Strathclyde Electrophysiology Software

Electrophysiology Data Recorder

WinEDR V3.0

User Guide

04/02/2009

Contents

1. 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 department of Physiology & Pharmacology, 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).

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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1. Introduction & Software Installation

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

- 8 analogue input channels.
- Continuous sampling-to-disc at rates up to 100 kHz.
- Stimulus generator.
-

Analysis

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

1.1. Installation procedure

If you wish to use WinEDR to digitise analogue signals (rather than just analyse existing or simulated data files) you must have one of the laboratory interface cards supported by WinEDR installed in your computer. You must also ensure that the interface card is appropriately configured. This may involve setting switches or jumpers on the card itself. With some interfaces the manufacturer's software support libraries must also be installed and configured before it can be used. The full installation procedure consists of the following steps:

1) Install the WinEDR software (see section 1.2).

2) Install the laboratory interface unit and software (see section 1.4, 1.5, or 1.6).

3) Configure WinEDR to work with the laboratory interface

4) Attach analogue input/output signal cables (see section 3).

1.2. Installing the WinEDR software

To install WinEDR:

1) Go to the web page http://spider.science.strath.ac.uk/sipbs/page.php?page=software and click the WinEDR V2.x.x Setup File option to download a self-extracting archive (WinEDR_Vxxx.exe) containing the WinEDR installation files. Store this file in a temporary folder (e.g. c:\temp) on your computer.

2) Open the temporary folder and double-click the archive to unpack the WinEDR setup program.

3) Start the installation program by double-clicking the program **Setup.**

The setup program creates the directory **c:\Program Files\Strathclyde University\WinEDR** and installs the WinEDR programs files within it. (You can change the disk drive and directory if you wish).

4) To start WinEDR, click the Microsoft Windows **Start** button and select **WinEDR V3.2.x** from the **WinWCP** group in the **Programs** menu.

1.3. 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 or XP operating system. A laboratory interface unit is also required to perform the analogue-digital (A/D) and digital-analogue (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 interfaces supported by the NI-DAQ and NIDAQ-MX libraries,
- Axon Instruments Digidata 1200, 1320 or 1440 Series
- Instrutech ITC-16 or ITC-18
- Biologic VP500

1.4. Cambridge Electronic Design interfaces

Cambridge Electronic Design Ltd., Science Park, Milton Rd., Cambridge CB4 4FE.
Tel. (01223) 420186, Fax. (01223) 420488 (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. There are 4 main types of CED 1401 in common use - CED 1401, CED 1401-plus, CED Micro-1401 and CED Power-1401. They all fully support WinEDR's features with the exception that only 4 analogue input channels are available on the Micro1401 and that the maximum sampling rate for the older CED 1401 is substantially less than the others.

1.4.1. 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) Insert the CED 1401 installation CD and run the program
SETUP
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

If the CED 1401 tests check out OK, run WinEDR and select from its main menu

Setup
Recording

Select **Cambridge Electronic Design** from the **Laboratory Interface** list box.

Note. The latest versions of the above software can be obtained from CED's Web site, **www.ced.co.uk**.

See **Troubleshooting** section if you have a CED 1401 with ±10V A/D or D/A ranges/

1.4.2. Signal input / output connections

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

WinEDR channel	**CED 1401/1401+**	**Micro 1401**
Analogue inputs		
Ch. 0	ADC Input 0	ADC Input 0
Ch. 1	ADC Input 1	ADC Input 1
Ch. 2	ADC Input 2	ADC Input 2
Ch. 3	ADC Input 3	ADC Input 3
Ch. 4	ADC Input 4	-
Ch. 5	ADC Input 5	-
Ch.6	ADC Input 6	-
Ch.7	ADC Input 7	-
Analogue outputs		**Micro/Power 1401**
Command voltage out	DAC Output 0	DAC Output 0
External trigger in	Event Input 4	Trigger In

1.4.3. Troubleshooting tips

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

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.

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.

Modified CED 1401s with ±10V A/D or D/A ranges

CED 1401s interfaces are supplied with ±5V A/D input and D/A output voltage ranges as standard. They can however be supplied (or modified by the user) to have ±10V ranges, either for the D/A outputs alone or for both A/D inputs and D/A outputs. WinWCP cannot detect these modifications but you can indicate to the software that ±10V ranges are in use by placing an appropriate “flag” file into the WinWCP program folder.

10V D/A Outputs If you have a CED 1401 with ±10V D/A outputs, create a file named **CEDDAC10V.TXT** (it does not need to contain anything) and place it into the folder **c:\Program File\Strathclyde University\WinWCP**.

