing the Channel Event List

Artifactual channel transitions arising from parts of the current recording corrupted by external interference or invalid for other reasons often have to be excluded from the channel dwell time and amplitude analysis. Individual states can be excluded from analysis by ticking the **Ignore State** tick box for that event



To automatically exclude a block of events, click the **Set Block** button to open the dialog box.



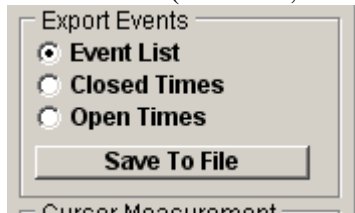
1. Select the type of state to be modified (**Closed**, **Open** or **All** for both types of state) from the **State** list.
2. Define the criteria to be used for selecting the events to be changed.  
To restrict changes to events with a specific range of durations, select **Duration** and enter a range of times in the box.  
To restrict changes to events with a specific range of amplitudes, select **Amplitude** and enter a range of amplitudes in the box.  
To restrict changes to events with a specific range of current standard deviations, select **St. Dev.** and enter a range of amplitudes in the box. (All selected criteria must be met before a change is made.)
3. Select the **Ignore State** (Ignored, Not Ignored) setting to be applied when an event is found to match the selection criteria.
4. Select the **All Events** option to apply the criteria to all events or select **Range** and enter a specific range of events
5. Click the **OK** button to begin the changes. The number of event matching the criteria are indicated in the status box as the search progresses.

**Note.** Events marked for exclusion from analysis remain within the channel state list and, consequently, continue to separate adjacent states. It is sometimes useful to completely remove Ignored events from the list and merge adjacent states into one. This can be done by clicking the **Delete Ignored** button.

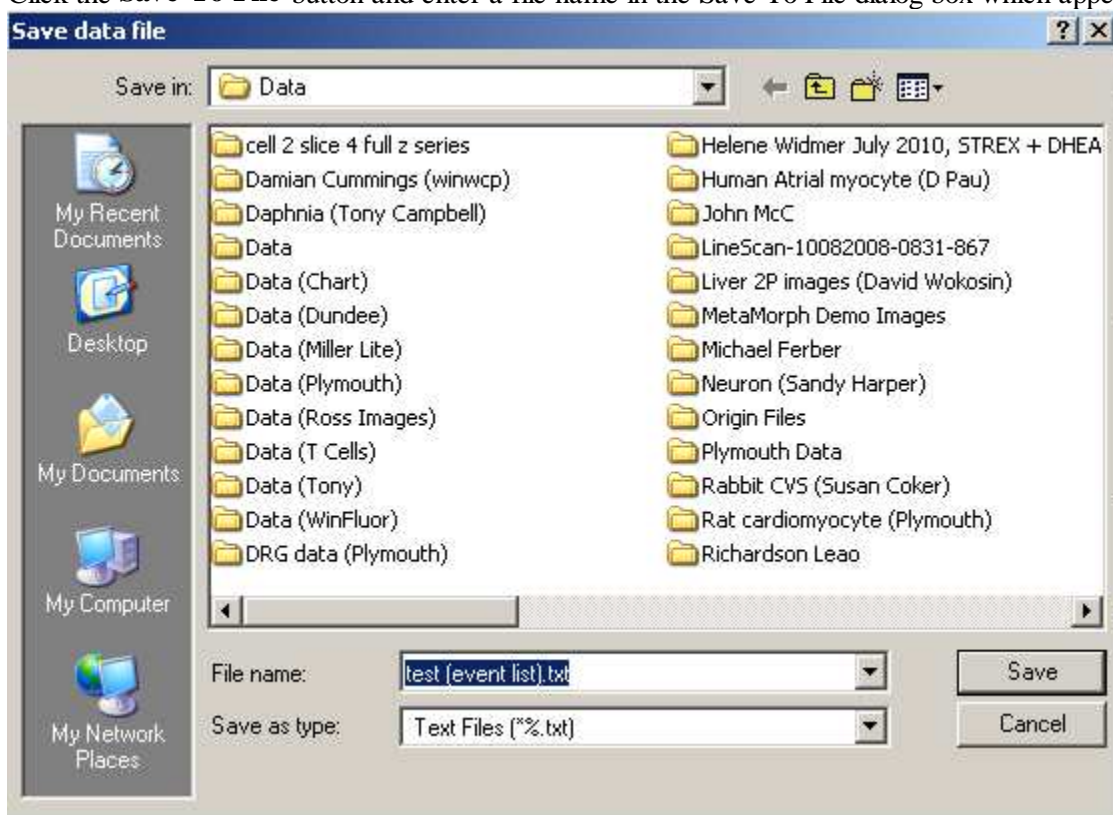
## Analysing Single-channel Currents > Detecting Channel Transitions > Exporting the Channel State List

To export the data stored in the channel state list to a text file:

1. Select **Open Times** or **Closed Times** to export the sequential list of channel open and closed dwell times produced by transition detection. Select **Event List** to export a list of channel dwell times with associated channel state (0=Closed, 1=Open).

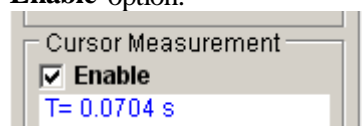


2. Click the **Save To File** button and enter a file name in the Save To File dialog box which appears.

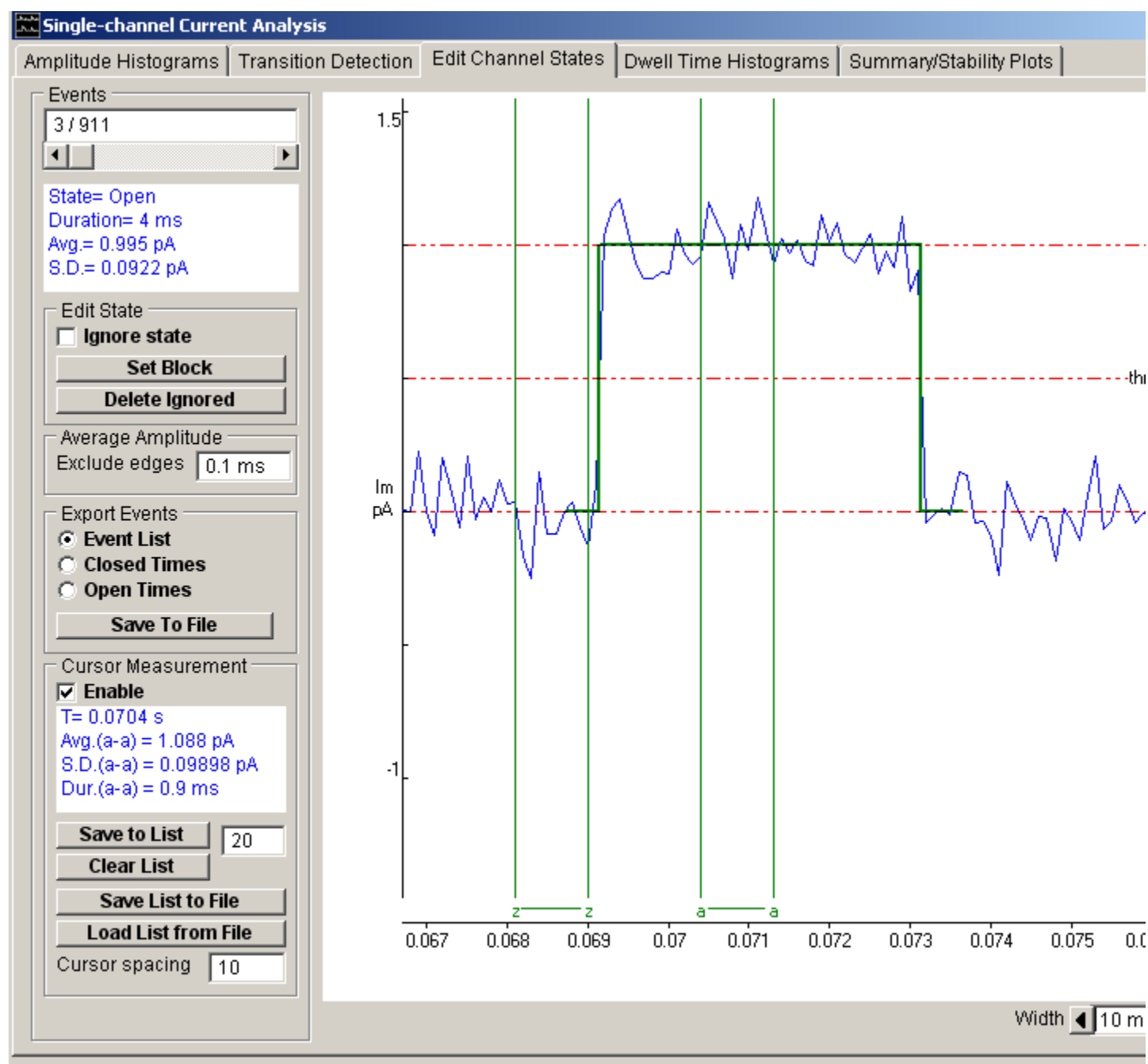


## Analysing Single-channel Currents > Detecting Channel Transitions > Cursor Measurements

Two pairs of linked cursors, **a-a** and **z-z**, can be used to make manual measurements of duration, average current amplitude and standard deviation, and save these to a list. This feature is enabled by ticking the **Cursor Measurement Enable** option.



The average current (difference between the average signal within the **z-z** and **a-a** cursors), duration (time between the **a-a** cursors) and standard deviation of the signal within the **a-a** region) are displayed in the **Cursor Measurement** box. Clicking the **Save to List** button saves the displayed measurements to a storage list.



The amplitude, duration and standard deviation measurements stored in this list can be plotted (vs. time) on the Stability Plot page. A histogram of the distribution of amplitude measurements can be plotted on the Amplitude Histograms page.

The cursor measurements list can be saved to an Excel-readable text file by clicking the **Save List to File** button and entering a file name. Previously saved files can be re-loaded into the measurements list by clicking the **Load List from**

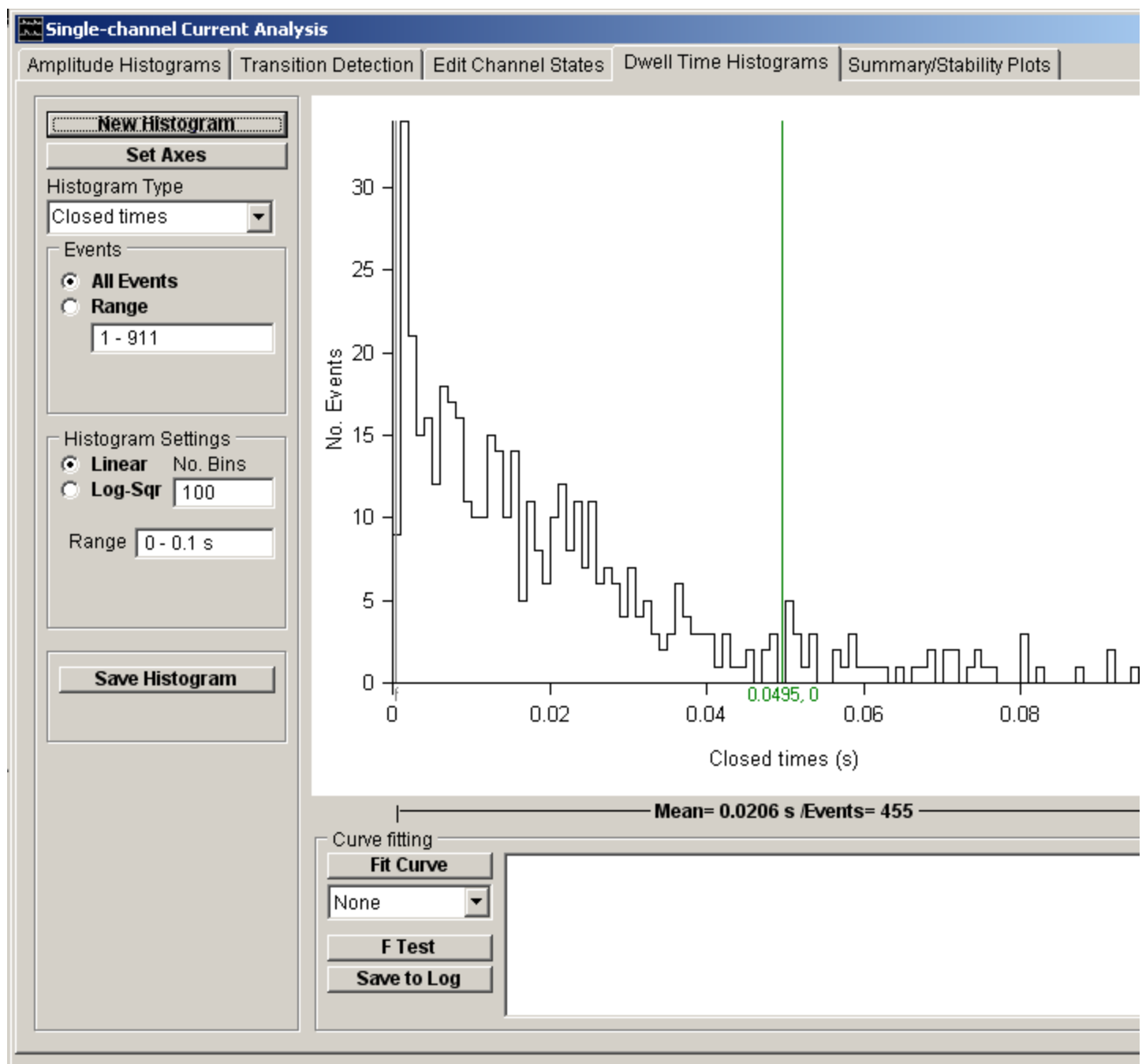
**File** button.

The default inter-cursor spacing is defined by the **Cursor spacing** box.

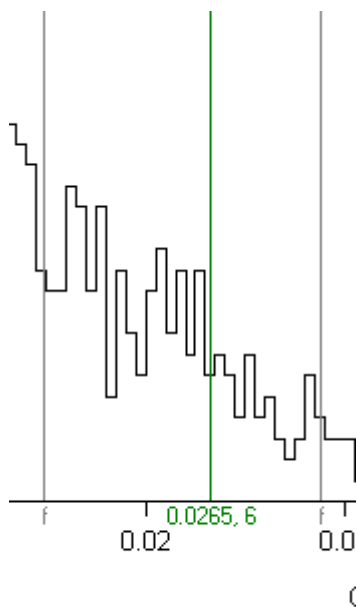


## Analysing Single-channel Currents > Channel Dwell Time Analysis > Analysis of Channel Dwell Times

The **Dwell Time Histograms** page provides tools for compiling and analysing histograms of the distribution of the channel open and closed times determined by the transition detection process. Dwell time histograms can be plotted with linear or logarithmic axes and mean state durations estimated by fitting one or more exponential probability density functions.



The height of each histogram bin indicates the number of samples falling within the limits of the bin. The number of events within each bin and the dwell time at its mid-point can be read out using the green readout cursor. The number of events contained in the bins falling within a selected range of dwell times, and the mean dwell time of that region, can be displayed by placing the pair of grey 'f--f' cursors at the limits of the region of interest.



**Mean= 0.0206 s /Events= 230**

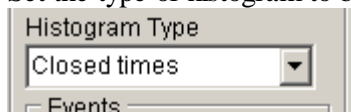
Seven basic types of histogram can be produced from the dwell time data in the channel state list.

- Open times: distribution of channel open times.
- Closed times: distribution of channel close times.
- Burst Length: distribution of the duration of bursts of openings, a burst being defined as a series of opening interspersed by short closures, terminated by a closure longer than a predefined period.
- Single open times: distribution of open times of channel openings which do not form part of a burst.
- Opens times within bursts: distribution of open times of channel openings which form part of a burst.
- Openings per burst: distribution of the number of channel openings within a burst.
- External file: distribution of times loaded from an external file.

## Analysing Single-channel Currents > Channel Dwell Time Analysis > Plotting a Dwell Time Histogram

To plot a dwell time histogram:

1. Set the type of histogram to be plotted using the **Histogram Type** list.

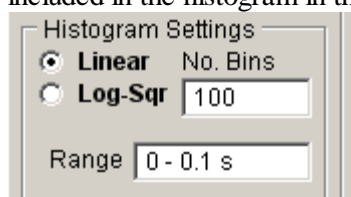


2. Select the **All Events** option to use all channel state events in the recording or select **Range** and enter the sub-range of events to be included.

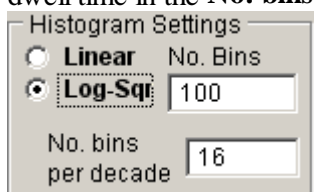


3. If Burst Length, or any of the other burst-related histogram types have been selected, enter the critical closed time value used to distinguish between intra- and inter-burst closed intervals in the **T.critical** box.
- 4.
5. **Histogram Settings:** Set the type of bin spacing to be used. Set the number of histogram bins in the **No. Bins** box.

Select the **Linear** option for fixed width bins and enter the lower and upper limits of the range of times to be included in the histogram in the **Range** box a



OR select the **Log-Sqr** option for logarithmically spaced bins and enter the number of bins per 10 fold change in dwell time in the **No. bins per decade** box.



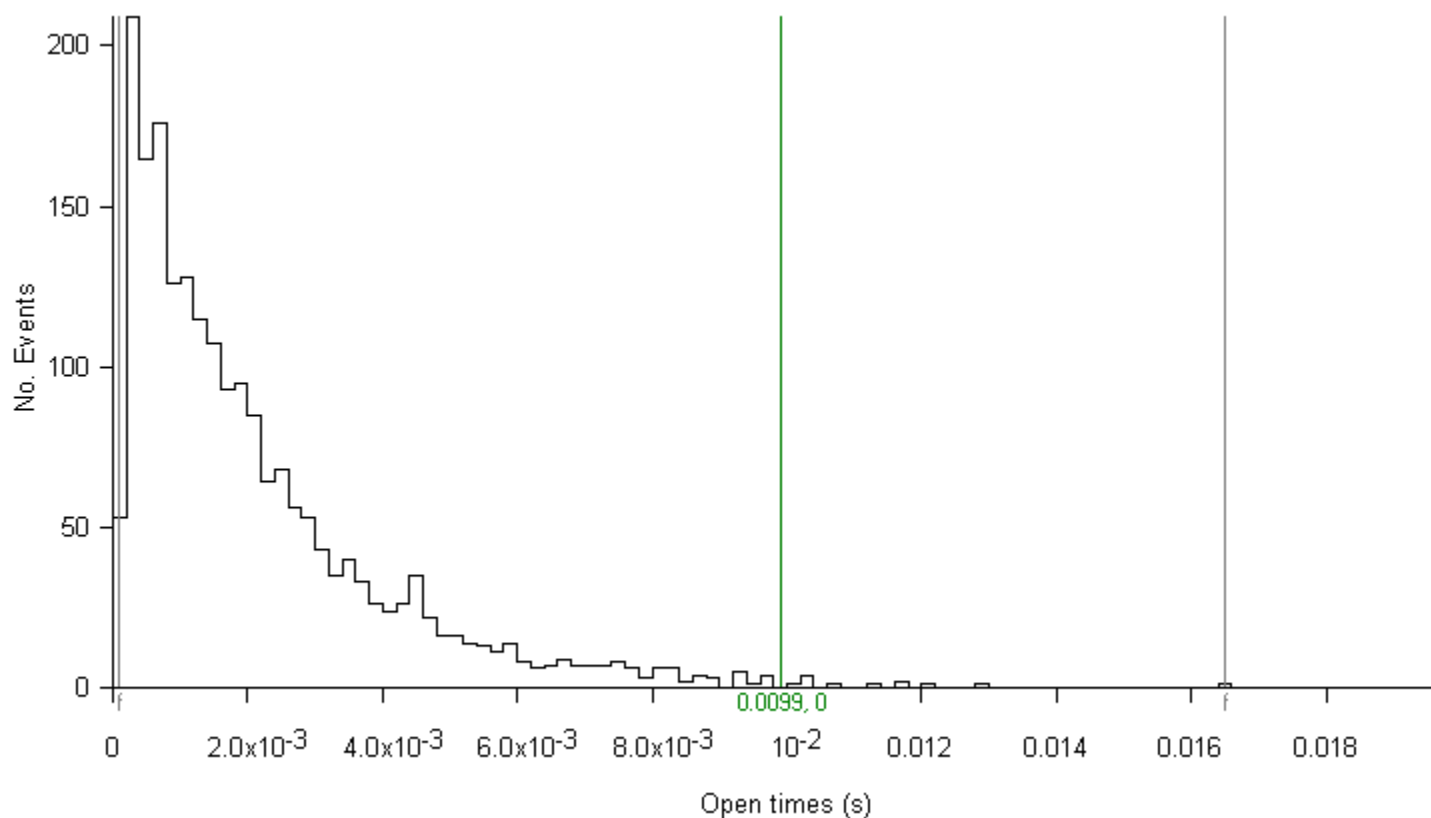
6. Click the **New Histogram** button to plot the histogram.

### Linear histograms with fixed width bins

A **Linear** dwell time histogram is compiled by counting the number of state dwell times which fall into a series of equal sized time bins spread over a given range of times. Each dwell time,  $t_{state}$ , is assigned a bin number,  $i_{bin}$ , according to the formula

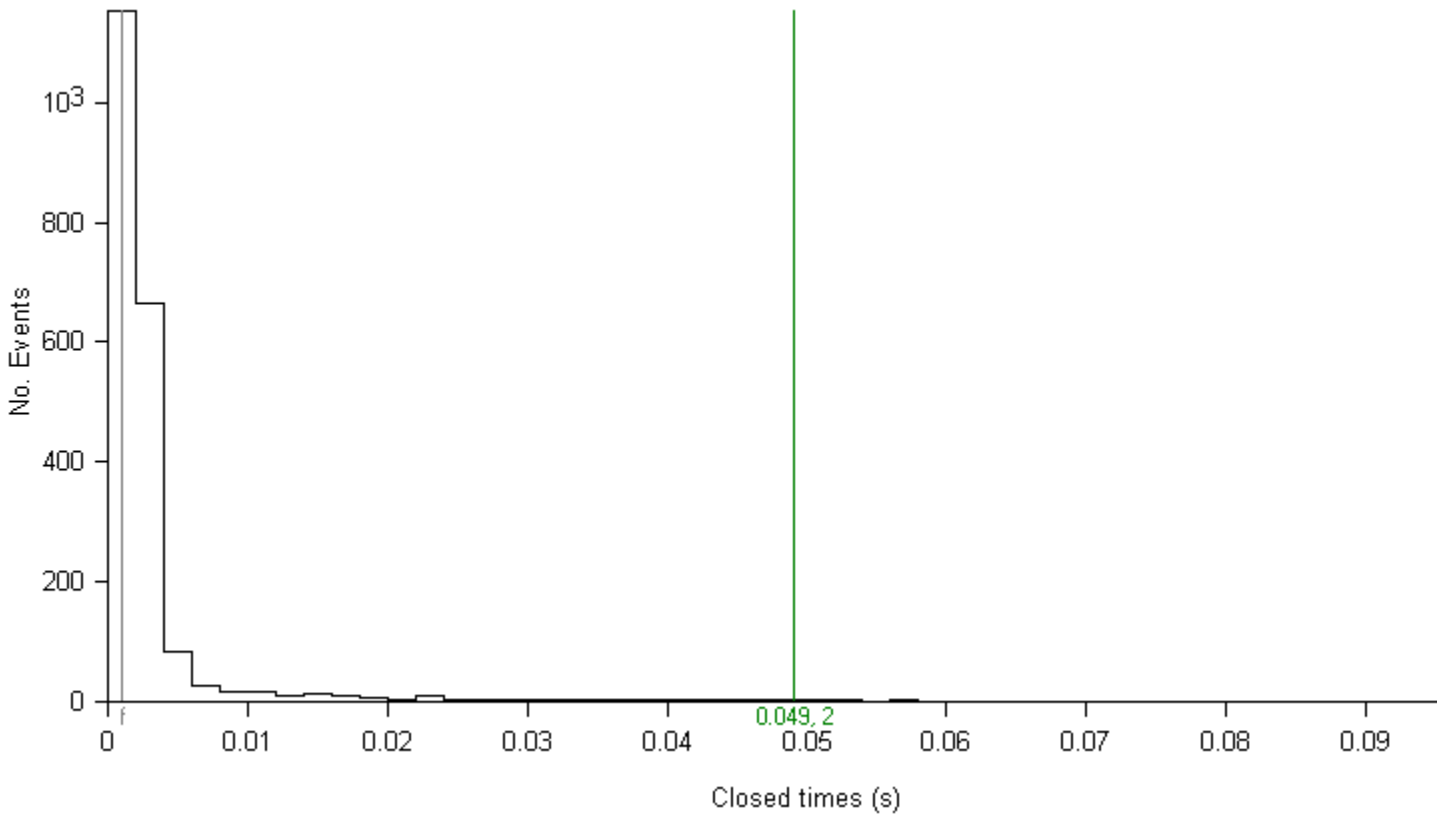
$$i_{bin} = Int\left(\frac{t_{state}}{w} + 1\right)$$

where  $w$  is the width of the bin, and  $Int$  means 'take the integer part of'. The exponential shape of the typical ion channel dwell time distribution is clearly apparent in a linear histogram.



### **Logarithmic histograms with variable bin widths**

Many channels, however, produce open or closed distributions consisting of more than one type of state with radically different mean dwell times. In such circumstances it proves difficult to adequately represent the whole distribution of times using a single fixed bin width. For example, a closed time distribution composed of two types of states, one with a mean dwell time of 1 ms and the other with a mean of 40 ms cannot be easily represented on a linear histogram, as shown in the example histogram (100 bins, 1ms bin width). The 1ms bin width is too large to adequately represent the distribution of the short closures and too small to accumulate significant numbers of the long events in any one bin.

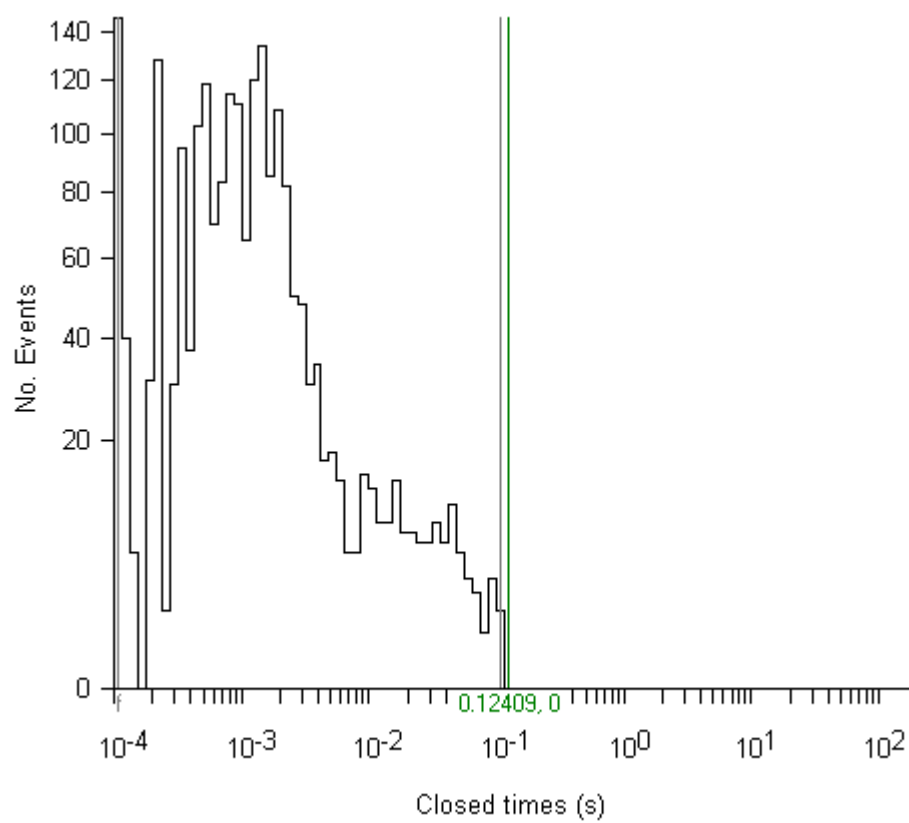


One solution to the binning problems encountered with multi-state distributions is to begin with narrow bin widths for brief events and to progressively increase bin width for subsequent bins. An approach like this, developed by Sigworth & Sine (1987), has found widespread use. Dwell times are allocated to bins according to the formula

$$i_{bin} = 1 + \text{Int}(bpd \cdot \log(n_d))$$

where *bpd* is the number of histogram bins per decade (10-fold) change in dwell time, and *nd* is the dwell time in units of sampling intervals. This algorithm results in a histogram consisting of a series of bins which logarithmically increase in width, from a bin size equal to the sampling interval, by the factor *bpd* every decade. By convention, the vertical axis of the Sigworth-Sine histogram is plotted using a square root scaling.

A typical logarithmic histogram is shown here (the same two-state, 1 ms, 40 ms closed time distribution used earlier). Each exponential now appears as a peaked distribution with the peak value occurring at the mean dwell time for that state.



## Analysing Single-channel Currents > Channel Dwell Time Analysis > Fitting exponential Functions to dwell time histograms

Dwell time distributions can usually be described in terms of a mixture of exponential probability density functions, where the probability  $p(t)$  of dwell time,  $t$ , being observed is given by

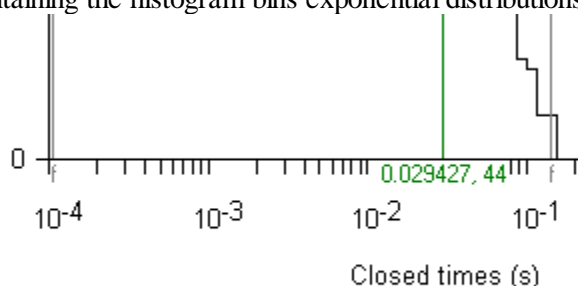
$$p(t) = \sum_{i=1}^{n_{\text{exp}}} \frac{A_i}{\tau_i} \cdot \exp\left(\frac{-t}{\tau_i}\right)$$

where  $n_{\text{exp}}$  is the number of exponential components in the mixture,  $A_i$  is the fraction of the total number of dwell times associated with component  $i$ , and  $\tau_i$  is the mean dwell time. Mixtures of up to 5 exponential p.d.f.s can be fitted to the dwell time histograms, using either an iterative least squares method.

### Fitting Exponential Probability Density Functions

To fit an exponential mixture to a histogram:

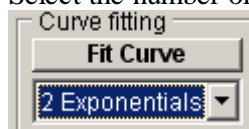
1. Define the range of dwell times containing the histogram bins exponential distributions to be fitted using the pair of



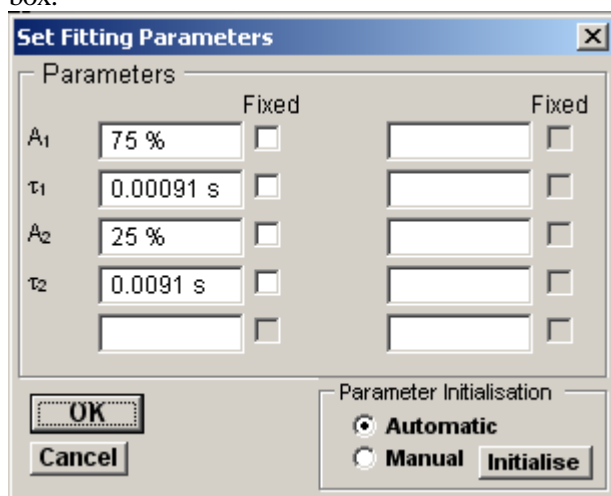
grey '|--|' region of interest cursors.

Mean= 0.00581 s /Events= 3857

2. Select the number of exponential components in the mixture to be fitted from the **Curve Fitting** list.

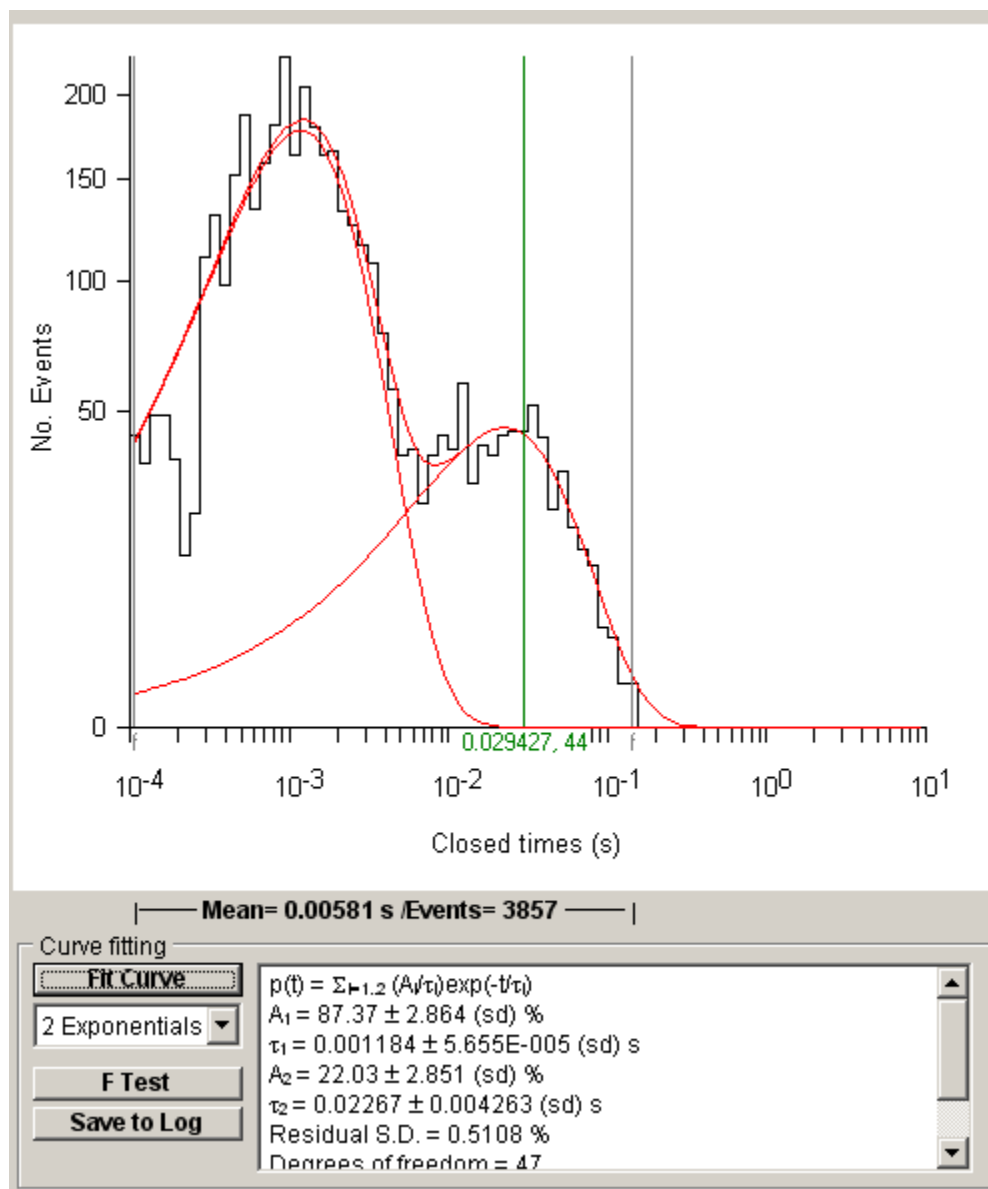


3. Click the **Fit Curve** button and enter an appropriate set of initial guesses for the exponential function parameters ( $A_i$ ,  $\tau_i$ ). A set of initial guesses are computed automatically, but it is often necessary to adjust these to better match the location and size of the observed distribution. E.g. set the mean dwell times to the peaks in a logarithmic histogram. Individual function parameters can also be fixed at their initial values by ticking its associated **Fixed** box.



Click the **OK** button to begin fitting.

The mixture and the best fitting individual exponential components are superimposed (in red) on the histogram. The values of the function parameters along with their standard errors are displayed in curve fitting results area at the bottom of the display.



**Note.** Standard error values computed by the curve fitting program are not true estimates of experimental standard error since they take no account of inter-cell or other variability. They tend only provide a **lower** bound to the estimate of the standard error in a parameter value.) The residual standard deviation ( $SD_{res}$ ) between the histogram data and the fitted curve provides a measure of the goodness of fit. The smaller the value of  $SD_{res}$  the better the fit.

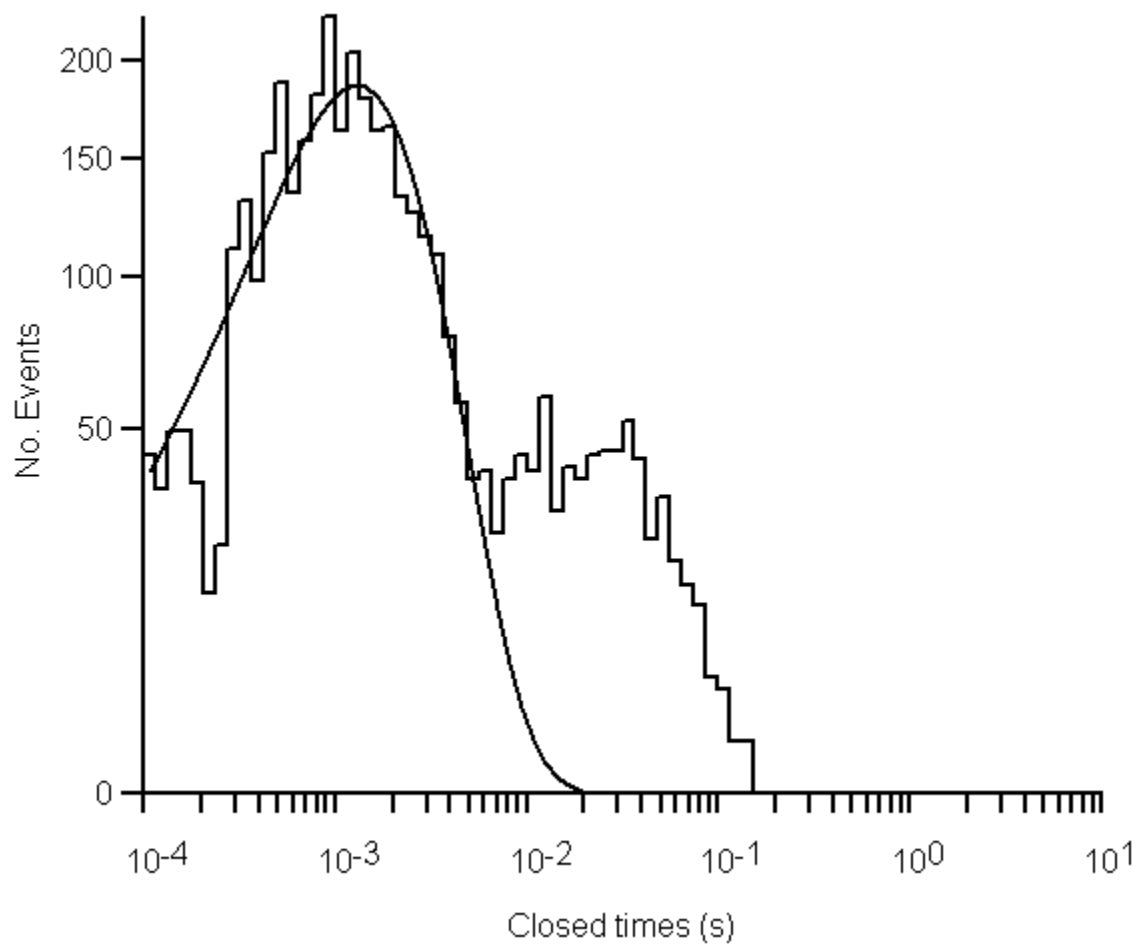
Iterative curve fitting is a numerical approximation technique, which is not without its limitations. In some circumstances, it can fail to converge to a meaningful answer, in others the best fit parameters may be poorly defined. It is important to make an assessment of how well the function fits the histogram before placing too much reliance on the parameters.