10V A/D Inputs If you have a CED 1401 with ±10V A/D inputs, create a file named **CEDADC10V.TXT** (it does not need to contain anything) and place it into the folder **c:\Program File\Strathclyde University\WinWCP**.

1.5. National Instruments interface cards

National Instruments UK, 21 Kingfisher Court, Hambridge Rd., Newbury, RG14 5SJ. Tel. (0635) 523545, Fax. (0635) 523154. OR National Instruments, 6504 Bridge Point Parkway, Austin, Texas 78730-5039. Tel. (512) 794 0100, Fax. (512) 794 8411.) (www.ni.com)

WinEDR is compatible with many of the 1200 Series (Lab-PC) and E-Series cards supplied by National Instruments. It has been tested with the Lab-PC, Lab-PC+, Lab-PC-1200, DAQ-Card-1200, PCI-MIO-16E-1, PCI-MIO-16E-4, and PCI-6024. (Note. It is not compatible with the Lab-PC 1200/AI and PCI-6023, which lack D/A output facilities.) Also, WinEDR's digital stimulus pulse generation facility is supported only by the 1200-series cards and the PCI-6025E. If digital output facilities are not required, the PCI-MIO-16E-4 can be recommended. It has a 500 kHz, 12 bit A/D sampling rate and programmable input voltage range (±5V, ±4V, ±2.5V, ±1V, ±0.5V, ±0.25V, ±0.1V, ±0.05V).

WinEDR controls the National Instruments interface cards via the company's NIDAQ interface library. NIDAQ must therefore be installed before WinEDR can use the interface card. WinEDR is compatible with NIDAQ versions 4.9-7.3, running under Windows 95, 98, NT, 2000 or XP.

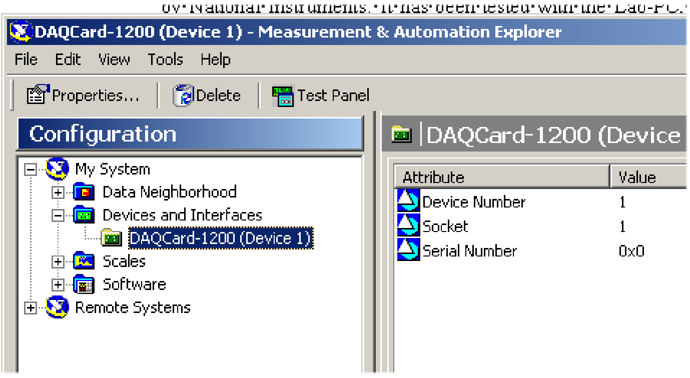
1.5.1. Software installation (LabPC/1200 & E Series cards)

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.

3) Reboot the computer.

4) Run National Instruments' **Measurement & Automation Explorer** program. You should find the card listed under **Devices & Interfaces**. (in NIDAQ V7.0 or later under the **Traditional NIDAQ Devices** option within **Devices & Interfaces**) The card must be installed as (**Device 1)** to work with WinEDR.

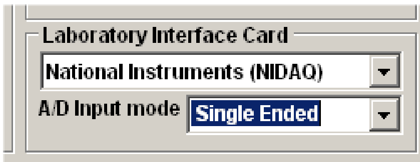


5) Click on the interface card entry in the Devices & Interfaces list then click the right hand mouse button and select **Test Panel** to check if the card is working.

6) If the tests check out OK, run WinEDR, and select from its main menu

Setup
Recording Parameters

Select **National Instruments (NIDAQ)** from the **Laboratory Interface** list box.



7) Set the **A/D Input** mode. If you are using a **BNC-2110** input/output box, select **Differential** or **BNC-2110 (Diff).** If you are using a **BNC 2090** input/output box, select **Single-Ended** or **BNC 2090 (SE). (Note.** The **SE/DI** switches on a BNC 2090 panel must be set to **SE** and the **NSRE**/RSE switch set to **NRSE)**

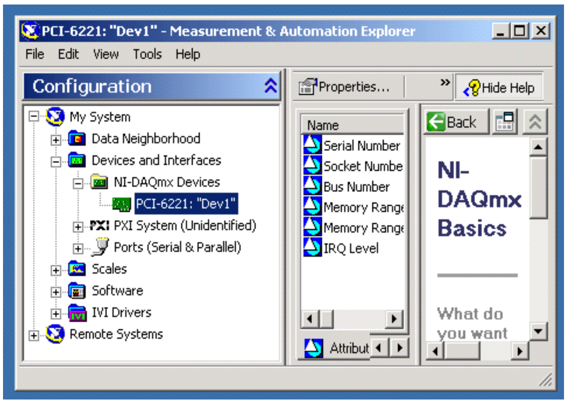
1.5.2. Software installation (M Series cards)

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.

3) Reboot the computer.