### Determining the required number of exponential components

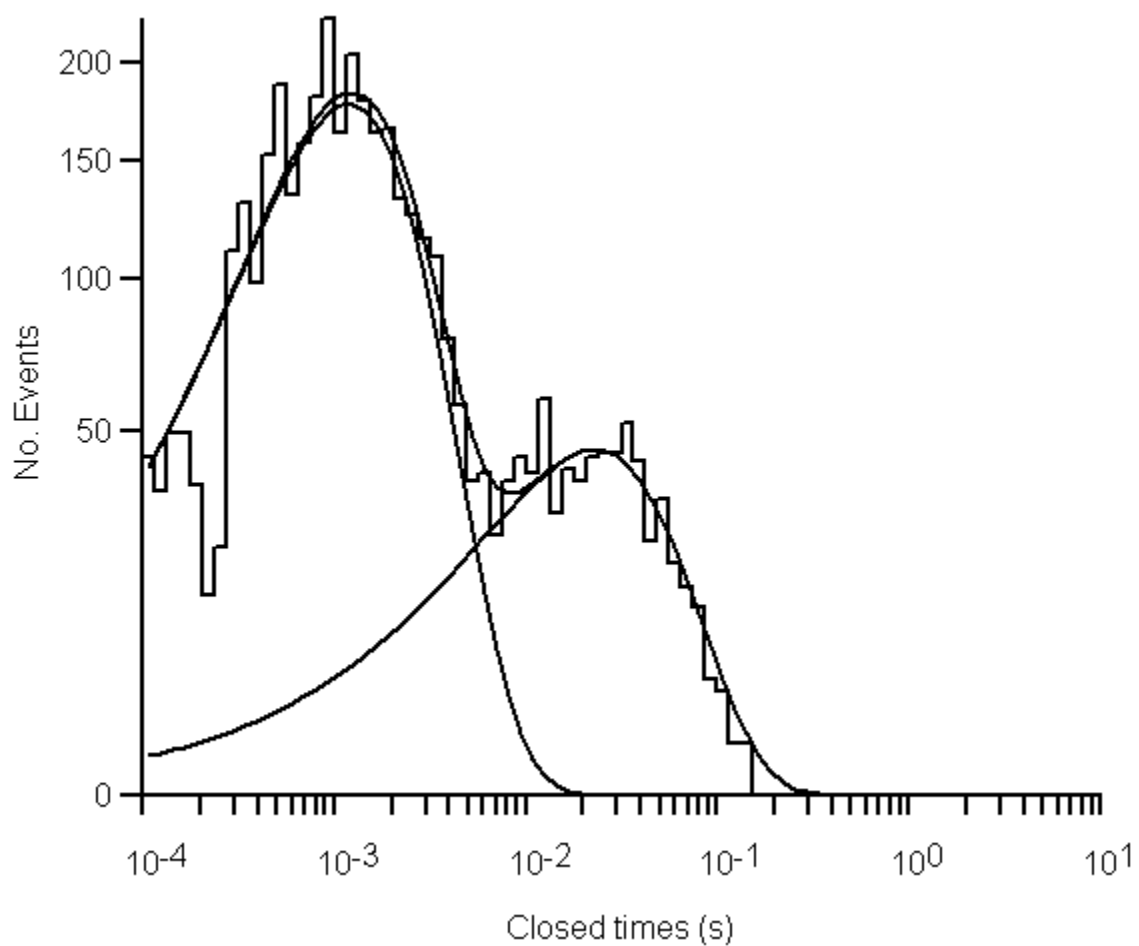
The number of components necessary to fit a dwell time distribution can be determined by fitting a series of exponential p.d.f. mixtures with increasing numbers of components, until no significant improvement in fit is observed. For example, the figures below show the results of fitting mixtures of 1, 2 and 3 exponential components.

The single exponential p.d.f. can be clearly seen to be a poor fit, with marked deviations between the fitted line and the data.

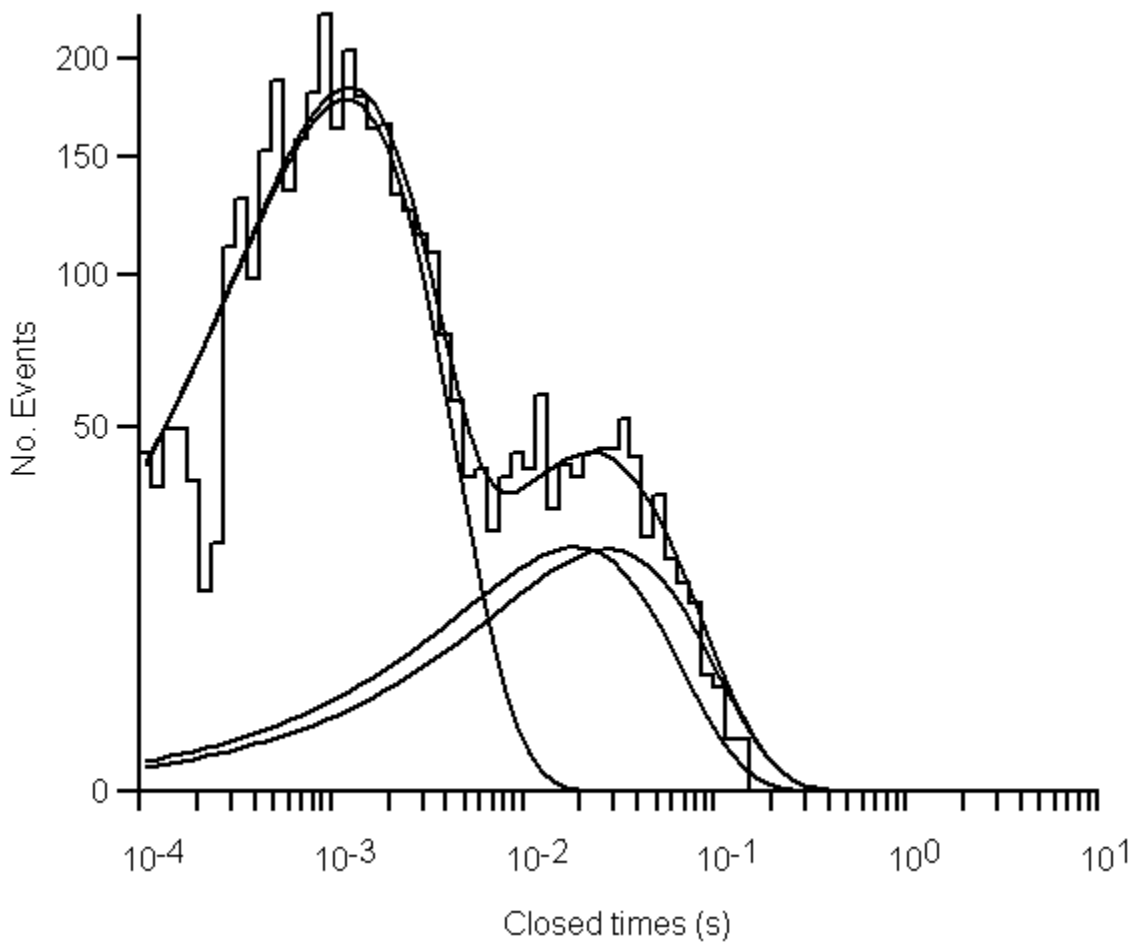




The mixture of two exponentials provides a qualitatively better fit, accounting for both peaks in the distribution. The residual standard deviation has also been reduced, indicating a better quantitative fit.



The mixture of three exponentials (c) also provides a qualitatively good fit, with a residual standard deviation similar to the two exponential fit. Two of the components appear to be very similar mean values



It is usual to choose the p.d.f. function with the least number of parameters, sufficient to provide a good fit. Additional components should only be included if they can be demonstrated to significantly improve the quality of fit. A quantitative estimate of the improvement in the quality of the fit, obtained by adding additional exponential components, can be obtained by computing the F statistic

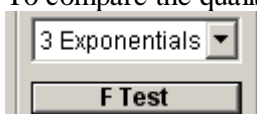
$$F_{a,b} = \frac{SSQ_a - SSQ_b}{SSQ_b} \times \frac{n - m_b}{m_a}$$

$SSQ_a$  and  $SSQ_b$  are the sums of squares of the residual differences between the histogram data and the expected values for two p.d.f. functions  $a$  and  $b$ , where function  $a$  contains fewer exponential component than  $b$ .  $m_a$  and  $m_b$  are the number of free parameters in each function and  $n$  is the number of histogram bins.  $SSQ$  can be calculated for each function from the residual standard deviation,  $SD_{res}$  and degrees of freedom,  $n_{free}$ , values returned as part of the curve fitting results.

$$SSQ = n_{free} \times SD_{res}^2$$

Large positive values of  $F_{a,b}$  indicate that extra exponentials in function  $b$  result in a better fit to the data than function  $a$ , small positive values indicate that the fit is little improved and negative that it is worse. The significance probability of the observed  $F_{a,b}$  value can be determined from a standard  $F$  distribution, with  $m_a$  and  $n - m_b$  as its degrees of freedom. Further details can be found in Horn (1987).

To compare the quality of fit of the series of p.d.f.s fitted to a histogram, click the **F Test** button



to open the F Test box.

F Test			
Results			
Exponentials	1	2	3
Residual SSQ	28.02	12.26	12.3
No. Parameters	2	4	6
Degrees of Freedom	49	47	45
F		30.19	-0.03605
p		0.0000	0.9805
A(1)	91.64 %	87.37 %	87.59 %
Tau(1)	0.001298 s	0.001184 s	0.001188 s
A(2)		22.03 %	10.83 %
Tau(2)		0.02267 s	0.01869 s
A(3)			10.7 %
Tau(3)			0.02791 s
<input type="button" value="Print"/> <input type="button" value="Clear Table"/>			

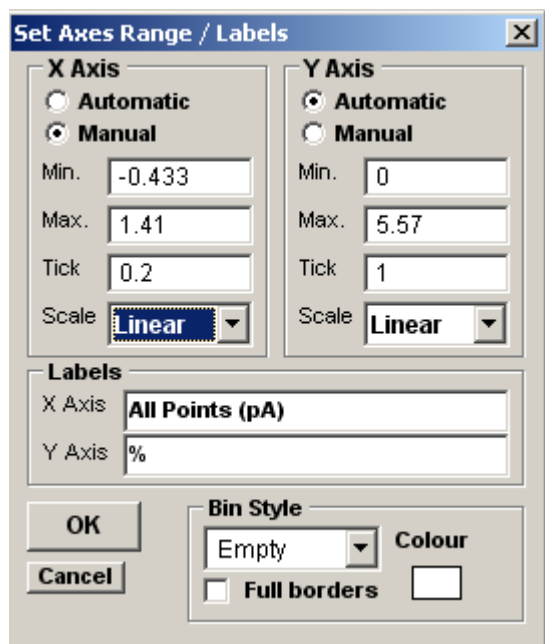
The residual sum of squares, number of function parameters and degrees of freedom for each function that has been fitted are tabulated.

For each successive exponential component the F statistic comparing its quality of fit with the previous one is computed along with its associated significance probability, p. A function is deemed to have significantly improved the quality of fit, if  $p \leq 0.05$ . In the example shown here, 2 exponentials provide a significantly better fit ( $p=0.0000$ ) than, but 3 exponentials do not provide a better fit than 2 ( $p=0.9805$ .)

Click the **Print** button to print a hard copy of the F test results table. The table of results can be cleared by clicking the **Clear Table** button.

## Analysing Single-channel Currents > Channel Dwell Time Analysis > Customising Histogram Plots

If you want to alter the X or Y axis range, scaling or labels on a histogram, click the **Set Axes** button to open the dialog box.



The dialog box is titled "Set Axes Range / Labels" and contains the following sections:

- X Axis:** Radio buttons for **Automatic** and **Manual** (selected). Fields for Min. (-0.433), Max. (1.41), and Tick (0.2). A **Scale** dropdown menu set to **Linear**.
- Y Axis:** Radio buttons for **Automatic** and **Manual** (selected). Fields for Min. (0), Max. (5.57), and Tick (1). A **Scale** dropdown menu set to **Linear**.
- Labels:** Text boxes for X Axis ("All Points (pA)") and Y Axis ("%").
- Bin Style:** A dropdown menu set to **Empty**, a **Colour** box, and a **Full borders** checkbox.
- Buttons:** **OK** and **Cancel** buttons.

You can change the X or Y axis limits by selecting the **Manual** Axis option and entering new values into the **Min**, **Max**, and **Tick** (spacing) boxes. An axis can be made **Linear** or **Logarithmic** by selecting the option from its **Scale** list. Labels for the X and Y axes can be entered into the **Labels** boxes.

The style of rectangle used to plot the histogram bins can be changed using the **Bin Style** options. Select **No Fill** to display bins as rectangular outlines, **Solid Fill** to fill the bins in with a solid colour, and **Hatched Fill** for bins filled with a diagonal lines. You can define the colour used for the solid fill, by clicking the **Colour** box, and selecting a colour from the palette. The **Full Borders** check box determines whether the outline is drawn completely around each bar, or just where bars do not overlap.

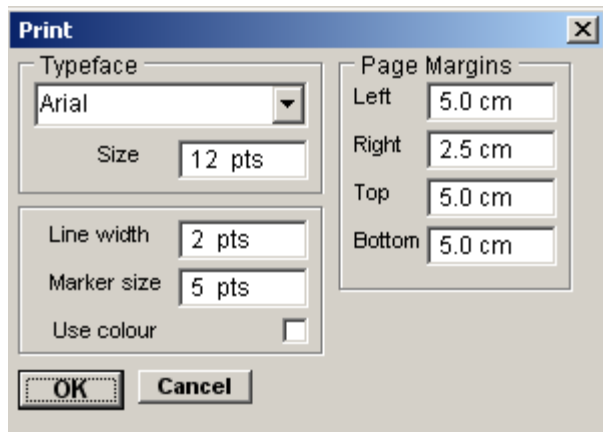
## Analysing Single-channel Currents > Channel Dwell Time Analysis > Printing Histograms

To print the displayed histogram, select

**File**

**Print**

To open the dialog box.



Click the **OK** button to print the graph.

## Analysing Single-channel Currents > Channel Dwell Time Analysis > Copying Histograms to the Clipboard

### Copying the histogram data points to the Windows clipboard

The numerical values of the X,Y data points which generate the histogram can be copied to the clipboard by selecting

**Edit**

**Copy Data**

The data is placed on the clipboard as a table of data values, in tab text format, defining the histogram. There are 4 values per row, and one row for every bin in the histogram. Each row has the format

```
<Bin Lower Limit> <tab> <Bin Mid-point> <Bin Upper Limit> <tab> <Bin Count> <cr> <lf>
```

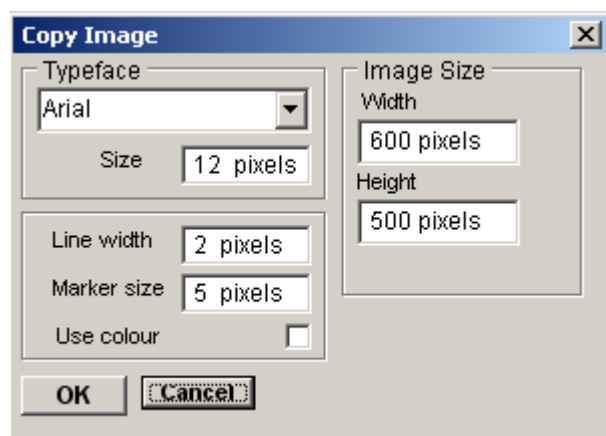
### Copying an image of the histogram to the Windows clipboard

An image of the histogram plot can be copied to the clipboard by selecting

**Edit**

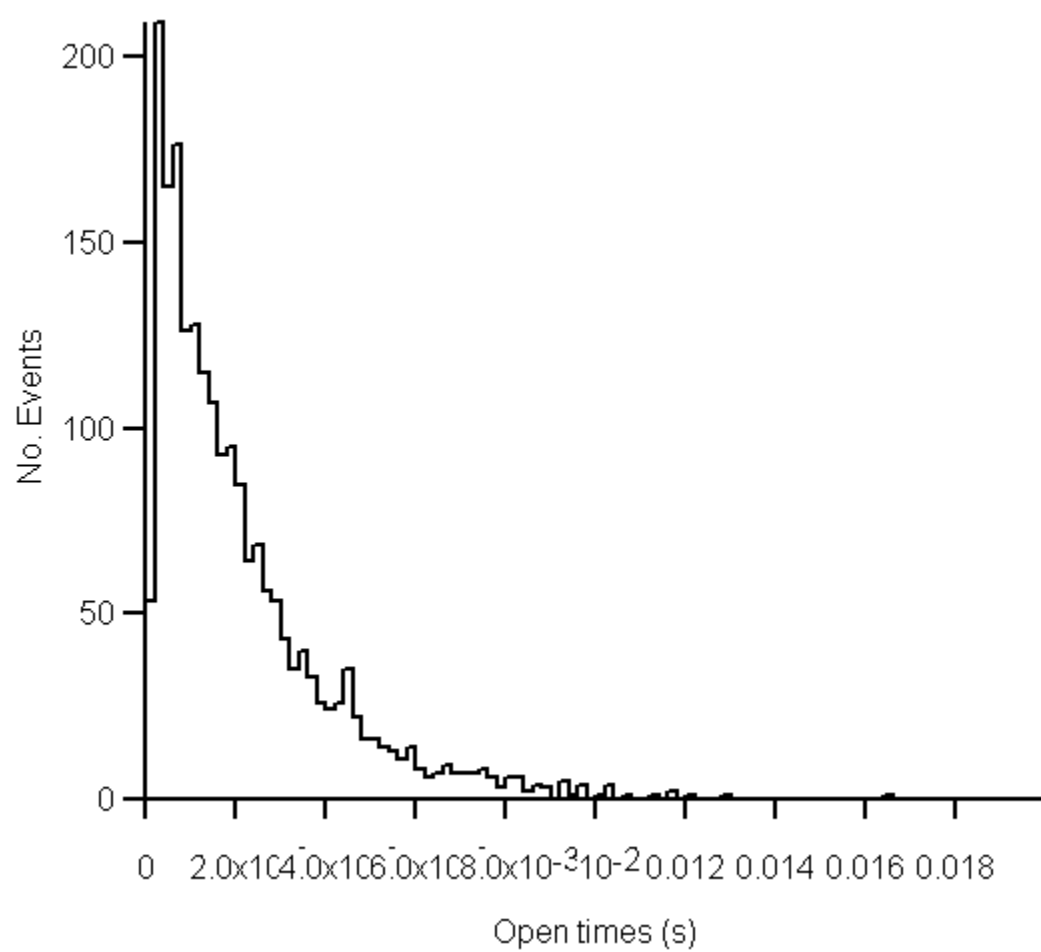
**Copy Image**

to open the dialog box.



The dimensions (pixels) of the bit map, which will hold the image, can be set using the Width and Height image size boxes. The size and style of the typeface can be set using the Typeface and Size boxes.

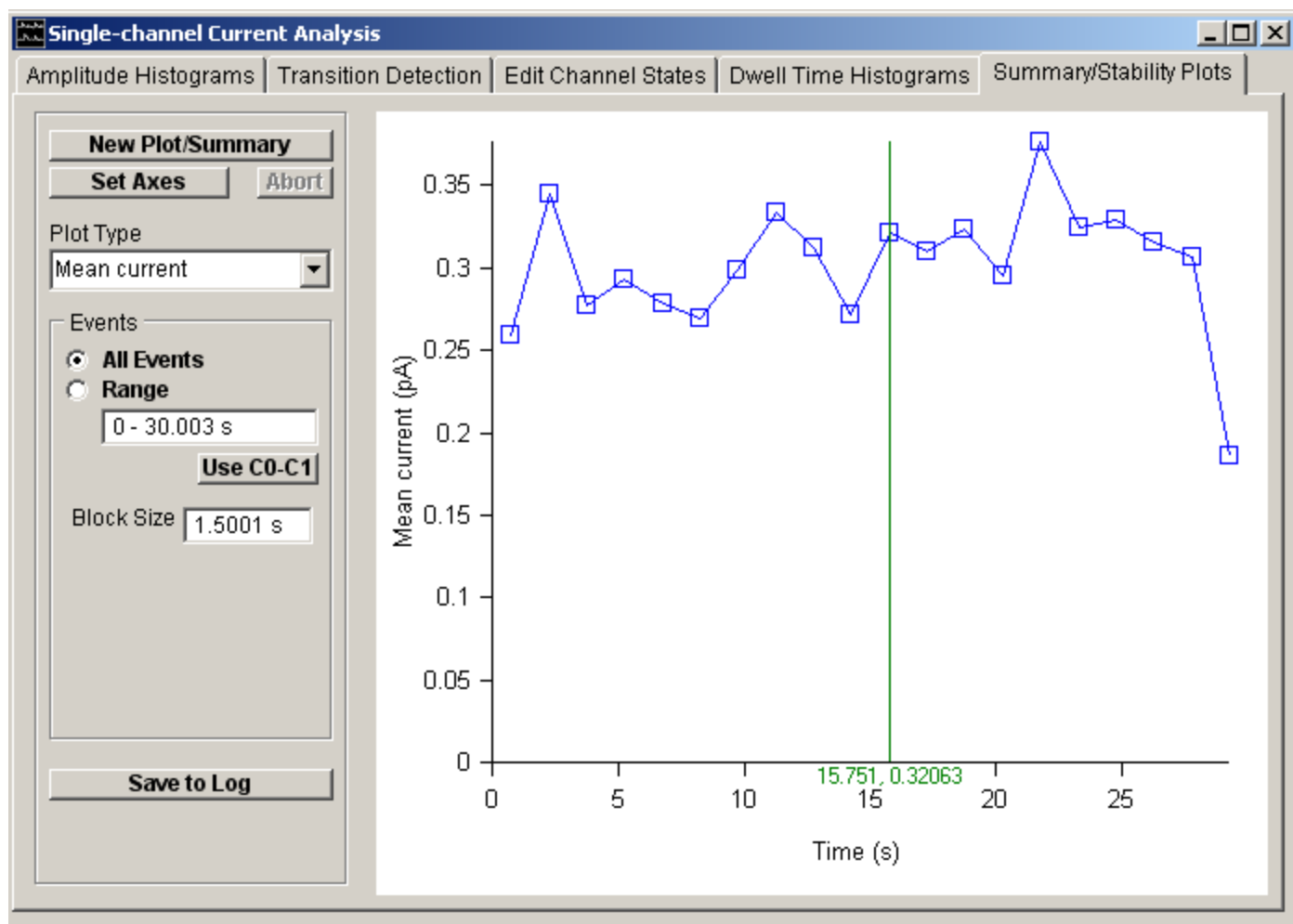
When the image parameters have been set, click the **OK** button to copy the image to the clipboard.