4) Run the National Instruments' **Measurement & Automation Explorer** program. You should find the card listed under the **NI-DAQmx Devices** option under **Devices & Interfaces**. The card name must be '**Dev1**' to work with WinEDR. (If necessary you can change the name of the card, by right-clicking and selecting **Change Name** from the pop-up menu.)

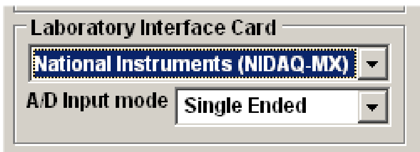


5) Click on the interface card entry in the Devices & Interfaces list then click the right hand mouse button and select **Test Panel** to check if the card is working.

6) If the tests check out OK, run WinEDR, and select from its main menu

Setup
Recording Parameters

Select **National Instruments (NIDAQ-MX)** from the **Laboratory Interface** list box.



7) Set the **A/D Input** mode. If you are using a **BNC-2110** input/output box, select **Differential** or **BNC-2110 (Diff).** If you are using a **BNC 2090** input/output box, select **Single-Ended** or **BNC 2090 (SE). (Note.** The **SE/DI** switches on a BNC 2090 panel must be set to **SE** and the **NSRE**/RSE switch set to **NRSE)**

1.5.3. Signal input / output connections

Signal input and output from National Instruments cards are made via a 50 or 68 way ribbon cable connector on the rear of the card. BNC socketed input/output panels (BNC-2090, BNC2110) are available from National Instruments for E or M-Series boards. Standard screw terminal panels with 50 way ribbon cable sockets can also be obtained from electronic component suppliers.

The input/output connections for 50 pin 1200- and 68 pin E- and M-series boards are tabulated below.

Lab-PC/1200 Series Cards

Lab-PC/1200 Cards		
Analogue Inputs	**I/O Panel**	**Screw terminal panel**
Ch. 0	ACH0	1,9 (signal,ground)
Ch. 1	ACH1	2,9 (See Note 1)
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
Analogue Outputs		
Ch. 0	DAC 0	10,11
Ch. 1	DAC 1	12,11 (See Note 2)
Trigger Inputs		
Ext. Sweep Trigger	EXTTRIG	38,50 (See Note 2)

NOTE 1. The Lab-PC/1200 card analogue inputs should be configured in the **RSE (Referenced Single Ended)** mode (using the National Instruments Measurements & Automation Explorer configuration program.)

NOTE 2 Analogue 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 Seal Test / Signal Monitor pulses to operate.

National Instruments E & M Series cards

National Instruments E & M Series cards		
Analogue Input	**I/O Panel**	**Screw terminal panel**
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
Analogue Output		
Ch. 0	DACOUT 0	22,55
Trigger Inputs		
Ext. Sweep Trigger	PFI0/TRIG1	11,44

NOTE 1. Connections can be made to E & M Series boards using either a BNC 2090 (19" rack mountable), BNC 2110 I/O panel or a screw terminal panel. Panels are connected to the interface via a 1 or 2 metre SH68-68 shielded cable. Depending upon what type of I/O panel is in use, the analogue inputs of the card should be configured as follows:

I/O Panel	Analogue Input mode
BNC 2090	RSE (Referenced Single Ended)
BNC 2110	DIFF (Differential)
CB68	RSE (Referenced Single Ended)

1.6. Axon Instruments Digidata 1200

Axon Instruments Inc. 3280 Whipple Road, Union City CA 94587 U.S.A. Tel (510) 675-6200 (www.axon.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). The Digidata 1200 is also supported by the pCLAMP electrophysiology software package. 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 6

1.6.1. 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) Click the Windows **Start** button and select **Install Digidata 1200 driver (Windows 95,98,ME)** or **Install Digidata 1200 driver (WindowsNT, 2000, XP)** (depending on your Windows version) from within the **WinEDR\Digidata 1200 Support** group in the **Programs** menu. Follow the instructions to install the Digidata 1200 Windows device driver. (WinEDR does not use the standard Axon Instruments Digidata 1200 device driver).

3) Reboot the computer.

4) Run WinEDR and select from its main menu

   Setup
   Recording

   Select
   Axon Instruments Digidata 1200

   from the **Laboratory Interface** list box.

1.6.2. 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.

WinEDR channel	**Name**	
Analogue inputs		
Ch. 0	Analogue In 0	
Ch. 1	Analogue In 1	
Ch. 2	Analogue In 2	
Ch. 3	Analogue In 3	
Ch. 4	Analogue In 4	
Ch. 5	Analogue In 5	
Analogue outputs		
Command voltage out	Analogue Out 0	
Trigger/Sync.		
External trigger in	Gate 3 (on rear)	See Note 1

1.6.3. Troubleshooting

There are two known problems which will prevent WinEDR from recording from a Digidata 1200's analogue 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

a) Change the Sound-Blaster MIDI port setting to a value higher than 33AH.

b) 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 6 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.