## Analysing Single-channel Currents > Stability Plots > Stability Plots

The validity of the channel dwell time analysis is dependent upon the channel gating being in a **stationary** condition throughout the recording. Stationarity means that the rate constants governing the transitions between channel states are constant and the mean open and closed state dwell times, and open state probability, do not vary during the recording. The **Summary/Stability Plots** page provides a set of tools for revealing the presence of trends in the single-channel current amplitudes or dwell times.



To create a stability plot :

1. Select the type of plot to be created from the **Plot Type** list.
2. Select the **All** option to use all sample points/events contained in the recording/event list or select **Range** and enter a time interval/event range to define a selected region.
3. Enter the number of averaging blocks that the analysis region is to be divided into in **No. Blocks** box.
4. Click the **New Plot** button to display the plot.

The values of the x,y points in the plot can be read out using the blue readout cursor.

The following parameters can be plotted:

- **Mean current:** Displays trends in mean currents. The digitised current signal is divided into a series of contiguous blocks, the mean current for each block is computed and plotted vs. the mid-point time for each block.
- **Open probability:** Displays the probability of a channel being open and is derived from the mean current plot, by dividing the mean block current by the single-channel current value (set on the Amplitude Histograms or Transition Detection page).

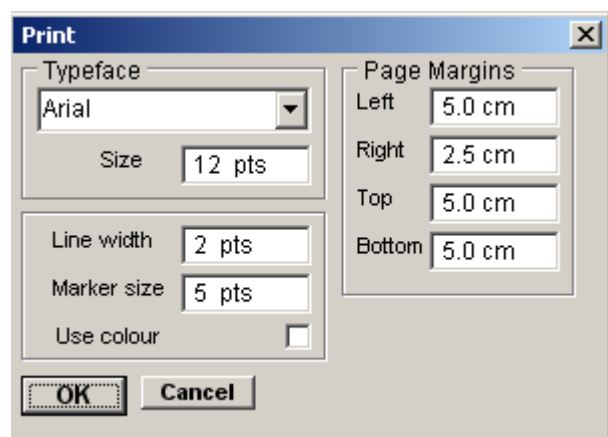
- **Open times:** Displays trends in the channel open state dwell time. The channel event list is divided into a series of contiguous blocks, the mean open state dwell time for each block is computed and plotted vs. the mid-point time for each block.
- **Closed times:** Displays trends in the channel closed state dwell time.
- **Single-channel currents:** Displays trends in the amplitude of the single-channel currents. The channel event list is divided into a series of contiguous blocks, the mean single-channel current for each block is computed and plotted vs. the mid-point time for each block.
- **Current vs Open times:** Displays the relationship between open state duration and average open state current. Dwell time and average current for the open states in the event list are displayed as a scatter plot.
- **Cursor Meas. (Avg):** Displays (vs time) the average current in the the manual cursor measurements lists.
- **Cursor Meas. (Dur):** Displays (vs time) the average duration in the the manual cursor measurements lists.
- **Cursor Meas. (S.D.):** Displays (vs time) the average standard deviation in the the manual cursor measurements lists.

## Analysing Single-channel Currents > Stability Plots > Printing Plots

To print the displayed plot, select

**File**  
**Print**

To open the dialog box.



Click the **OK** button to print the graph.

## Analysing Single-channel Currents > Stability Plots > Copying Plots to the Clipboard

### Copying the plot data points to the Windows clipboard

The numerical values of the X,Y data points which generate the plot can be copied to the clipboard by selecting

**Edit**

**Copy Data**

The data is placed on the clipboard as a table of data values, in tab text format, defining the histogram. There are 4 values per row, and one row for every bin in the histogram. Each row has the format

```
<X> <tab> <Y> <cr> <lf>
```

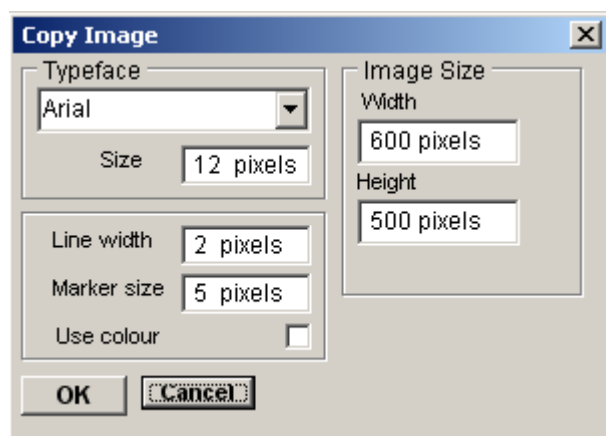
### Copying an image of the plot to the Windows clipboard

An image of the histogram plot can be copied to the clipboard by selecting

**Edit**

**Copy Image**

to open the dialog box.



The dimensions (pixels) of the bit map, which will hold the image, can be set using the Width and Height image size boxes. The size and style of the typeface can be set using the Typeface and Size boxes.

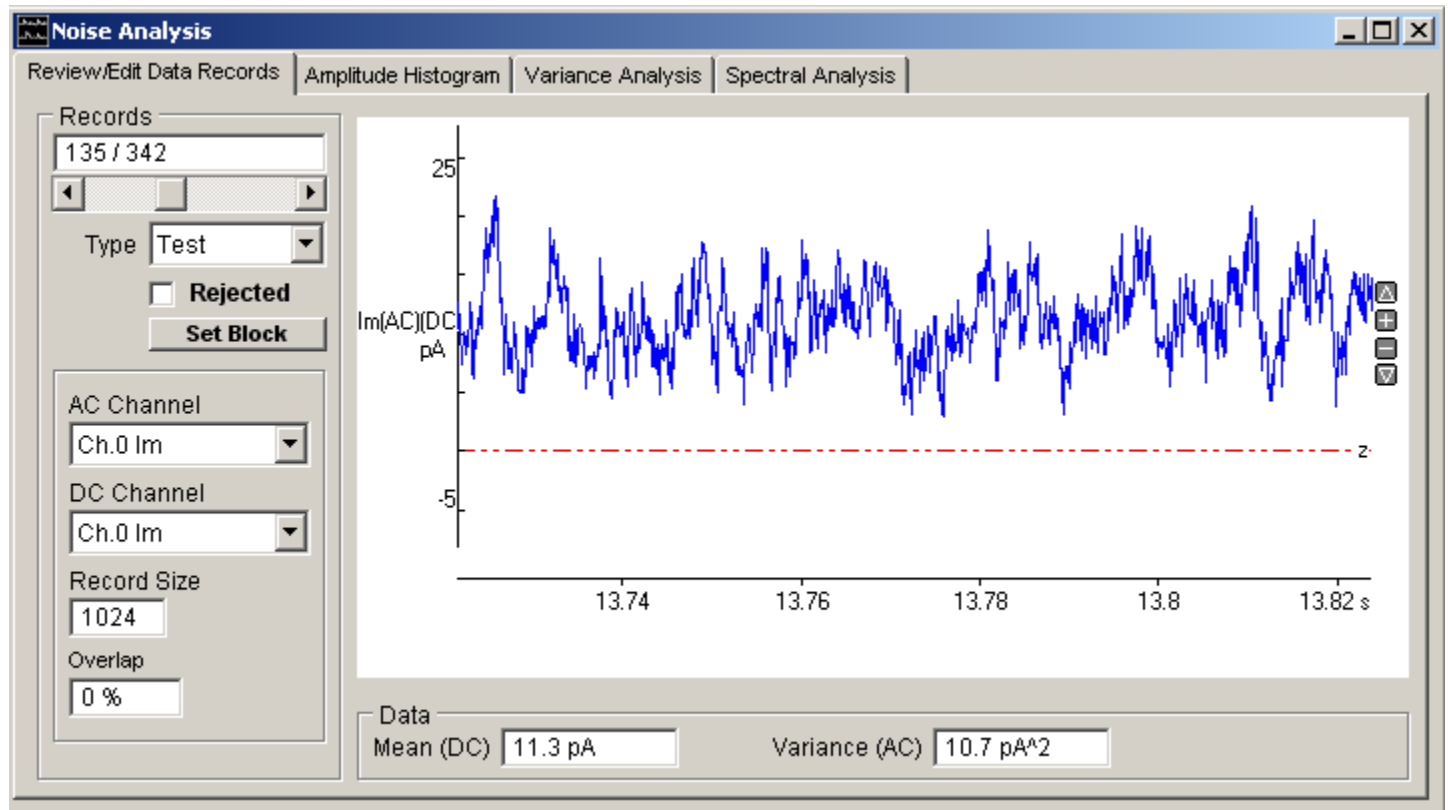
When the image parameters have been set, click the **OK** button to copy the image to the clipboard.

## Noise Analysis > Noise Analysis

To analyse ionic current noise, select

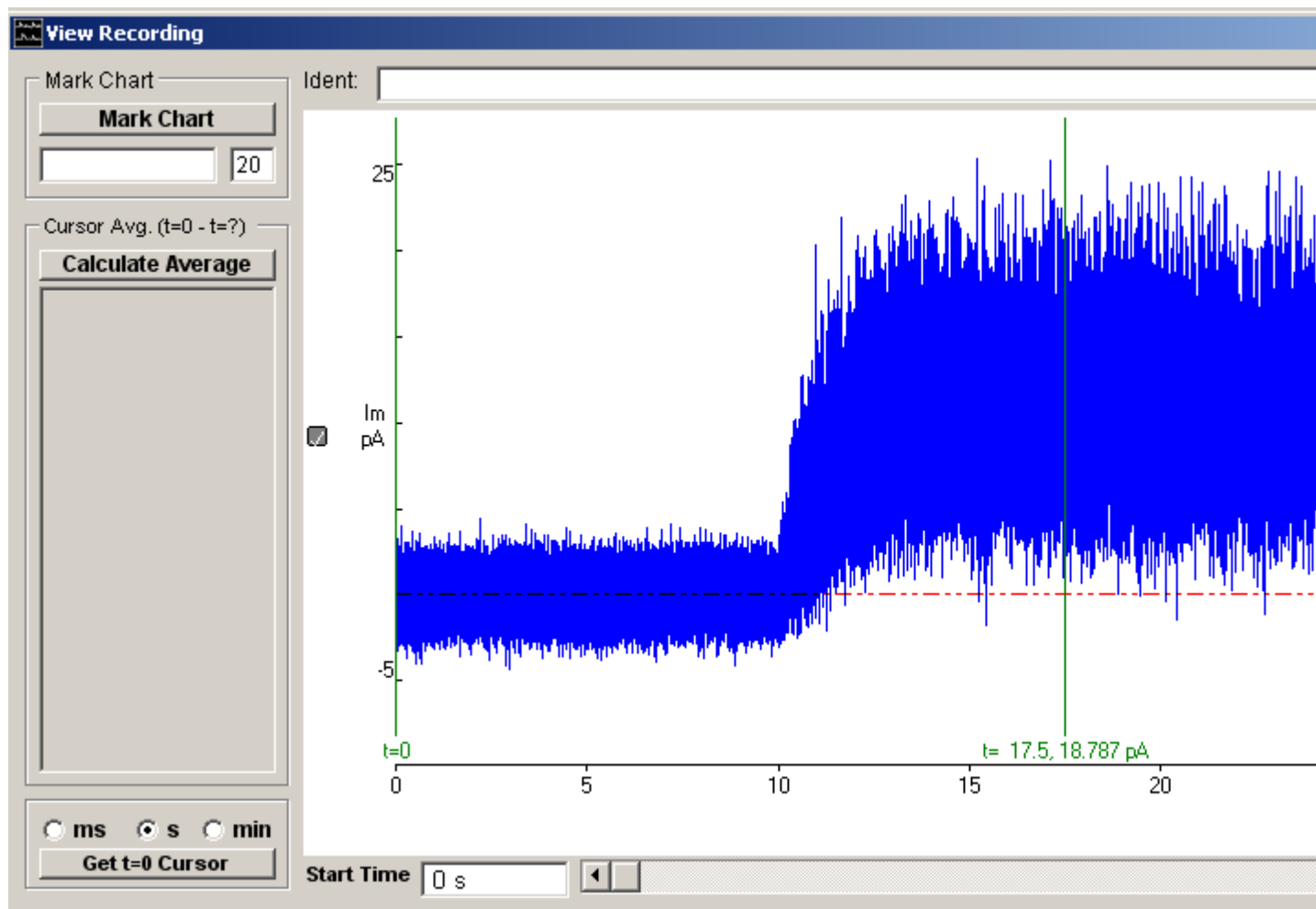
### Analysis Noise Analysis

to open WinEDR's module for analysing the random fluctuations in ion channel currents under stationary or semi-stationary conditions.



The module is split into 3 pages each associated with a specific noise analysis operation. The **Variance Records** page provides tools for the partition of the continuous digitised signal into variance records, rejection of artefact and classification of records. The **Variance Analysis** page is used to plot the mean, variance and other parameters computed from variance records against each other. The **Spectral Analysis** page is used to plot the average power spectrum of the signal.

Noise analysis is most commonly applied to the whole cell currents activated by the opening of receptor-activated ion channels.. The figure below shows a typical recording. Recording begins before the agonist is applied in order to obtain a sample of the background noise, the agonist is applied after 10 s and both the mean current and the amplitude of its fluctuations can be seen to increase to a steady state.



A 'stationary' random signal is one whose basic statistical parameters (mean, variance, power spectrum) remain constant during the period of the recording. A semi-stationary signal is defined as one where the statistical parameters are not constant, but varying at a rate much slower than the intrinsic fluctuations. A discussion of the theory and practice of noise analysis can be found in Defelice (1981), Eisenberg et al. (1984) or Dempster (1992, 2001).

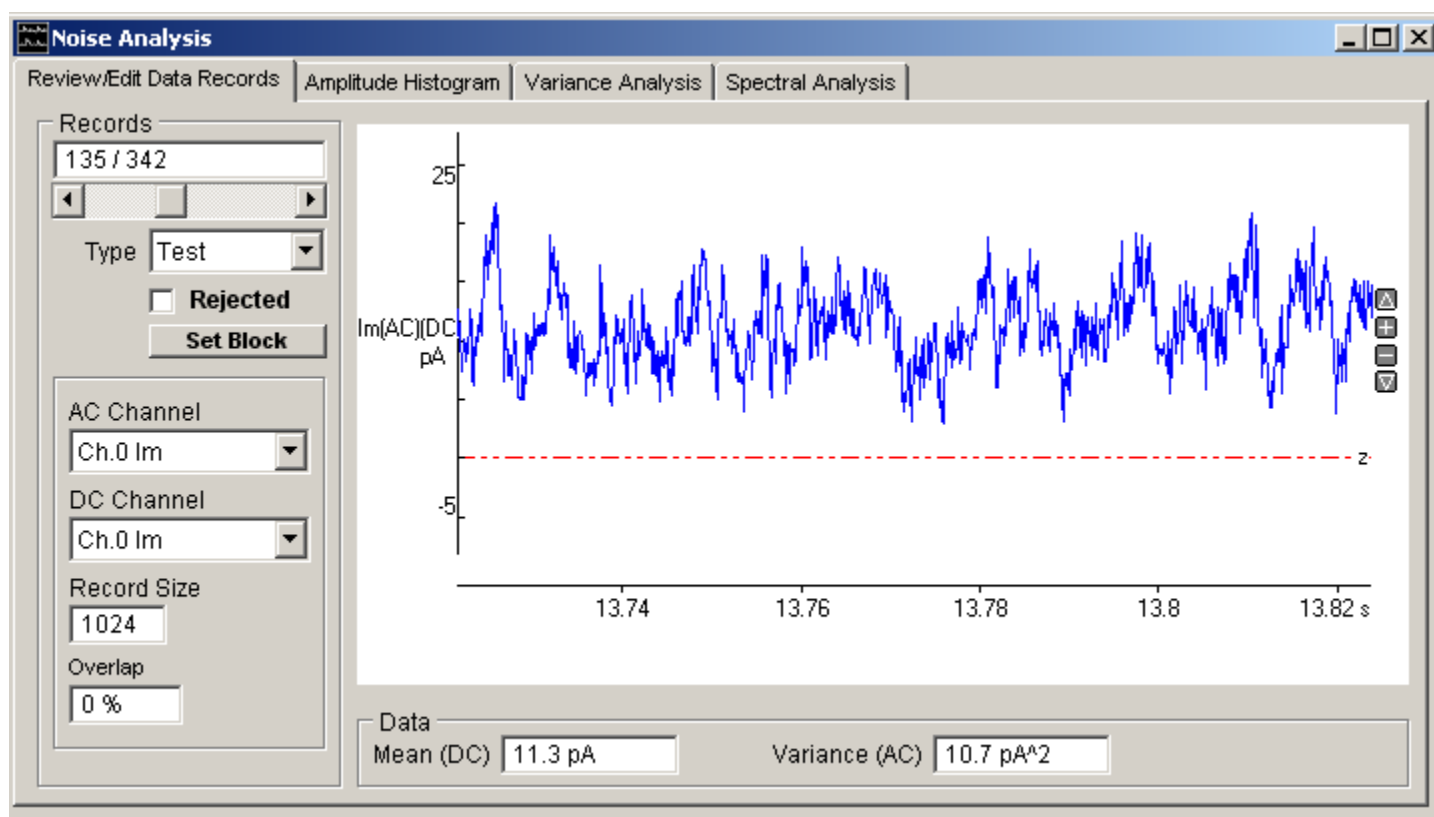
## Noise Analysis > Signal Conditioning

The analogue signal conditioning requirements for signals intended for noise analysis are somewhat stricter than for other applications A low-pass anti-aliasing filter with sharp cut-off characteristics (Butterworth or Chebyshev) is essential. (Suitable filters include the Frequency Devices 901 or Kemo VBF-8) The cut-off frequency of this filter should be set to slightly less than half of the digital sampling rate.

In some studies, e.g. endplate noise at the neuromuscular junction, the current fluctuations are a small fraction of the mean current. In these circumstances, it may be necessary to use a high-pass filter to separate out the noise from the mean current signal, amplify by X10-X100, and digitise it on a second input channel (See Dempster 2001).

## Noise Analysis > Selection & Inspection of Variance Records

Select the **Variance Records** page to select the signal channel(s) to be analysed, define the size of the variance record, and to visually inspect, edit and classify the recorded signals.



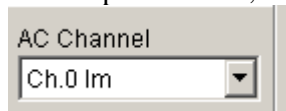
Variance records are displayed individually on the screen, selectable using the **Records** scroll bar or box. The record mean signal level and variance are displayed at the bottom of the screen.

Set up for a variance or power spectrum analysis using the following procedure :

1. If there is more than one input channel, select the channel containing the basic, DC-coupled, current signal with the **DC Channel** list.

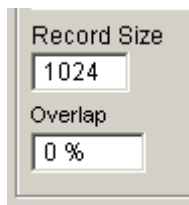


2. If the current noise has been acquired in an additional high-gain, AC-coupled channel, separate from the DC-coupled channel, select this channel with the **AC Channel** list.