1.7. Axon Instruments Digidata 1320/22

Axon Instruments Inc. 3280 Whipple Road, Union City CA 94587 U.S.A. Tel (510) 675-6200.

The Digidata 1320 Series interface consists 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. The Digidata 1320 Series is currently supported by WinEDR under Windows 95, 98 and NT.

1.7.1. 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 its main menu

Setup
Recording

Select
Axon Instruments Digidata 1320

from the **Laboratory Interface** list box.

1.7.2. *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.

WinEDR channel	**Name**
Analogue inputs	
Ch. 0	Analogue In 0
Ch. 1	Analogue In 1
Ch. 2	Analogue In 2
Ch. 3	Analogue In 3
Ch. 4	Analogue In 4
Ch. 5	Analogue In 5
Ch.6	Analogue In 6
Ch.7	Analogue In 7
Analogue outputs	
Command voltage out	Analogue Out 0
Trigger/Sync.	
External Trigger In	Trigger In (Start)

1.7.3. *Troubleshooting*

When multiple analogue 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.

1.8. Molecular Devices Digidata 1440A

Molecular Devices Corporation, Sunnyvale, California 94089, USA (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 (8 used by WinEDR). It has a fixed input and output voltage range of ±10V and supports 4 analogue output channels (2 used by WinEDR) and 8 digital output channels. The Digidata 1440A is currently supported by WinEDR under Windows 2000, XP and Vista.

1.8.1. 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) Install the WinEDR software.

5) Run WinEDR and select from its main menu

Setup
Recording sweep

to open the Setup dialog box then select

Axon Instruments Digidata 1440

from the **Laboratory Interface** list box.

1.8.2. 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		
Analogue 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	
Analogue Output		
Ch. 0	Analog Output 0	
Ch. 1	Analog Output 1	
Trigger Inputs		
Ext. Sweep Trigger	Start	

1.9. Instrutech ITC-16/18

The Instrutech ITC-16 and ITC-18 interfaces consist of self-contained, 19” rack-mountable, mains-powered digitiser unit 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 analogue input channels, 4 analogue outputs (2 used by WinWCP) and 8 digital outputs. The ITC-16 is a 12 bit resolution device, the ITC-18 is 16 bit.

Both devices are currently supported by WinWCP under Windows 95, 98, NT and 2000. The ITC-16 and ITC-18 are manufactured by Instrutech Inc., 20 Vanderventer Ave., Suite 101E, Port Washington, New York 11050-3752 U.S.A. Telephone: (516) 883-1300. (www.instrutech.com)

1.9.1. Instrutech ITC-16/18 – I/O Panel Connections

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

WinWCP channel	**Name**
Analogue inputs	
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
Analogue outputs	
Command voltage out	DAC Output 0
Trigger/Sync.	
External trigger in	Trig In
Digital Out	
Dig. 0	TTL Out 0
Dig. 1	TTL Out 1
Dig. 2	TTL: Out 2
Dig. 3	TTL Out 3

1.9.2. Installing software support for the Instrutech ITC-16/18

WinWCP uses Instrutech’s device interface library (ITCMM.DLL) for the ITC-16/18 family. Details for steps (1)-(3) can be found in the Instrutech Data Acquisition Interface user manual.

Installation Procedure

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)

4) Reboot the computer.

5) Run the Instrutech test program installed with the device driver to test whether the software installed OK.

6) Run WinWCP and select from its main menu

   Setup
   Recording

   Select
   Instrutech ITC-16/18

   from the **Laboratory Interface** list box.

1.9.3. Instrutech ITC-16/18 : Troubleshooting

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

1.10. Biologic VP500

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 WinWCP.

The current implementation of the WinWCP software supports

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

The VP500 is manufactured by Bio-Logic - Science Instruments SA, 1, rue de l' Europe, F-38640 - CLAIX – France (www.bio-logic.fr)

1.10.1. 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.

1.10.2. Installing software support for the Biologic VP500.

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

Installation Procedure

1) Install the National Instruments NIDAQ software, supplied with the GPIB interface card and reboot.

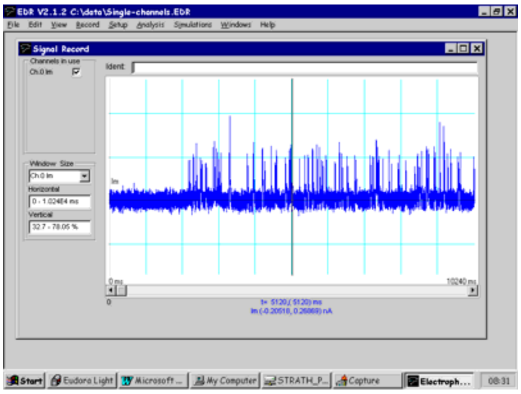
2) Install the GPIB card into the host computer and reboot.