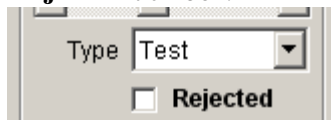


3. Enter the number of samples in the variance calculation record in the **Record Size** box (32-8192). (**Note.** The record size is constrained to be a power of 2.) The larger the size of the variance record the more accurately the variance can be calculated. This is, however, at the expense of the time resolution of changes in variance. The size of the record also determines the low frequency limit of the power spectrum. [Optional] Enter the record overlapping factor in the **Overlap** box (0%, 25%, 50%, 75%). The default setting, an overlap factor of 0%, corresponds to contiguous, independent records. Increasing the overlap factor, increases the number of available records improving the temporal resolution at the expense of sharing samples between records.

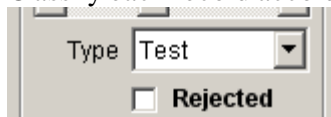




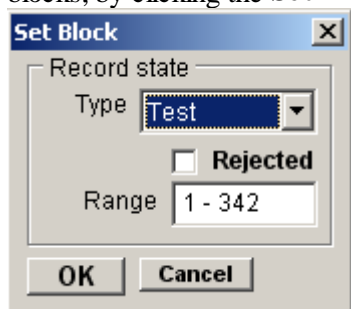
4. Inspect each individual variance record and reject those containing artefacts from analysis by ticking the **Rejected** tick box.



5. Classify each record according to what type of signal it contains - Background or Test – by setting its **Type**.



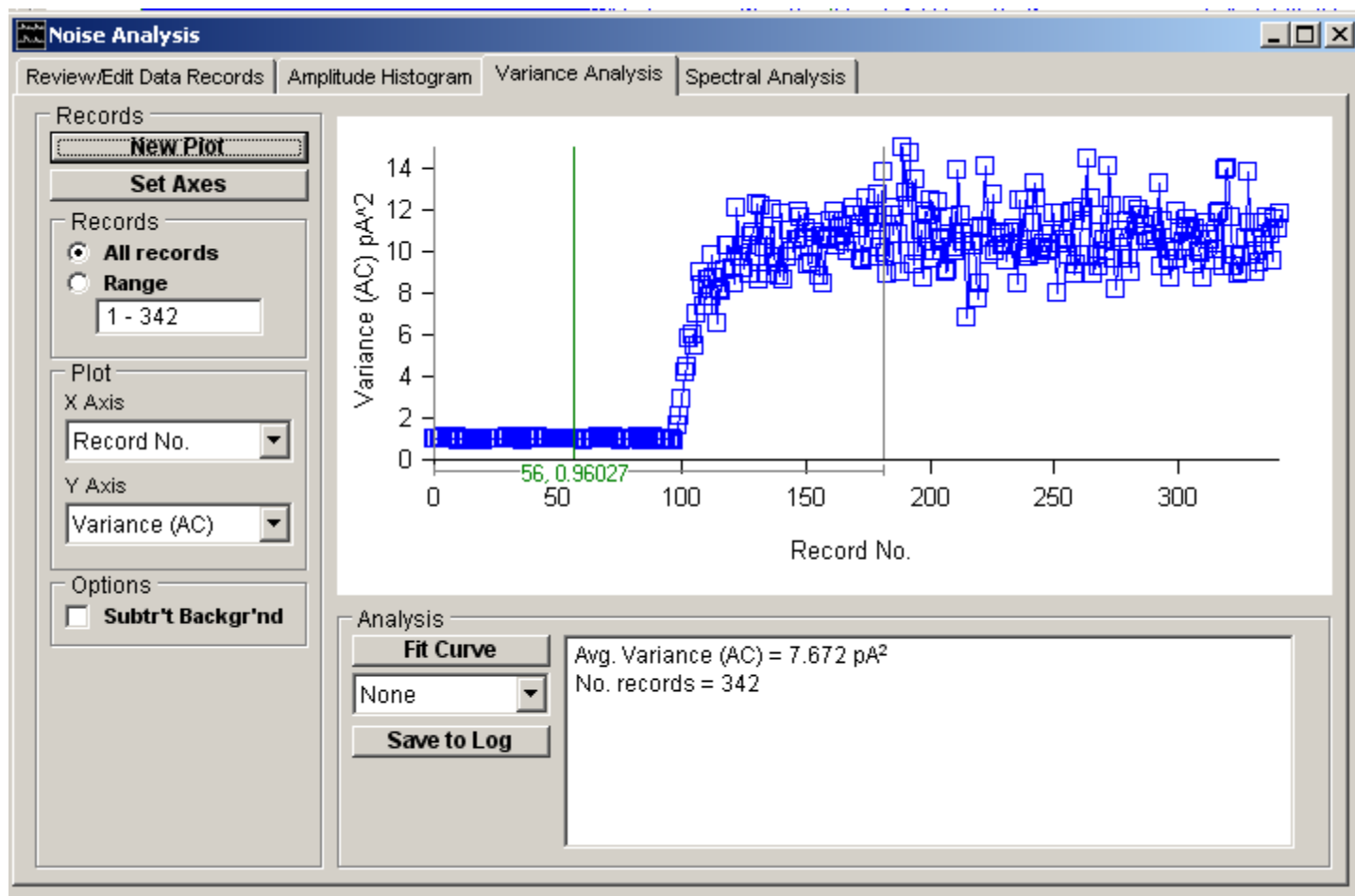
Records containing only noise from sources other than the ion channels under study (e.g. instrumentation noise) are classed as **Background**. Records containing the ion channel noise under investigation are classed as **Test**. (Background and Test records are processed separately, allowing the averaged variance/power of the background noise to be subtracted from the ion channel noise.) Records can either be classified individually or in blocks, by clicking the **Set Block** button to open the Set Block dialog box.



6. If necessary, set the DC Channel zero current level by dragging zero level cursor up or down. (**Note**. The zero current level is usually set to the mean signal level that exists within the Background records.

## Noise Analysis > Analysis of Signal Variance

Select the **Variance Analysis** page to display and analyse the mean current, variance and other parameters computed from the variance records.



To select and plot a pair of variables :

1. Define the variable to be plotted on the X axis, by selecting it from the **X Axis** variable list.
2. Define the variable to be plotted on the Y axis, by selecting it from **Y Axis** list.
3. Select the **All Records** option to plot all available variance records or select **Range** and enter a specific range of records. (**Note**. Records marked as Rejected and Background records are excluded.)
4. Select the **Subtr't Backg'd** option to subtract the average variable value within the Background records from the plotted data
5. Click the **New Plot** button to plot the graph. Five basic parameters are computed from each variance record and can be plotted against each other.

Five basic parameters are computed from each variance record and can be plotted against each other:

**Mean (DC)** is the mean current computed with the formula

$$I_{mean} = \frac{\sum_{i=1}^N I(i)}{N}$$

where  $I(i)$  is the  $i^{th}$  of the  $N$  samples in the variance record.

**Variance (AC)** is the current variance, <sup>2</sup>, computed using the formula

$$\sigma^2 = \frac{\sum_{i=1}^N (I(i) - I_{mean})^2}{N - 1}$$

**St Dev (AC)** is the standard deviation (the square root of Variance (AC)).

**Skew (AC)** is the current skew, computed using the formula

$$Skew = \frac{\sum_{i=1}^N (I(i) - I_{mean})^3}{N - 1}$$

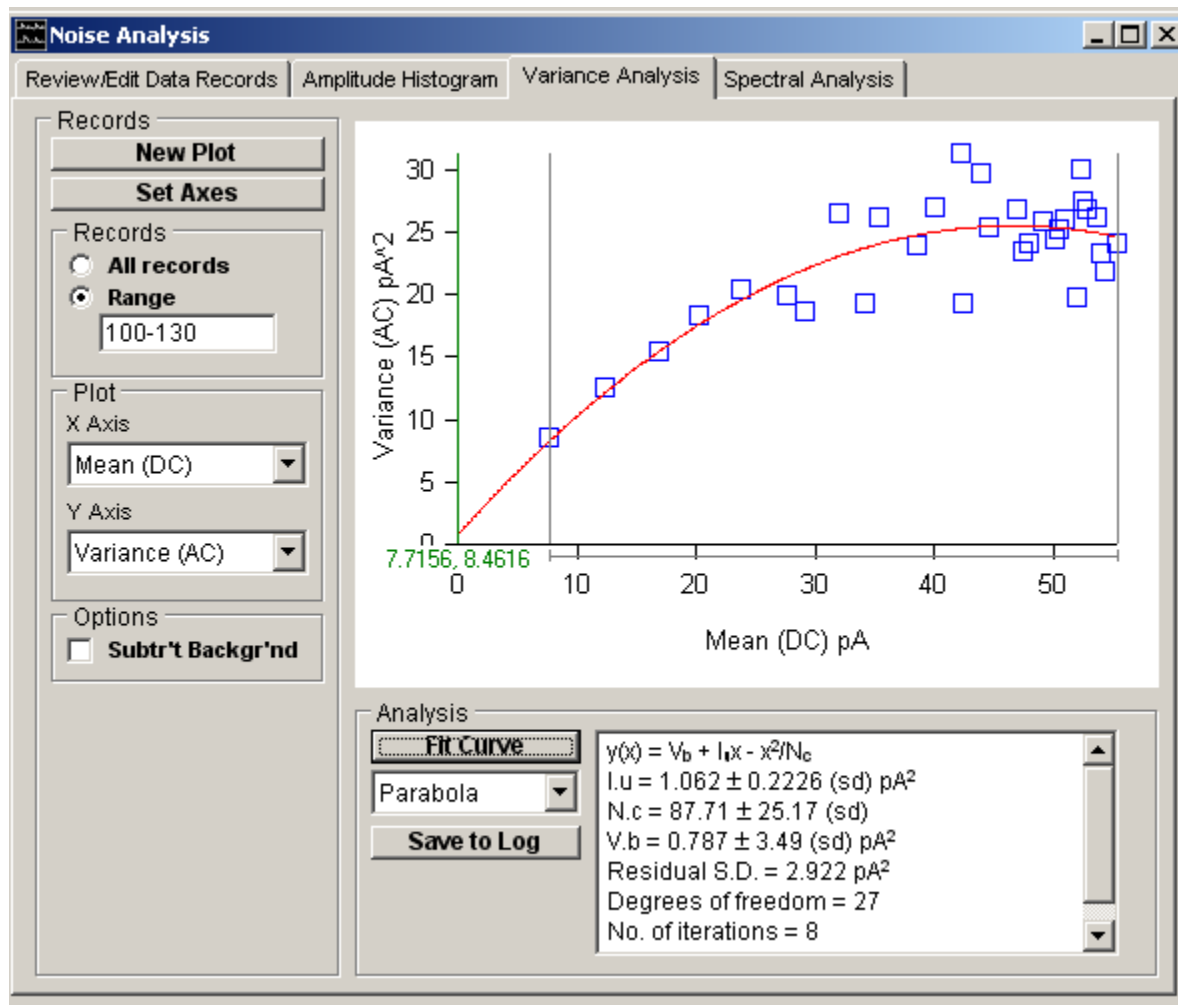
**Median Freq. (AC)** is median frequency of the power spectrum calculated from each variance record. (

**Note.** Variables marked (AC) are computed from the designated AC signal channel (See Variance Records page), if one exists. Variables marked DC are computed from the DC channel.)

## Noise Analysis > Variance-Mean Current Plots

To plot a variance-mean current curve and estimate single-channel current and number of channel by fitting a parabolic curve:

1. Select a range of variance records from a period of the recording where the mean current is changing.
2. Select **Mean Current (DC)** from the **X Axis** list and **Variance (AC)** from the **Y Axis** list.
3. Click the **New Plot** button to plot the variance-mean graph.
4. Select the region of the graph to fitted, using the **|--|** analysis region cursors.
5. Select **Parabola** from the Fit Curve list to fit the parabolic equation to the plot.
6. Click the **Fit Curve** button. Set the initial parameter guesses (optional) and click the **OK** button.



The best-fit single-channel current ( $I_u$ ), number of channels ( $N_c$ ) and background variance ( $V_b$ ), unrelated to channel activity, along with estimated standard error and residual standard deviation are displayed in the fitting results box.

### Theoretical Background

An estimate of the single-channel current and the number of channels in the cell (or patch) can be obtained by plotting current variance vs. mean current. For a cell containing a population of  $n_c$  ion channels, each capable of passing a current,  $I_u$ , and a probability,  $p$ , of being open at any given time, the mean current for the whole cell is

$$I_m = I_u \cdot n_c \cdot p$$

The variance of the current fluctuations is

$$\sigma^2 = I_u^2 \cdot n_c \cdot p \cdot (1 - p)$$

These two equations can be combined to provide a parabolic relationship between  $\sigma^2$  and  $I_m$ ,

$$\sigma^2(I_m) = I_u^2 \cdot I_m - \frac{I_m^2}{n_c}$$

Estimates of  $I_u$  and  $n_c$  can thus be obtained from plots of current variance vs. mean current by fitting the above using non-linear curve fitting.

Examples of the use of variance-mean plots and a discussion of the issues involved can be found in Cull-Candy et al (1988), Dempster (1992, 2001).

## Noise Analysis > Power Spectra

The power spectrum is a measure of the frequency distribution of the signal variance. Analysis of the amplitude and shape of the power spectrum provides a means of estimating both single-channel current amplitude and channel gating kinetics. The averaged spectrum is obtained by computing the individual power spectrum for each variance record using the fast Fourier transform, averaging the corresponding frequency components from each record, and [optionally] averaging adjacent frequency components. By convention, the power spectra is then plotted using log-log axes.

Select the **Power Spectrum** page to compute and plot the averaged power spectrum for a series of variance records.

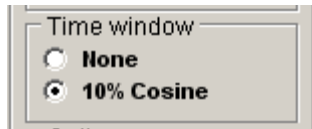
To plot a power spectrum :

1. Select the **All Records** option to include all available variance records in the spectrum or select **Range** and enter a specific range of records. (**Note**. Records marked as Rejected and Background records are excluded.)

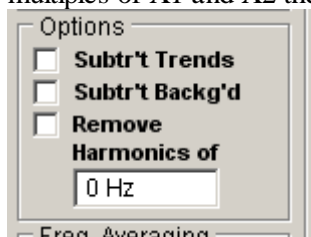
2. **Freq. Averaging:** Select the type of averaging to be applied to adjacent spectral frequencies. Select **None** for no averaging. Select **Logarithmic** to logarithmically increase the number of adjacent frequencies averaged with increasing frequency. Select **Linear** to average a fixed number of adjacent frequencies throughout the frequency range, the number entered in the **No. Freqs.** box.



3. **Time Window:** Select the time window to be applied to the data in each variance record. Select **None** for no time window. Select **10% Cosine** to taper the first and last 10% of data points using a cosine bell function.

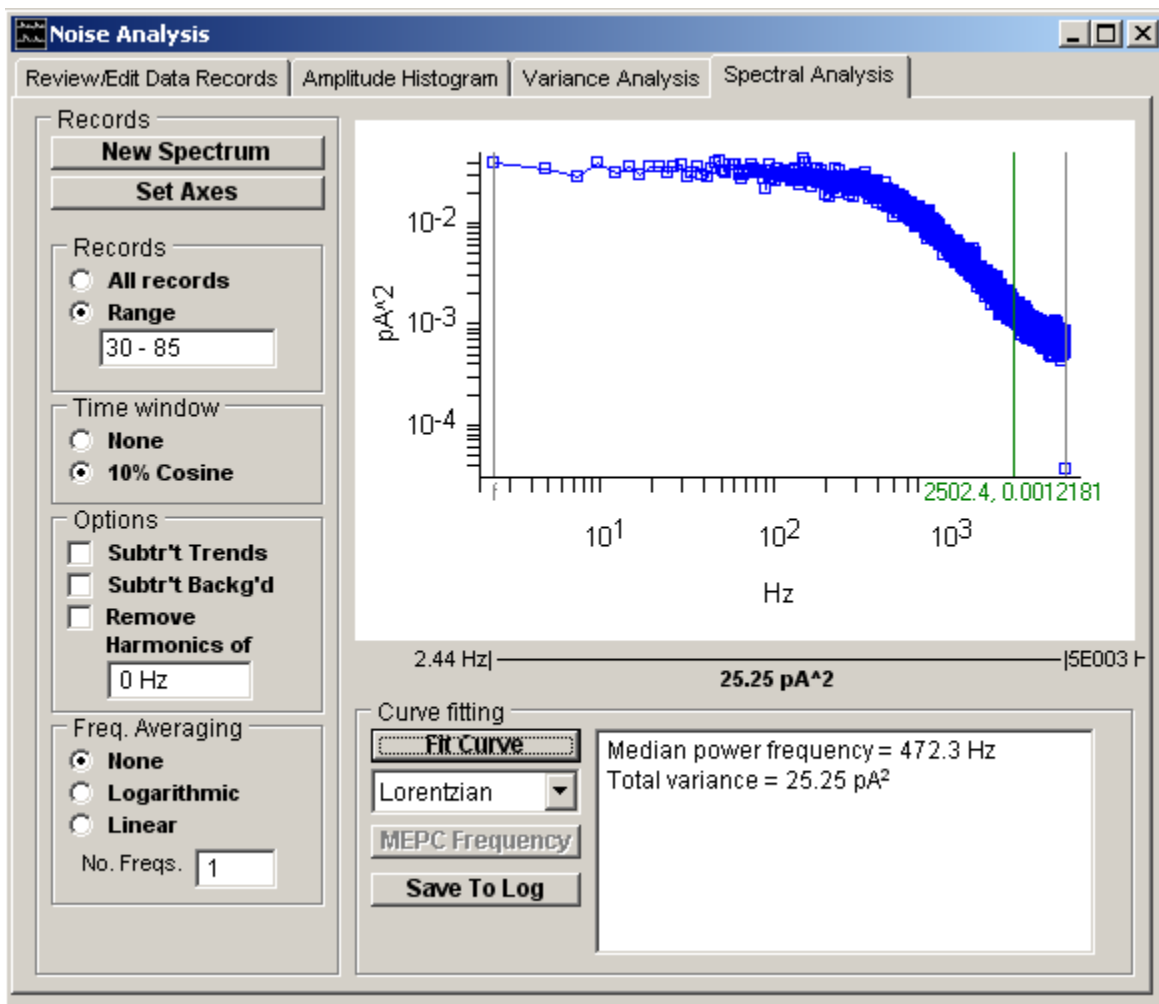


4. **Options:** Tick the **Subtr't Backg'd** option to subtract the averaged power spectrum of the Background records from the spectrum of the Test records. (The background spectrum is also plotted ( symbols).)  
 Tick the **Subtr't Trends** option to remove any linear trends from the data points within each variance record before computation of its power spectrum.  
 Tick the **Remove Harmonics of** option and enter a base frequency in the box to remove frequencies at multiples of X1 and X2 the base.



5. Click the **New Spectrum** button to compute and plot the averaged spectrum.

The spectral power at each frequency component can be displayed using the blue readout cursor. The average variance contained within a range of frequencies indicated by the |--| region of interest cursors is also displayed. The total variance obtained by integrating the spectrum and the median power frequency (frequency below/above which 50% of the variance lies) is displayed in the results box.



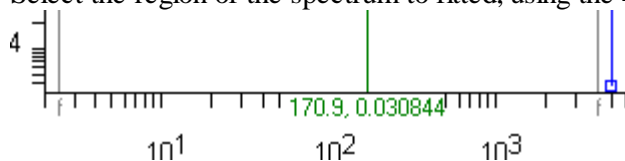
A detailed discussion of the computation of power spectra can be found in Dempster (1992 or 2001).



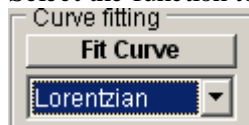
## Noise Analysis > Fitting Curves to Power Spectra

To fit a Lorentzian function to a power spectrum :

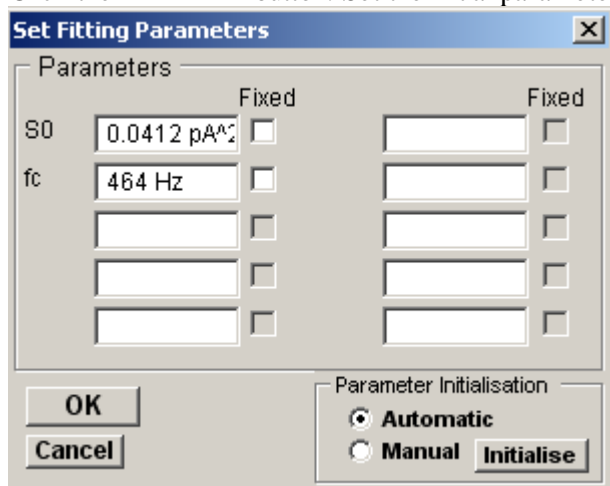
1. Select the region of the spectrum to fitted, using the **f--f** region of interest cursors.