3) Check using the National Instruments Measurement & Automation Explorer program that the GPIB has been detected and is functioning correctly.

4) Once the device driver installation procedure has been completed, run WinWCP and select the **Recording** item from the **Setup** menu.

5) Select **Biologic VP500** from the **Laboratory Interface** list box.

2. Using WinEDR - An Overview



WinEDR consists of a variety of program modules for recording and analysing electrophysiological signals. These modules are accessed via the main program menu on the program's title bar and appear as independent sub-windows enclosed within the main WinEDR window.

The **File** menu provides the standard Windows functions for creating, opening and closing data file, printing and import and export to non-native data formats.

The **Edit** menu permits data to be copied to the Windows clipboard.

The **View** menu provides options for displaying the recorded signals.

The **Record** menu invokes the digital recording module for recording analogue signals to disk and the seal test module for monitoring the sealing of patch pipettes to cells.

The **Setup** menu provides options for setting; the numbers of analogue channels to be recorded, recording sweep duration and other parameters; voltage-clamp command voltage stimulus patterns; and controlling external amplifiers.

The **Analysis** menu provides access to a range of analysis modules, which can be applied to the digitised signals stored on file. These include:

- A miniature synaptic current detection module
- A noise analysis module
- An amplitude histogram module
- A single-channel current analysis module

The **Simulations** menu provides access to modules for creating simulated synaptic currents, ionic current noise and single-channel currents.

The **Windows** menu selects between active windows.

The **Help** menu provides access to the WinEDR Help files.

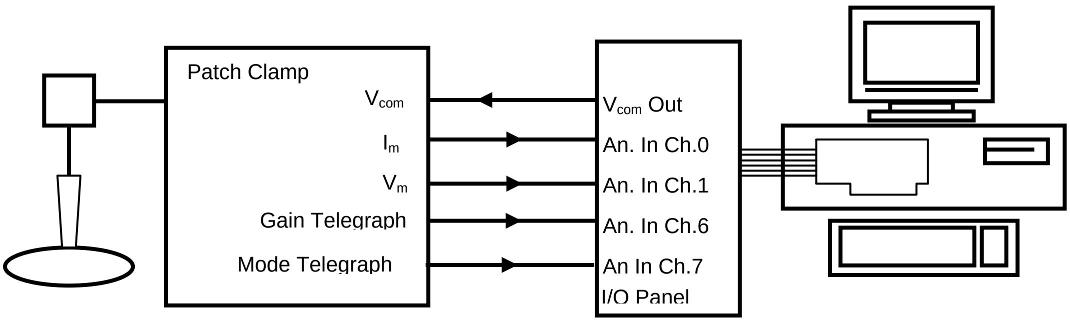
3. Connecting WinEDR to your experiment

The first step in making a digital recording is to connect the signal outputs from your electrophysiological amplifier to the appropriate analogue inputs of the A/D converter in your laboratory interface unit. If you plan to use the computer to apply voltage pulse stimuli to the cell, you must also connect a D/A converter output to the command voltage input of the voltage-clamp. You may also have to supply a digital trigger pulse to initiate each recording sweep.

Depending upon the absolute levels produced by the recording device, you may need to amplify the signal to make it compatible with the input requirements of your A/D converters. You may also need to low-pass filter the signal and/or apply a DC offset to the signal, before it can be digitised. Two typical recording situations are discussed below.

3.1. Example 1 - Connecting WinEDR to a patch clamp.

One of the most common applications for WinEDR is recording from, and controlling, a whole-cell patch clamp experiment. Two analogue channels are normally recorded, membrane current and voltage, and computer-generated voltage pulses are applied to the patch clamp command voltage input to stimulate the cell. The patch clamp is connected to the computer as follows



Consult the signal connections table for your particular laboratory interface, in sections 1.4.2, 1.5.3 or 1.6.2, and make the following connections.

The membrane **current** (I_m) output from the patch clamp is connected to WinEDR input channel **Ch.0**.

The membrane **potential** (V_m) is fed into WinEDR input channel **Ch.1**.

Voltage stimulus pulses generated at the WinEDR **Command voltage output** are passed to the **Command voltage input** (V_{com}) of the patch clamp.

The patch clamp **gain telegraph** output is connected to WinWCP input channel **Ch.7**. (Note. Some patch clamps do not support gain telegraphs. Others (e.g. Axon Multiclamp 700A/B or the Biologic VP500 communicate gain information via USB or other communications lines.)