2. Select the function to be fitted (**Lorentzian, 2 Lorentzians**) from the Fit Curve list.

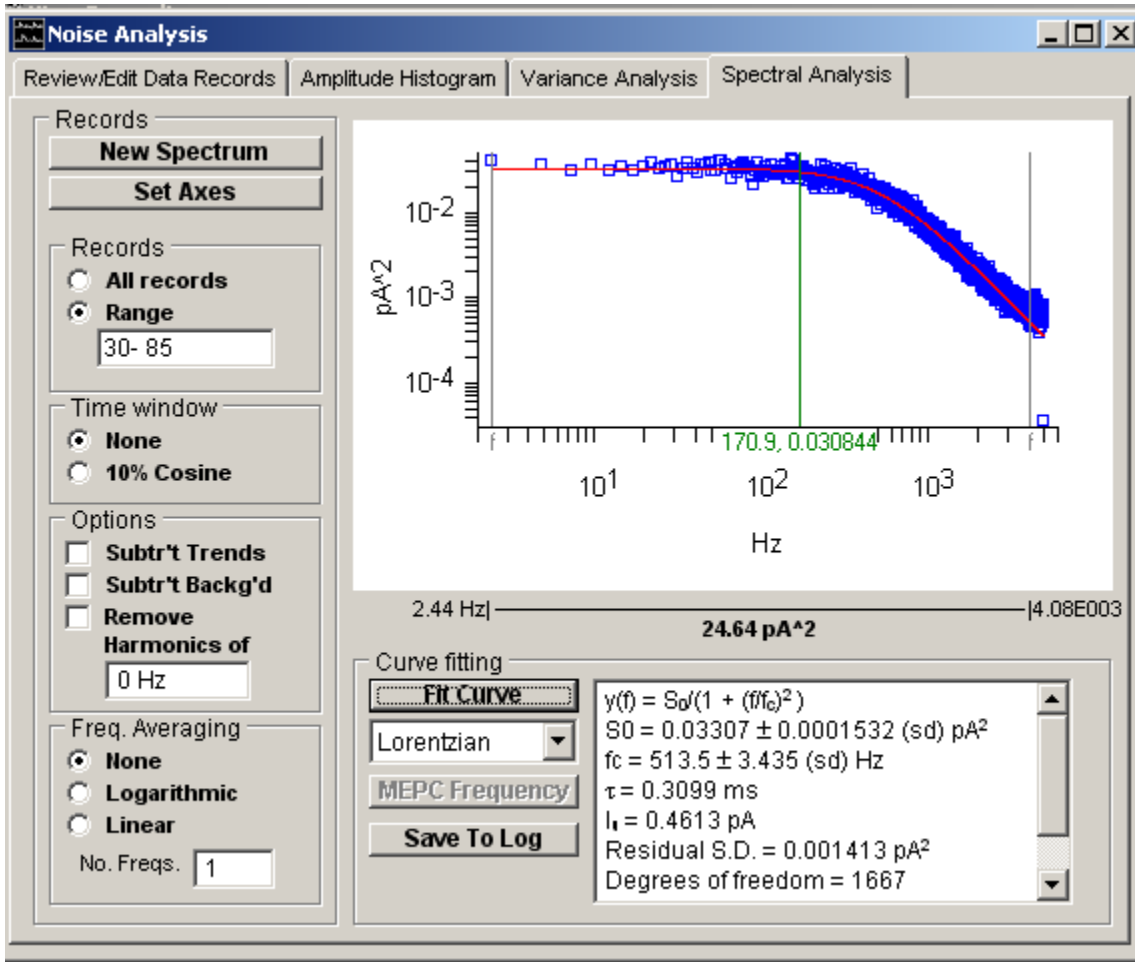


3. Click the **Fit Curve** button. Set the initial parameter guesses (optional)



and click the **OK** button.

The best fitting curve is superimposed upon the spectrum and the best fit function parameters (zero frequency spectral power,  $S_0$  and corner frequency,  $f_c$ ) along with the parameters standard errors and residual standard deviation are displayed in the results box. The single-channel current,  $I_u$ , and channel gating time constant are also displayed.



## Lorentzian Functions

The spectra of ion channel fluctuations have characteristic shapes represented by the sum of one or more Lorentzian functions,

$$S(f) = \frac{S_0}{1 + (f/f_c)^2} \quad (1)$$

At low frequencies, the function tends to a constant value,  $S_0$  (units  $I^2/\text{Hz}$ ). At high frequencies the spectrum decays in proportion to  $1/f^2$ . The rate of ion channel opening and closure determines the corner frequency,  $f_c$ , at which the power has declined to  $S_0/2$ . Channels which rapidly flicker between short open and closed states produce higher frequency noise and spectra with higher corner frequencies. For a channel with two states (open and closed),  $f_c$  is related to the ion channel gating time constant,  $\tau$ , and the mean open and closed times,  $t_{open}$ ,  $t_{closed}$ , by the formula,

$$\tau = \frac{1}{2\pi \cdot f_c} = \frac{1}{1/t_{open} + 1/t_{closed}} \quad (2)$$

If  $t_{closed} \gg t_{open}$  then  $\tau \approx t_{open}$ .

If a channel has more than two states then its noise spectrum will consist of a mixture of  $n-1$  Lorentzian functions for  $n$  channels state. The relationship between channel dwell times and corner frequencies of the Lorentzian components is more complicated in these cases (See Ruff, 1977).

If the spectrum has been recorded under conditions where the open channel probability is small (e.g.  $p \approx 0.1$ ), the single-channel current and conductance can also be computed from the power spectrum. For small  $p$  values the second term in the eqn. 2 can be neglected leading to a direct relationship between current variance and mean current

$$\sigma^2 = I_u \cdot I_m \quad (3)$$

Since the spectrum is the frequency distribution of the variance, the variance can be calculated from the integral of the

Lorentzian function.

$$\sigma^2 = \frac{f_c \cdot S_0}{2} \quad (4)$$

By combining eqns. 3 and 4 the single-channel conductance can be calculated from the Lorentzian curve fitted to the ionic current power spectrum.

$$I_u = \frac{f_c \cdot S_0}{2 \cdot I_m} \quad (5)$$

(Note. In these circumstances no information can be derived concerning the total number of channels.)

### **Miniature endplate current noise**

It is also possible to analyse the current fluctuations associated with the miniature endplate currents under conditions of the high frequency release evoked by increasing the K ion concentration of the bathing solution. The MEPC noise spectrum can be modelled with the three parameter ( $F_r$ ,  $F_d$ ,  $A$ ) function,

$$S(f) = \frac{A}{(1/F_d + 1/F_r)f^2 + f^4 / (F_r^2 \cdot F_d^2)} \quad (6)$$

Eqn. 6 can be fitted to a spectrum by selecting **MEPC Noise** from the fitting function list. Parameters  $F_r$  and  $F_d$  are associated with the MEPC rise and decay time course while  $A$  is associated with the overall noise amplitude. The time constants for MEPC rise and decay,  $\tau_r$  and  $\tau_d$ , can be computed from these.

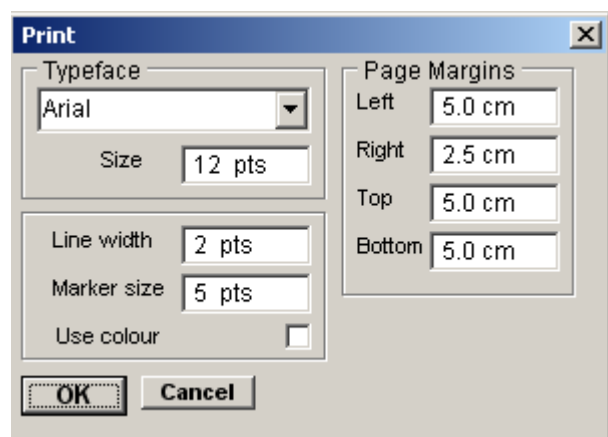
After the  $\tau_r$  and  $\tau_d$ , time constants have been established by fitting eqn. 6, to the MEPC noise power spectrum, the frequency of MEPC release can be computed by clicking the **MEPC Frequency** button (on the Variance Analysis page).

## Noise Analysis > Printing Plots or Spectra

To print the displayed plot, select

**File**  
**Print**

To open the dialog box.



Click the **OK** button to print the graph.

## Noise Analysis > Copying Plots to the Clipboard

### Copying the plot data points to the Windows clipboard

The numerical values of the X,Y data points which generate the plot can be copied to the clipboard by selecting

**Edit**

**Copy Data**

The data is placed on the clipboard as a table of data values, in tab text format, defining the histogram. There are 4 values per row, and one row for every bin in the histogram. Each row has the format

```
<X> <tab> <Y> <cr> <lf>
```

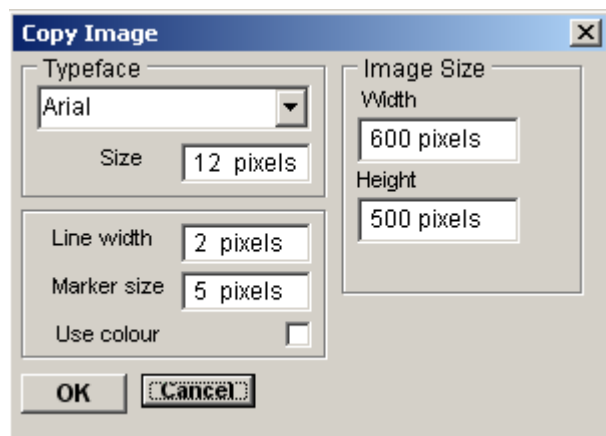
### Copying an image of the plot to the Windows clipboard

An image of the histogram plot can be copied to the clipboard by selecting

**Edit**

**Copy Image**

to open the dialog box.



The dimensions (pixels) of the bit map, which will hold the image, can be set using the Width and Height image size boxes. The size and style of the typeface can be set using the Typeface and Size boxes.

When the image parameters have been set, click the **OK** button to copy the image to the clipboard.

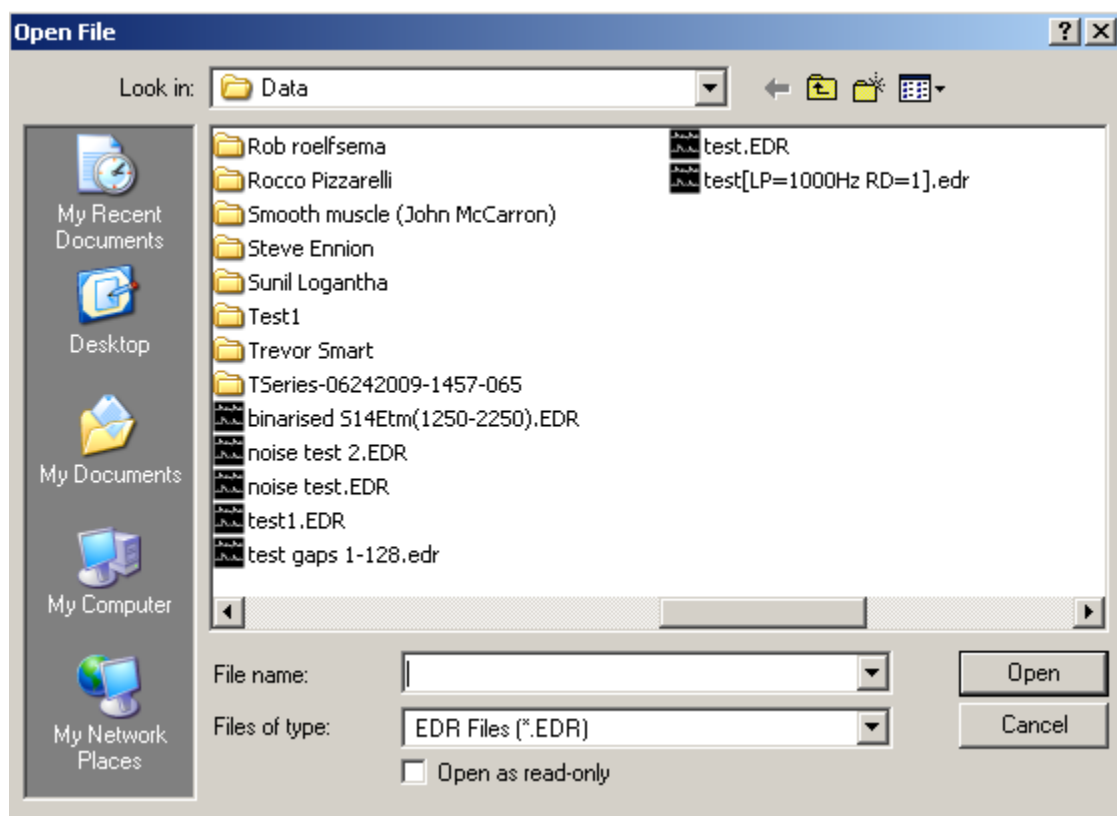
## Data Files > Opening an EDR Data File

WinEDR uses its own custom data file format for storing digitised signal records. These files are identified by the file extension “.EDR”

To load a previously created EDR data file, select:

**File**  
**Open**

to display the dialog box.



Select the disk drive and folder from the **Look In** list. A list of available EDR files will be displayed.

Select one of the file names, then click the **OK** button to open the data file for display and analysis.

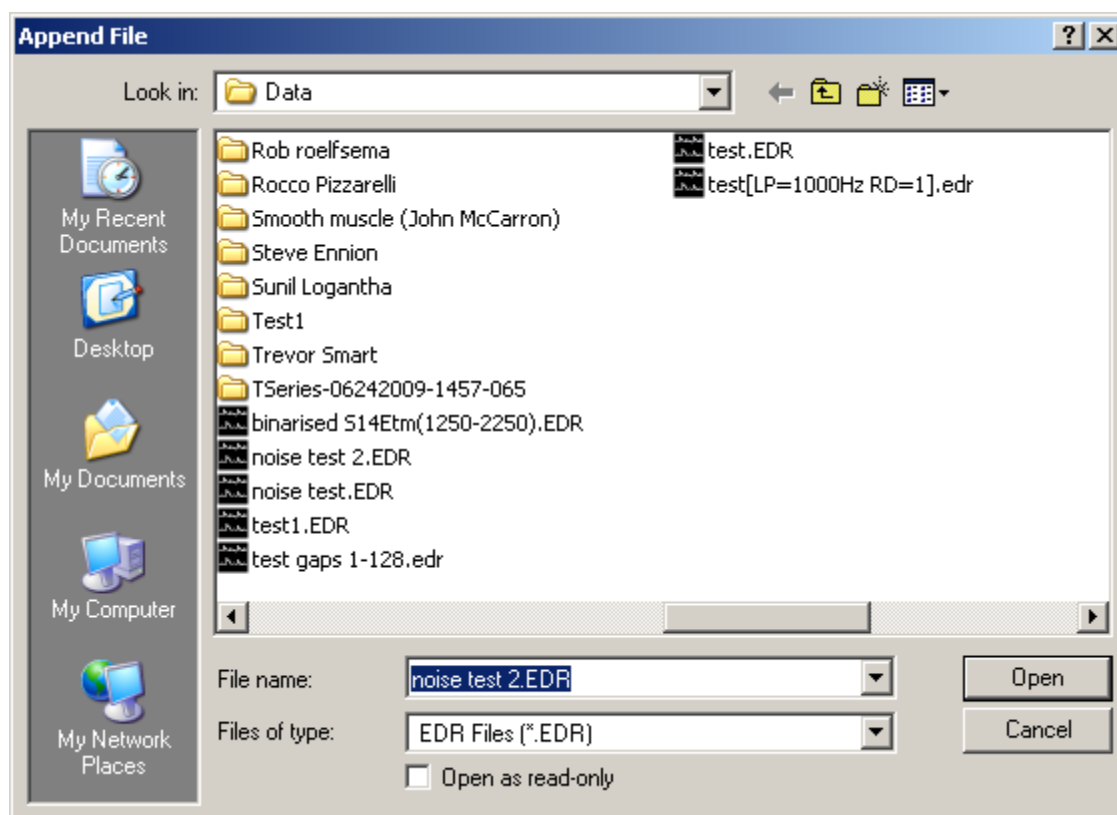
## Data Files > Appending an EDR Data File

To append records from an EDR data file on to the end of the currently open data file, select

**File**

**Append..**

To display the dialog box.



Select the disk drive and folder from the **Look In** list. A list of available WCP files will be displayed. Select one of the file names, then click the **OK** button to append the records from this file on to the end of the currently open the data file.

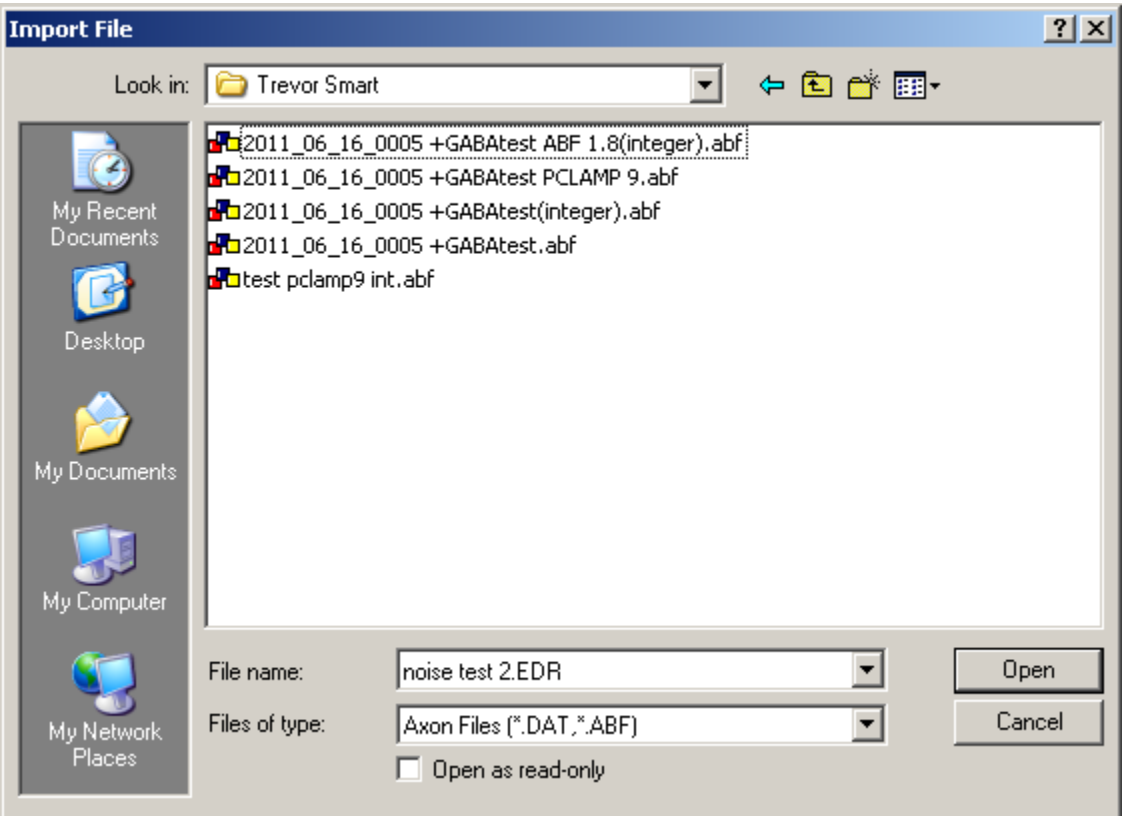
**Note.** You can only append files which have compatible records with the same number of channels.

Data Files > Importing Other Data File Formats

To import records from a non-EDR data file, select

File  
Import..

To display the dialog box.



Select the disk drive and folder from the **Look In** list. Then select the type of data file to be imported from the **Files of Type** list. A list of available files in that type are displayed.

Select one of the file names, then click the **OK** button to import the data into a .WCP format file.

**Note.** The original file is not changed. A new .EDR format file is created with the same name as the imported file but with the extension.EDR.

The currently supported data file formats are listed in the table below.