The patch clamp **mode telegraph** output is connected to WinWCP input channel **Ch.6**. (Note Currently only required for Axon Axopatch 200 or Cairn Optopatch patch clamps.)

The table below contains the required connections for the range of patch and voltage clamps currently supported by WinWCP.

WinWCP	Signal Inputs		Telegraphs		Command Voltage O/P
	Analog In 0	Analog In 1	Analog In 6	Analog In 7	Analog Out 0
Axopatch 1D	Scaled Output	10 Vm	-	Gain Telegraph	Ext. Command
Axopatch 200	Scaled Output	10 Vm (VC mode) Im (CC mode) **See Note.1**	Mode Telegraph	Gain Telegraph	Ext. Command
Multiclamp 700A	Scaled Output	Raw Output	-	- **See Note 2**	Ext. Command
Multiclamp 700B	Primary Output	Secondary Output	-	- **See Note 2**	Ext. Command
RK400	Iout	10 x Vm	-	-	Voltage Command Input
VP500	-	-	-	-	-
Heka EPC-8	Current Monitor	Vcomm Monitor	-	See table below	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	-	Gain Telegraph	External ÷50

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
WinWCP Digital Input	0	1	2	3	4	Gnd

Note 1. When the Axopatch 200 is switched from voltage- to current-clamp mode, the Scaled Outy signal to Analog In 1 of WinWCP is changes automatically from membrane current to voltage. To retain a current signal, Analog In 1 of WinWCP must be switched manually from 10 Vm to Im.

Note 2. WinWCP obtains channel gain information from the Axon Multiclamp commander software. Multiclamp Commander must be started up and running **before** WinWCP is started.

4. Configuring WinEDR for a recording session

WinEDR digitises analogue signals from your experiments as a single continuous, gap-free, record. Up to 8 separate input channels can be acquired.

Before making a digital recording for the first time, you must do the following :-

1) Create a data file to hold your recordings.

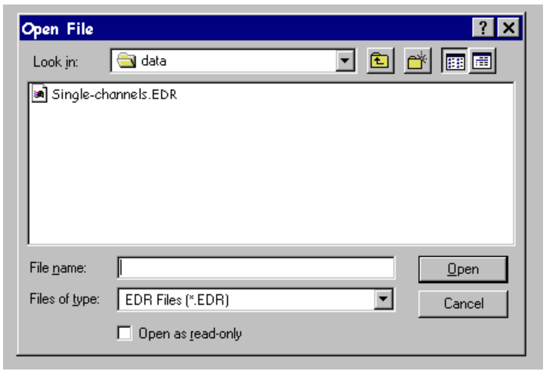
2) Define the number of analogue input channels, sampling interval etc. for the recording.

4.1. Creating a data file

To create a new data file to hold your recordings, select from the menu

File
New

To get the **New Data File** dialog box, shown here.



Select the disk and folder into which the file is to be placed using the **Save In** list box. WinEDR data files have the extension extension ".edr"

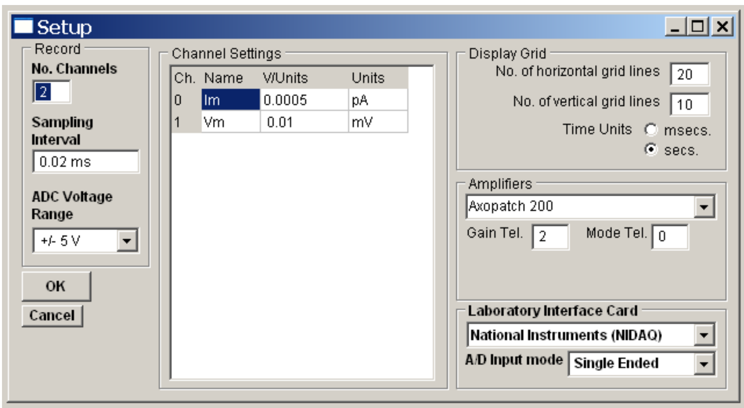
.

4.2. Setting recording parameters.

To set the number of channels to be recorded, recording duration and other parameters select the option

Setup
Recording Parameters

to display the Setup dialog box.



4.2.1. No. Channels

Sets the number of analogue input channels you intend to record from. WinEDR supports a maximum of 8 channels. Channels are always acquired in sequence from Ch.0 upwards, i.e. No. Channels=1, selects Ch.0; No. Channels=2 selects Ch.0 & Ch.1 etc.

4.2.2. Sampling Interval

Sets the time interval between A/D samples taken from each input channel. **Note**. It is important to choose a sampling interval small enough to ensure that a sufficient number of samples are acquired during the most rapidly changing phases of the signals being recorded. Also, to avoid aliasing artefacts, the analogue signals should be low-pass filtered to remove frequency components greater than half of the sampling rate (i.e. reciprocal of the sampling interval).