Importable Data File Formats		
Axon Instruments	*.abf *.dat	Axon Instruments ABF (Axon Binary File) format files produced by the PCLAMP or AxoScope programs. Note. ABF V2.0 format file (produced by PCLAMP V10) are currently supported. PCLAMP V10 files must be saved as ABF V1.8 (Integer) format to be imported into WinEDR.
Cambridge Electronic Design CFS	*.cfs *.dat	Cambridge Electronic Design CFS (CED Filing System) format files.
CDR Files	*.cdr	Strathclyde Electrophysiology Software CDR program (an MS-DOS based electrophysiology package) data files.
Chart Files (* *.CHT)	*.cht	Strathclyde Chart data files.



HEKA	*.asc	ASCII format aata records exported from the Heka PULSE software.
Igor Binary Files	*.ibw	IBW (Igor Binary Wave) files produced by the IGOR Pro software package.
PAT Files	*.pat	Strathclyde Electrophysiology Software PAT single-channel analysis program data file
Physionet WDBF (*HEA)	*.hea	Physionet WDBF format data files.
PoNehMah Files	*.pro	PoNehMah cardiovascular analyser software data files.
SCAN Files	*.sca	Strathclyde Electrophysiology Software SCAN program data files.
SPAN Files	*.spa	Strathclyde Electrophysiology Software SPAN spectral analysis program data files.
Wave File	*.wav	WAV format sound files.
WCD Files	*.wcd	Strathclyde Electrophysiology Software WinCDR program data files.

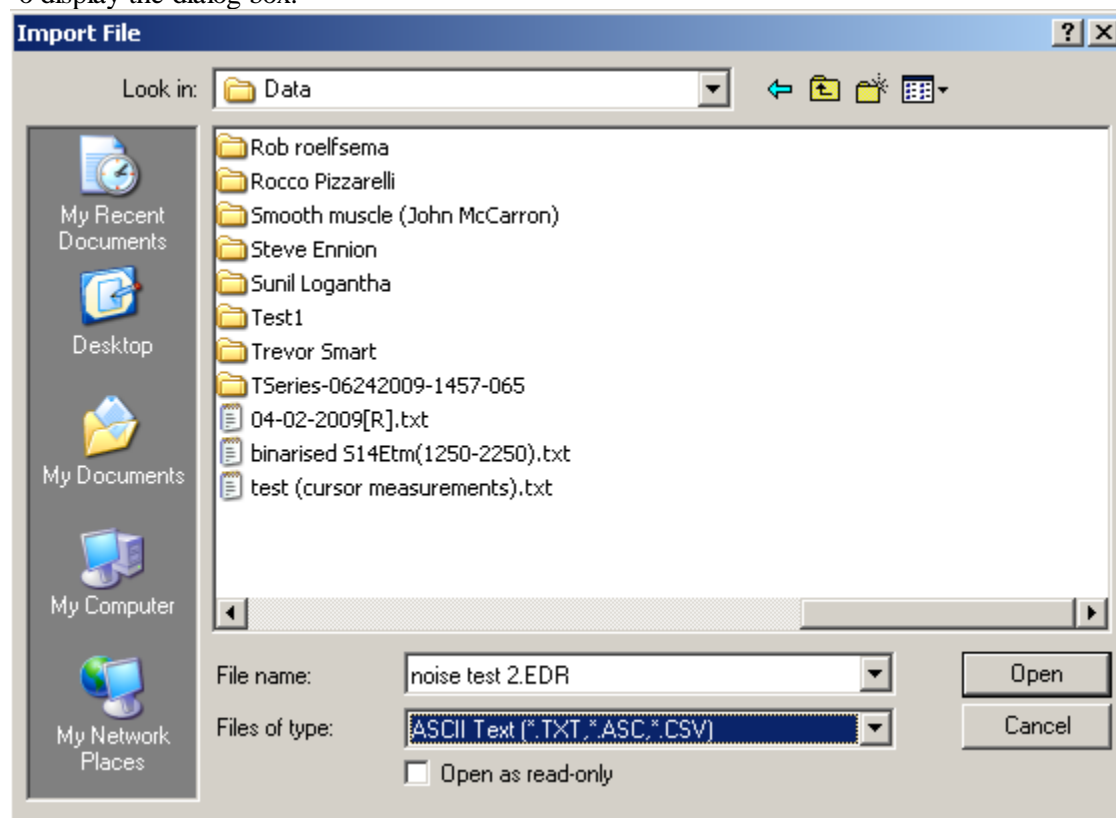
## Data Files > Importing from ASCII Text Files

To import records from a text file containing tables of numbers in ASCII format:

1. Select

**File  
Import..**

to display the dialog box.



Select the disk drive and folder from the **Look In** list. Then select **ASCII Text (\*.txt, \*.asc)** from the **Files of Type** list. A list of available files in that type are displayed.

2. Select one of the file names, then click the **OK** button to open the ASCII Import dialog box

**ASCII Import**

-0.002 -0.0611  
-0.00195 -0.06115  
-0.0019 -0.0611  
-0.00185 -0.06115  
-0.0018 -0.06115  
-0.00175 -0.06115  
-0.0017 -0.0611

Column Separator  
☒ **Tab** ☐ **Comma** ☐ **Space**

No. of title lines to be skipped

Time  
☐ **Time Data in Col. 0**  
☒ **User Defined**  
Sampling Interval

Units  
☐ **msecs** ☒ **secs** ☐ **mins**

**Channels**

Ch.	Name	Units
0	Vm	mV
1		
2		
3		
4		
5		
6		
7		
8		
9		

**OK** **Cancel**

which allows you to view the format of the data to be imported and to specify how it should be imported.

3. **Column Separator:** Select the character used to separate data columns in the file (<tab>, comma or single space character).
4. **No. of title lines to be skipped:**The first data row(s) in the table often contain labels or identification information, which should not be treated as samples. To skip one or more of these lines, enter the number to skipped in the **No. of title lines to ignore** box.
5. **Sample times.** If the first column in the table contains samples times, select the **Time Data in Col.0** option to derive the sampling interval from the times of successive rows. Select the units that the time data is expressed in from the **Units** list. If no sample time data is available, select **User Defined** and enter the sampling interval into the **Sampling Interval** box.
6. **Time Units:** Select the units of the time data column (secs, msecs, mins) from the **Units** list.
7. **Channels:** Enter the names and units for each channel into the **Channels** table.
8. Click the **OK** button, to import the data when the import settings are complete.

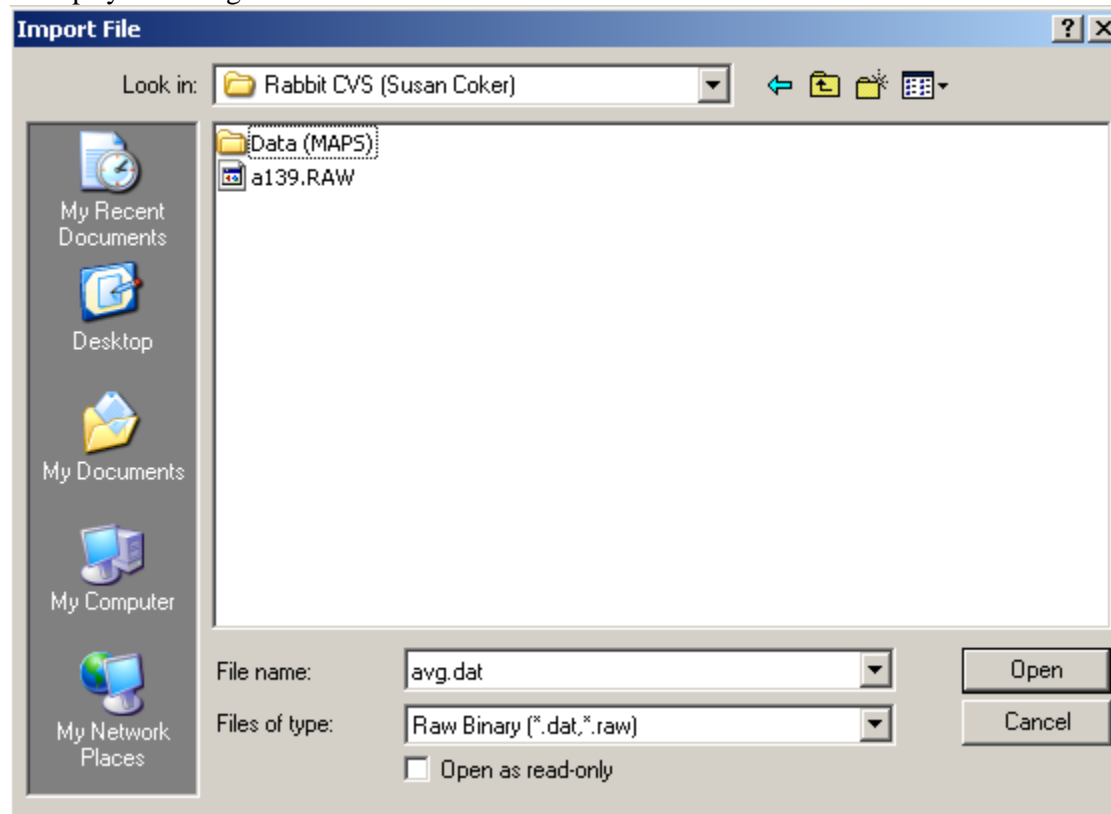
## Data Files > Importing from Raw Binary Data Files

To import records from a text file containing tables of numbers in ASCII format:

1. Select

**File  
Import..**

to display the dialog box.



If the type of data file being imported does not match any of the known formats, the import module reverts to its general purpose **Binary** import mode. The import module assumes that the data has the general format



At the beginning of the file, there is a block of **file header** data which contains the information on the number of records in the file, size of record, number and scaling of channels. This is followed by a **data block** containing the A/D converter samples stored as 16 bit integers. If more than one input channel has been digitised, samples are interleaved within the data block (e.g. Ch.0,Ch.1,Ch.2,Ch.0,Ch.1,Ch.2,...).

Given information on the size of each of the file header block and the number of channels, the import module can extract the signals from the file. These details of the data file structure can often be obtained from the user manuals associated with the software, which created the data files. (Note that the sampling interval and other scaling information is discarded by the binary import module.)

**Raw Binary Import**

**File Description**

File header size (bytes)

No. of signal channels

Read in blocks of

Sample Format

☐ Float ☒ Integer

No. of bytes/sample

Max. Value

Sampling interval

☒ msecs ☐ secs ☐ mins

**Channels**

Ch.	Name	Units/bit	Units
0	Vm	0.001	mV

OK Cancel

The **Import settings** must be set up to match the characteristics of the file and data being imported.

1. Enter the size of the file header in the **File header** box. (If no file header exists, set this value to zero.)
2. Enter the number of analog data channels in the file in the **No. of signal channels** box.
3. Provide names, scaling factors and units for each data channel in the Channels table.
4. Set the number of time points read from the file in a single operation in the **Read in blocks of** box (usually set to 512).
5. Specify the format of the analog data samples to be imported. Select the **Float** option and **No. of bytes/samples** to 4 for 4 byte floating point samples or **Integer** for binary integer samples and the size of the sample in **No. of bytes/samples**. Enter the upper limit of the sample value in the **Max. Value** box.
6. Enter the time interval between adjacent samples within each channel in the **Sampling interval** box. Select the units of the time interval from the **Time units** list.
7. Click the **OK** button, to import the data when the import settings are complete.

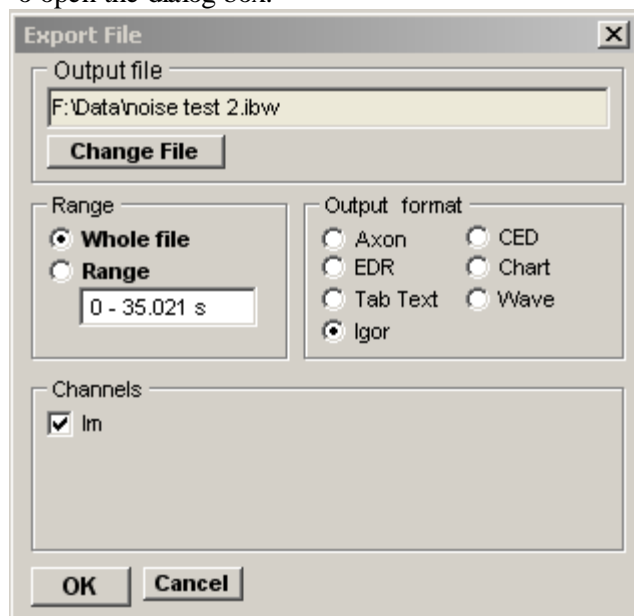
## Data Files > Exporting to Other Data File Formats

EDR data files can also be exported in a number of data file formats.

To export the currently open data file, select

**File**  
**Export**

to open the dialog box.



1. **Output Format:** Select the data format of the file to be exported to from the list of options.
2. **Range:** Select the **Whole File** option to export all data in the currently open EDR data file, or select **Range** and enter the range be exported.
3. **Channels:** Tick the signal channels to be exported.
4. **Output File:** To change the name/folder of the exported file, click the **Change File Name** button and select a new folder/name.
5. Click the **OK** button to create the export file.

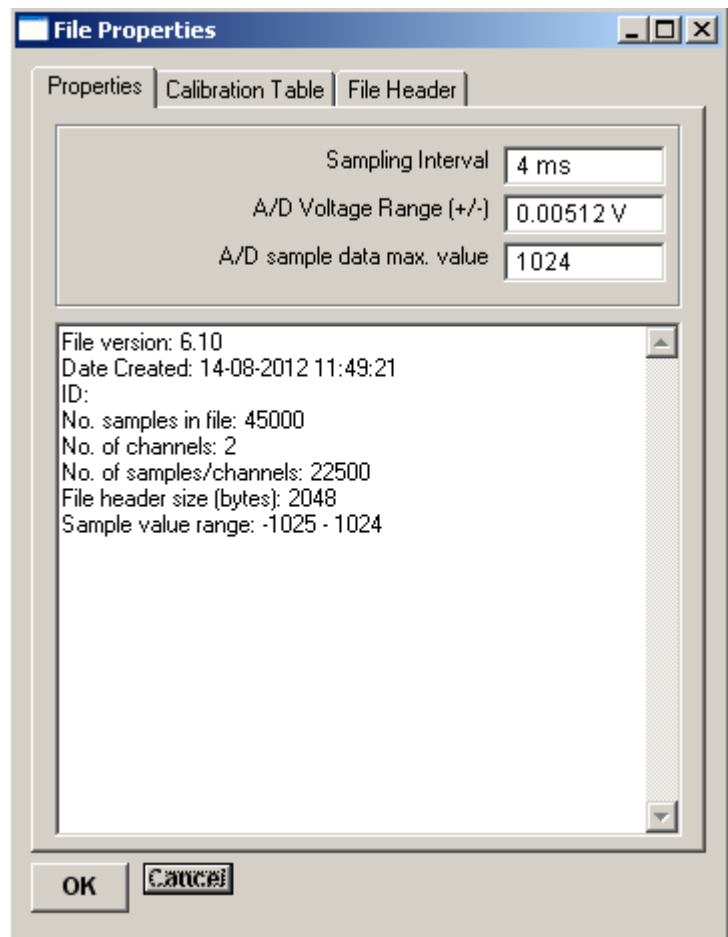
The currently supported export file formats are listed in the table below.

Export Data File Formats		
Axon Instruments	*.abf	Axon Instruments ABF (Axon Binary File) V1.8 format files.
Cambridge Electronic Design CFS	*.cfs *.dat	Cambridge Electronic Design CFS (CED Filing System) format files.
ASCII Text	*.txt	Tab-delimited columns of ASCII text.
WCP	*.wcp	Strathclyde Electrophysiology Software WinEDR data file format.
EDR	*.edr	Strathclyde Electrophysiology Software WinEDR data file format.
Igor Binary Files	*.ibw	IBW (Igor Binary Wave) files produced by the IGOR Pro software package.

## Data Files > Inspecting File Properties

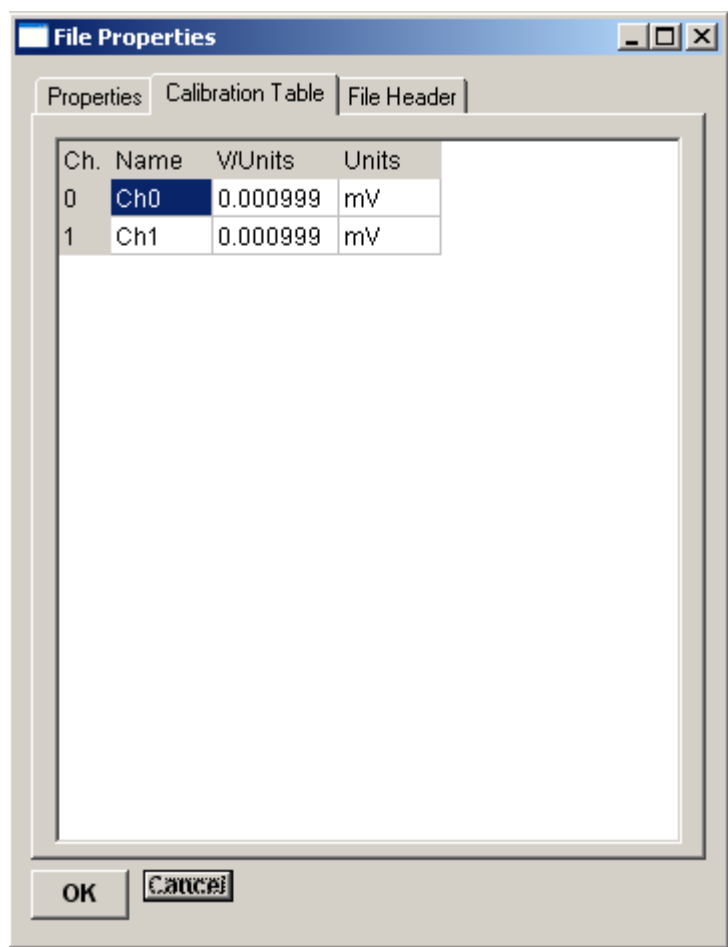
To inspect or change the data scaling, units and sampling rate properties of a data file, select **File Properties** to display the File Properties dialog box

### Properties Page



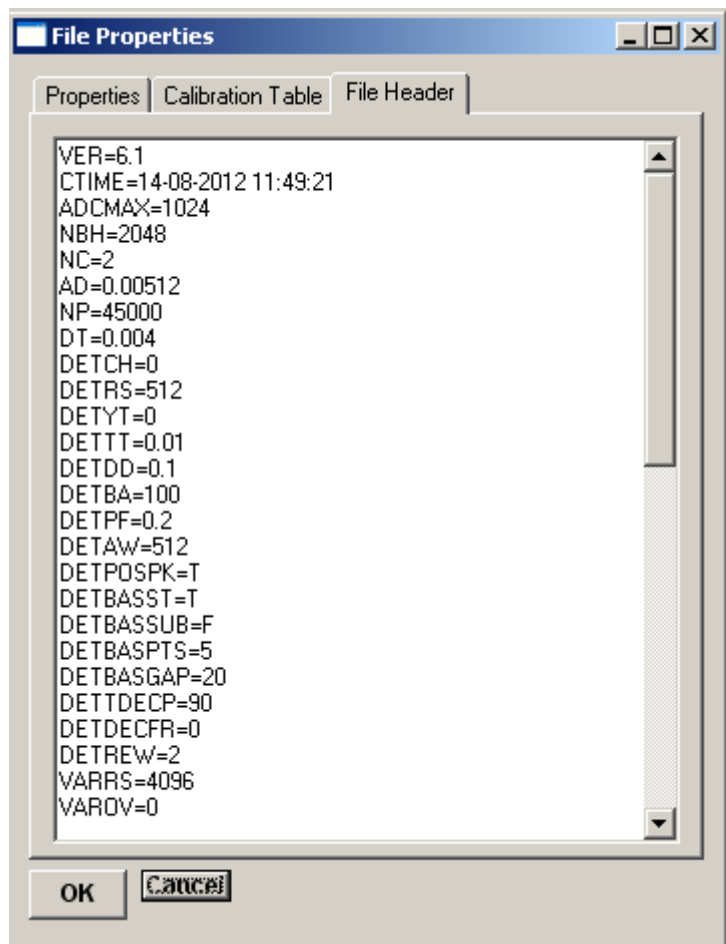
The Properties pages shows the no. of samples, sampling interval, no. of data channels and other parameters of the data stored in the file. The sampling interval, A/D Converter Voltage Range and the upper limit of the integer binary A/D converter samples can be edited by the user.

### Calibration Table Page



The Calibration Table pages shows the name, units and scaling factor (in Volts/unit) of each data channel within the file and allows them to be edited by the user.





The File Header pages shows the contents of the EDR file header. It cannot be edited.

## Data Files > EDR File Structure

The EDR data file is designed to store up to 12 channels of 16 bit integer binary records of digitised analogue signals, the associated scaling information required to reconstitute actual signal levels.

EDR files consists of a 2048 byte **header block** at the beginning of the file which contains a list of ASCII-format keywords, detailing the number of records in the file, record size, scaling factors etc. It is followed by the **data block** containing the digitised A/D samples.

### Header Block

The header block contains the information needed to allow a program to determine the number of channels, samples etc. in the file. It is usually the first block to be read when a file is opened. File parameters are stored as ASCII text in the form of keywords, one word per line, as follows

KEY= <value> <cr> <lf>

where <value> is a number or text depending on the parameter and <cr> <lf> are the carriage return and line feed characters.

A typical header block (from a file with 2 channels) contains the following keywords.

VER=6.4 <cr><lf>	EDR file version number
NC=2 <cr><lf>	No. of analogue input channels
NP=102400 <cr><lf>	No. of A/D samples in data block
NBH=2048 <cr><lf>	No. of bytes in file header block
AD=5.0000 <cr> <lf>	A/D converter upper limit of voltage range (V)
ADCMAX=4095 <cr><lf>	Maximum A/D sample value
DT=.1600 <cr><lf>	A/D sampling interval (s)
YN0=Im <cr> <lf>	Channel 0 name (n=0 .. NC-1)
YU0=nA <cr> <lf>	Channel 0 units
YCF0=0.0001 <cr> <lf>	Channel 0 calibration factor V/units
YAG0=10.0 <cr> <lf>	Channel 0 gain factor
YZ0=1024 <cr> <lf>	Channel 0 zero level (A/D bits)
Y00=0 <cr> <lf>	Channel 0 offset into sample group in data block
YN1=Im <cr> <lf>	Channel 1 name (n=0 .. NC-1)
YU1=nA <cr> <lf>	Channel 1 units
YCF1=0.01 <cr> <lf>	Channel 1 calibration factor V/units
YAG1=1.0 <cr> <lf>	Channel 1 gain factor
YZ1=1024 <cr> <lf>	Channel 1 zero level (A/D bits)
Y01=1 <cr> <lf>	Channel 1 offset into sample group in data block
TU=ms <cr> <lf>	Time units
ID= Cell 1 <cr> <lf>	Experiment identification line
BAK=T1 <cr> <lf> BAK=T indicates a .BAK file exist	

### Event detector parameters

DETC=0 <cr><lf>	Event detector channel
DETRS=1024 <cr><lf>	No. samples in event detector record
DETYT=500 <cr><lf>	Event detector amplitude threshold (A/D units)
DETTT=5E-3 <cr><lf>	Event detector.time threshold (s)
DETTDD=5-E2 <cr><lf>	Event detector dead time (s)
DETTBA=5E-2 <cr><lf>	Event detector.baseline averaging time (s)
DETTPT=10.0 <cr><lf>	Event detector pretrigger percentage

### Single-channel analysis parameters

VARRS=1024 <cr><lf>	No. samples in variance record
VAROV=0 <cr><lf>	Percentage overlap of variance records
VARTR=0.0005 <cr><lf>	MEPC rise time (MEPC freq. analysis) (s)
VARTD=0.005 <cr><lf>	MEPC decay time (MEPC freq. analysis) (s)
UNITC=1.0 <cr><lf>	Single channel current
DWTTH=50 <cr><lf>	Transition detection threshold (%)

Note. It should not be assumed that the keywords will follow any particular order.

### Data block

The data block contains the digitised signals, stored in the form of 16 bit binary integers. Each A/D sample takes up 2 bytes of space. The size of the data block is determined by the number of channels and number of samples per channels in the record

$$N_{bytes} = 2 \cdot N_{channels} \cdot N_{samples} \quad A.2$$

If there is more than one A/D input channel, samples are interleaved within the data block. For example, for 2 channels,

$$Y01 \ Y11 \ Y02 \ Y12 \ ..... \ Y0nsamples \ Y1nsamples \quad A.3$$

Different laboratory interfaces supported by WinEDR return multi-channel A/D samples in different orders. The channel interleaving order for a data file is specified by the YOn= channel keyword in the file header block.

### Scaling from A/D unit to physical units

The calibrated signal level ( $y_{cal}$ ) in the appropriate channel units can be reconstructed (for channel n) using information stored in the header block, using,

$$y_{cal} = (y_{adc} - YZn) \times \frac{AD}{YCFn \times YAGn \times (ADCMAX + 1)}$$

where  $AD$  is the maximum positive limit of the A/D converter voltage range, ADCMAX is maximum A/D sample value, YCFn is the channel calibration factor, YAGn amplifier gain and YZn zero level.

## Simulations > Postsynaptic Currents

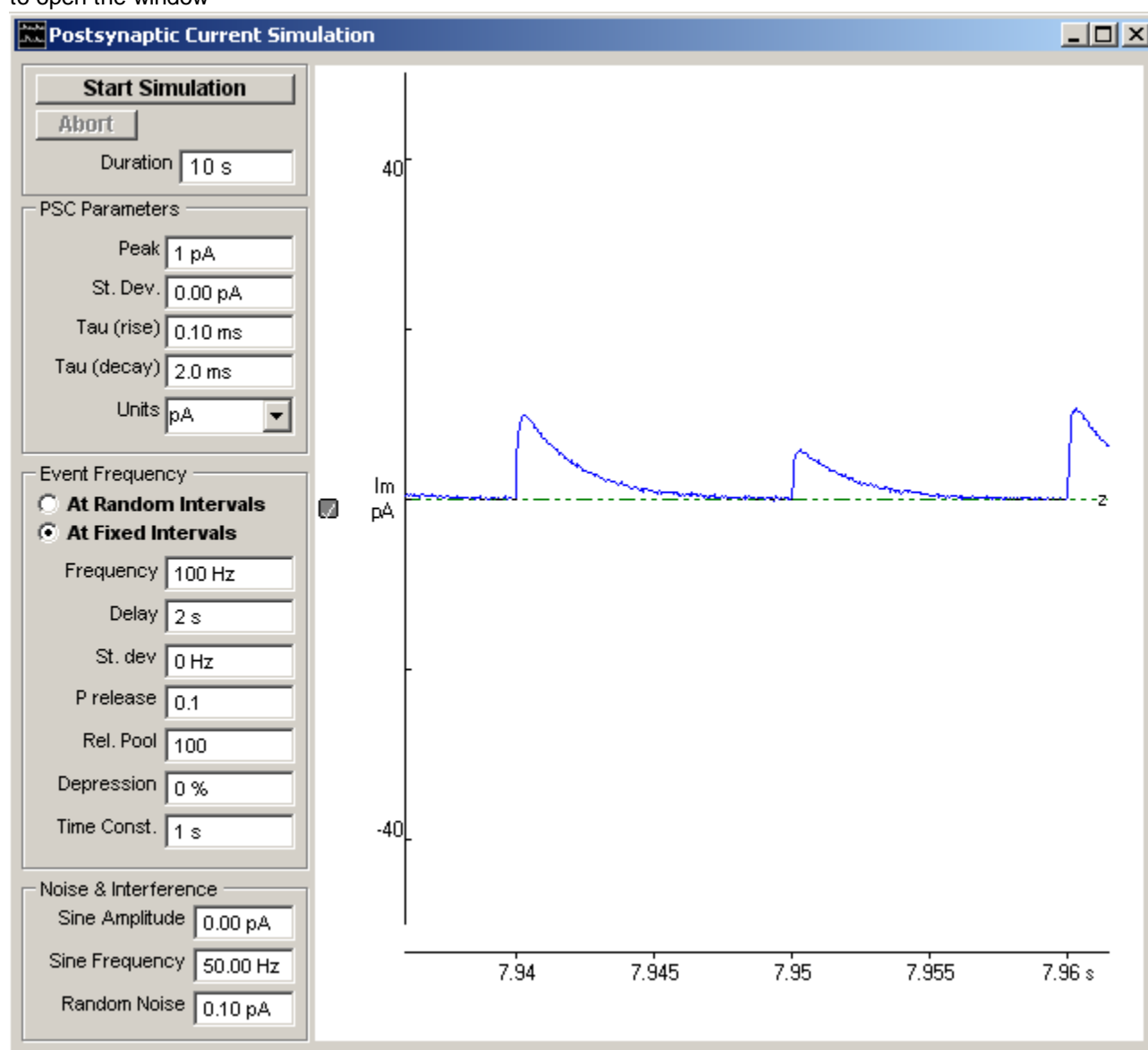
The post-synaptic current module generates series of post-synaptic currents (PSCs) which can be used to test WinEDR's [signal detection module](#) and the [MEPC frequency estimation](#) procedure in the noise analysis module.

The PSCs are modelled as signals with a rapid exponential rise and slower exponential decay, occurring at fixed intervals, or random time intervals governed by an exponential distribution. Gaussian background noise and sine wave interference can be added to the signals to test the abilities of the event detection system.

To create a data file containing simulated mPSCs:

1. Create a new data file to hold the records, by selecting  
**File**  
**New**  
and entering the name of a new data file.
2. Select  
**Simulations**  
**Post-synaptic currents**

to open the window



3. Enter the time period over which PSCs are to be generated in the **Duration** box.

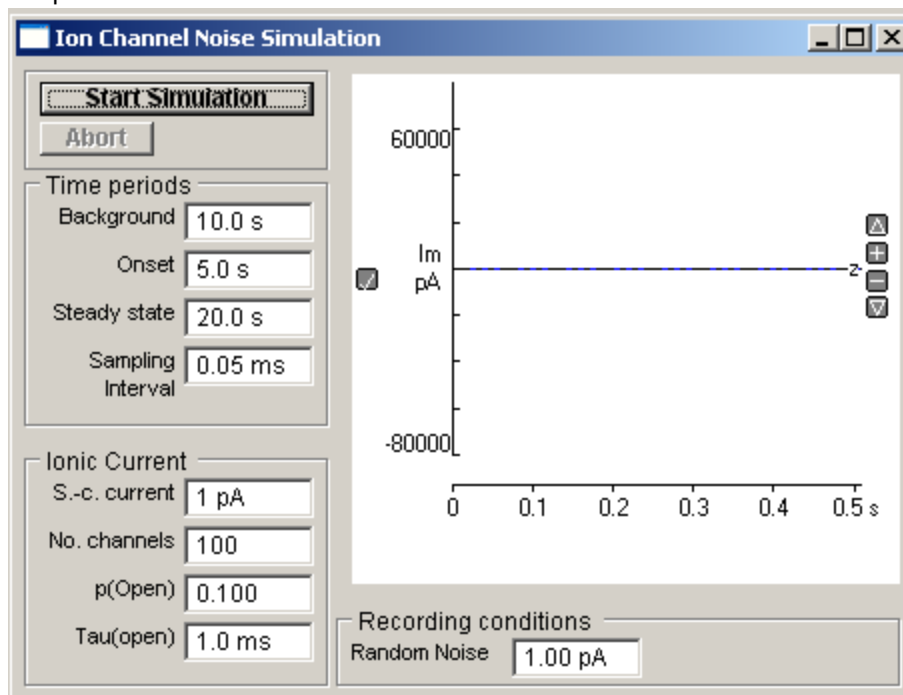
4. Enter the PSC peak amplitude in the **Peak** box.
5. Enter the standard deviation of the inter-event variation in PSC peak amplitude in the **St. Dev.** box.
6. Enter the time constant of the PSC rising phase in the **Tau (rise)** box.
7. Enter the time constant of the PSC decay phase in the **Tau (decay)** box.
8. Select the current units from the **Units list**.
9. Select the **At Random Intervals** option to generate PSCs at random intervals or **At Fixed Intervals** for fixed intervals
10. Enter the rate of occurrence of PSCs in the **Frequency** box.
11. *At Fixed Intervals options:* Enter the quantal release probability for each stimulus into the **P release** box and the number of quanta available for release in the releasable pool the **Rel. Pool** box. Set the percentage depression or facilitation during the fixed frequency train of stimuli in the **Depression** box (0%=no depression, 100 = 100% depression during the train, -100 = 100% facilitation) and the time constant of depression in the **Time Const.** box. To randomly vary the fixed interval frequency enter a non-zero value into the Frequency **St Dev.** box..
12. To delay the start of the PSC series enter the required delay in the **Delay** box.
13. Enter the standard deviation of the signal background noise in the **Random Noise** box.
14. To apply a slow sine wave baseline drift to the recording, enter a non-zero value in the Sine Wave **Amplitude (pk-pk)** box and its frequency in the **Frequency** box.
15. Click the **Start Simulation** button, to start the simulation run.

## Simulations > Ion Channel Noise

The ion channel noise simulation generates the random fluctuations in current associated with the open/close gating of a population of receptor-activated ion channels. The model simulates the application of agonist, the rise of current during a period of increasing agonist concentration and the fluctuations and about the steady-state mean current. Channel gating is simulated using a simple two state model. The simulated current noise can be used to test the operation of the [noise analysis module](#).

To create a data file containing simulated ionic current noise:

1. Create a new data file to hold the records, by selecting **File**  
**New**  
and entering the name of a new data file.
2. Select **Simulations**  
**Ion Channel Noise**  
to open the window

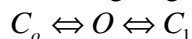


3. Enter the duration of the initial period before the application of agonist, during which background noise is recorded in the **Background** box
4. Enter the onset time during which agonist concentration is rising in the **Onset** box.
5. Enter the period of time that the agonist concentration is in a steady-state in the **Steady-state** box.
6. Enter the single-channel current amplitude in the **S-c current** box.
7. Enter the number of ion channels in the cell in the **No Channels** box.
8. Enter the steady-state probability of a channel begin open in the **P(Open)** box.
9. Enter the mean channel open time in the **Tau (open)** box.
10. Enter the standard deviation of the signal background noise in the **Random Noise** box.
11. Click the **Start Simulation** button, to start the simulation run.

## Simulations > Single-channel Currents

The single-channel current module simulates the currents associated with the opening and closure of a single (or small number of) ion channel(s).

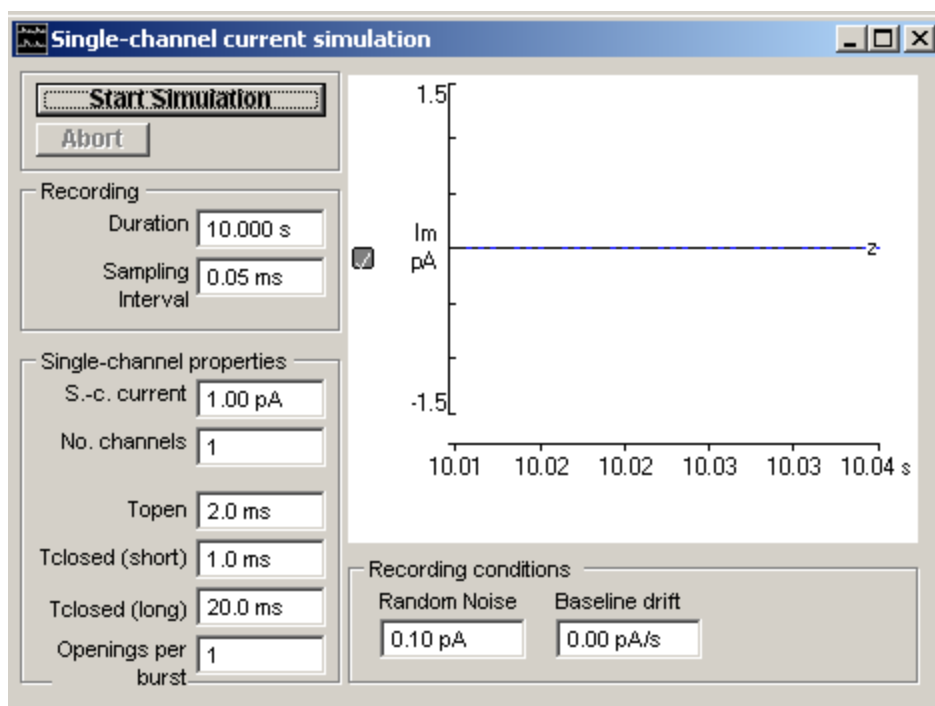
Channel gating is modelled using a 3-state model with one open state and two closed,



Channel openings occur in bursts with the kinetics of the channels determined by the mean dwell times in each of the 3 states.

To create a data file containing simulated single-channel currents:

1. Create a new data file to hold the currents, by selecting  
**File**  
**New**  
and entering the name of a new data file.
2. Select  
**Simulations**  
**Single-channel currents**  
to open the window



3. Enter the duration of the simulated recording in the **Duration** box.
4. Enter the time interval between simulated A/D samples in the **Sampling Interval** box.
5. Enter the single-channel current amplitude in the **S-c current** box.
6. Enter the number of active ion channels in the patch in the **No Channels** box.
7. Enter the mean channel open time in the **Topen** box.
8. Enter the mean number of channel opening per burst of openings in the **Openings per burst** box.
9. Enter the mean intra-burst closed time in the **Tclosed (short)** box and the mean inter-burst closed time in the **Tclosed (long)** box.
10. Enter the standard deviation of the background noise in the **Random Noise** box.
11. [Optional] To apply a linear trend to the signal baseline level enter a non-zero value into the **Baseline drift** box.

12. Click the **Start Simulation** button, to start the simulation run.



## COM Automation Interface > COM Automation Interface

WinEDR implements a COM automation server which allows its recording and seal test functions to be controlled from VBSCRIPT batch files or from applications such as Matlab which supports COM automation.

The name of the WinEDR automation object is **WinEDR.AUTO** and is opened by the VBSCRIPT command

```
set W = CreateObject("winedr.auto")
```

### Recording functions

Recording can be started/stopped, data files created/opened and the recording trigger mode and stimulus pulse protocols selected. The recording methods and properties are listed below.

Recording Methods & Properties		
.NewFile("filename.wcp")	Method	Creates a new data file with the supplied name
.OpenFile("filename.wcp")	Method	Opens a pre-existing data file with the supplied name
.RecordDuration	R/W Property	Reads/sets duration of recording (s)
.StartRecording	Method	Starts recording to disk.
.StopRecording	Method	Stops recording to disk.
.HoldingVoltage	R/W Property	Reads/sets the selected DAC channel holding voltage (V) applied to the cell. E.g. W.HoldingVoltage = -0.06
.DAChannel	R/W Property	Reads/sets the selected D/A channel to which .HoldingVoltage is applied.
.TriggerMode	R/W Property	Read/sets the recording sweep trigger mode. 'F'=Free run, 'E'= External trigger.
.NumTriggerSweeps	R/W Property	Read/set number of triggered sweeps to be acquired when recording with .TriggerMode='E'
.StimulusProtocol	R/W Property	Read/sets the selected stimulus pulse protocol. e.g. W.StimulusProtocol = "prot01"
.StartStimulus	Method	Start stimulus selected by .StimulusProtocol

.Status	Read Only Property	Reads the current operational status of WinEDR. (0= idle, 1=seal test running, 2=recording to disk)
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## Seal test functions

WinWCP's seal test function can be initiated via the command interface and used to apply test pulses to cells and calculate the cell membrane conductance, capacity, access conductance and pipette seal resistance. These measurements can be read via the command interface while the seal test is running.

The seal test commands are listed below:

Seal Test Methods and Properties		
.StartSealTest	Method	Displays the seal test window and applies the seal test pulse.
.SealTestPulseAmplitude	R/W Property	Reads/sets the amplitude (Volts) of the seal test pulse (e.g. W.SealTestPulseAmplitude= 0.01)
.SealTestPulseDuration	R/W Property	Reads/sets the duration amplitude (S) of the seal test pulse (e.g. W.SealTestPulseDuration= 0.01)
.SealTestSmoothingFactor	R/W Property	Set cell parameters smoothing factor (0.1 - 1.0) ' 1 = no smoothing, ' 0.1 = maximum smoothing (equivalent to averaging over 10 pulses)
.Vm	Read Only Property	Reads the most recent cell holding potential (V) measurement, computed by the seal test.
.Im	Read Only Property	Returns the most recent cell holding current (A) measurement, computed by the seal test.
.Ga	Read Only Property	Reads the most recent cell access conductance (S) measurement.
.Gm	Read Only Property	Reads the most recent cell membrane conductance (S) measurement.
.Cm	Read Only Property	Reads the most recent cell capacity (F) measurement.

.Rseal	Read Only Property	Reads the most recent pipette seal resistance () measurement.
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A file (WinEDR VBSCRIPT Example.vbs)containing VBSCRIPT example code can be found in the c:\winedr folder.

## References > References

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