4.2.3. A/D Converter Voltage Range

Defines the measurable voltage range of the A/D converter. The range of possible options depends upon the laboratory interface in use. The CED 1401, for instance, only has a single sensitivity ±5V, other interfaces such as the Axon Instruments Digidata 1200 have 4 programmable input sensitivities: ±10V, ±5V, ±2.5, and ±1.25V.

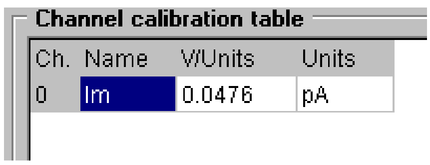
In order to accurately measure the amplitude of an analogue signal it is important to ensure that it spans a significant proportion (30-50%) of the A/D converter's input voltage range. By changing the voltage range you can adapt the sensitivity of the A/D converter to best match the amplitude of the signals from your experiment.

4.2.4. Time Units

Determines whether time measurements are presented in units of seconds or milliseconds.

4.2.5. Channel Calibration Table

WinEDR can display the signals stored in each input channel in the units appropriate to each channel. In order to do this correctly, the names, units and scaling information for each channel must be entered into the **Channel Calibration Table**. There are 3 entries in the table for each analogue channel.


Channel calibration table

Ch.	Name	V/Units	Units
0	Im	0.0476	pA

Names contains a 1-4 letter name used to identify the source of the channel (e.g. Vm, Im).

Units defines the measurement units of the signal (e.g. mV, pA etc.).

V/Units defines the scaling factors relating the voltage level at the inputs of the A/D converter (in V) to the actual signal levels in each channel (in the channel units).

For instance, if the membrane voltage output of your patch clamp 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 patch clamp voltage output is 0.01 Volts per mV).

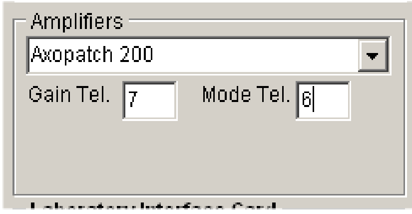
In the case of patch clamp current channels, the V/Units value is determined by the current gain setting which is usually a switchable value, e.g. if the current output was set at 20 mV/pA, and the channel units were pA, the V/Units settings would be 0.02.)

A typical setup for a patch-clamp experiment, recording current and voltage channels is shown below. Current is recorded in channel 0, which is named Im, and has units of pA.

	Name	**V/Units**	**Units**
Ch.0	Im	0.02	pA
Ch.1	Vm	0.01	mV

4.2.6. Amplifiers

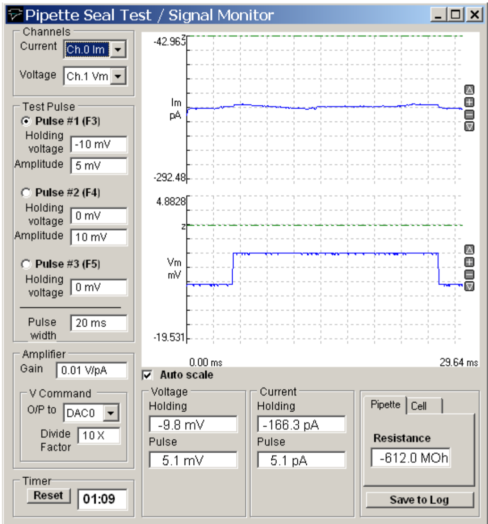
WinEDR can automatically determine the current and voltage gain factor for a number of patch clamp amplifiers and use it as the calibration factor for input channels Ch.0 & Ch.1. If a CED 1902 computer-controllable amplifier is in use, this option can also be used to read its gain setting. To enable this facility,



1) Select your amplifier from the **Amplifiers** list.
2) If required, connect an unused input channel to the gain telegraph output of the patch clamp, and enter the number of that channel in the **Gain Tel.** Box. (Note. The VP500, MultiClamp and CED 1902 do not require this.)
3) If required, connect an unused input channel to the current/voltage clamp mode telegraph output of the patch clamp, and enter the number of that channel in the **Mode Tel.** box. (Note. Only the Axopatch 200 and Cairn Optopatch currently require a mode telegraph connection)

Note. The channel calibration table settings for channels Ch.0 (current) & Ch.1 (voltage) are automatically configured with appropriate name, units and V/Units scaling when a patch clamp amplifier is selected from the **Amplifiers** list. (Patch clamp current and voltage outputs must be connected to analogue input channels Ch.0 & Ch.1 of the laboratory interface.)

5. Monitoring input signals & patch pipette seal test



After a data file has been created and input channel parameters defined, you can monitor the signals appearing on each channel using the signal monitor/pipette seal test module. This module 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.

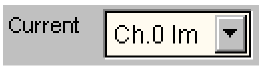
5.1. Display scaling

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.

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.

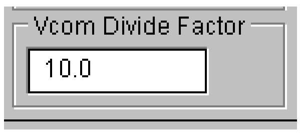
5.2. Select current and voltage channels

Select the input channels, which contain the current and voltage signals, by selecting the current channel in the current list.



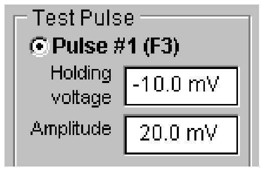
and the voltage channel in the voltage list

5.3. Command voltage divide factor



Most voltage and patch clamp amplifiers divide down their command voltage input signals by some factor in the range 10-50. Enter the scaling factor into the **Vcom divide factor** box. WinEDR uses this factor to scale the stimulus voltage output to the D/A converter to obtain the correct voltage at the cell. (Note. Axon Instruments amplifiers require a divide factor of 50 while the Heka EPC-7 patch clamp requires a divide factor 10.)

5.4. Cell holding voltage and test pulses



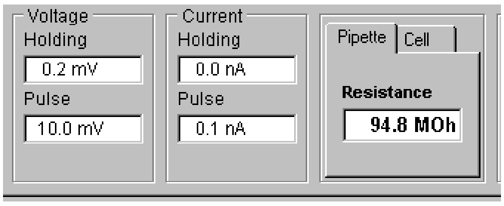
You can control the holding voltage applied to the cell and the amplitude and duration of a test voltage pulse by selecting one of two available test pulse types (Pulse #1, Pulse #2) or a holding voltage level without a pulse (Pulse #3).

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 both pulses is defined by the **pulse width** box

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

5.5. 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.

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

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

where V_{pulse} and I_{pulse} are the steady-state voltage and current pulse amplitudes. The **Cell** option displays the cell membrane conductance, G_m, capacity, C_m, and access conductance, G_a, computed from

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

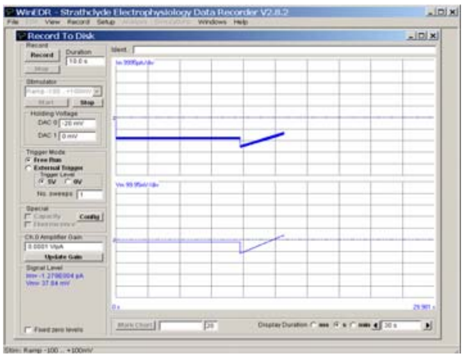
$$G_m = \frac{I_{pulse}}{\left(V_{pulse} - \frac{I_{pulse}}{G_a}\right)}$$

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

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

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

6. Making a recording



To make a recording of signals from your experiment, select

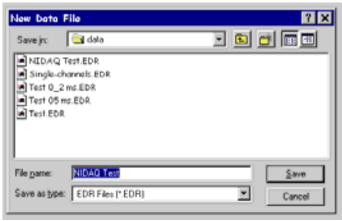
Record
Record to disk

to open the recording module. 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.

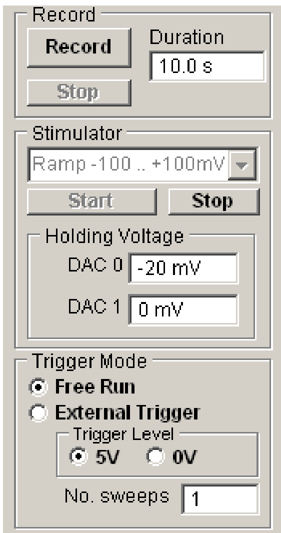
To make a recording of the incoming signal:



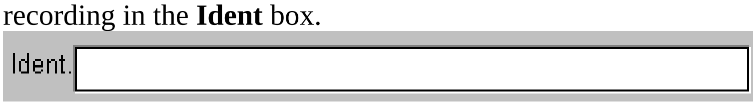
1) Create a new file to hold the recorded data by selecting

   File
   New data file

   to open the **New Data File** box. Select the disk and folder into which the file is to be placed using the **Save In** list box. Enter the name of the file in the **File name** box, WinEDR data file names have the extension ".edr"



2) Enter the required duration of the recording into the **Duration** box.
3) Enter the cell holding potential to be used during the recording sweep in the **DAC 0 Holding Voltage** box.
4) If required, select a stimulus protocol from the **Stimulator** list
5) (**Optional**) Enter a line of text identifying the purpose of the recording in the **Ident** box.



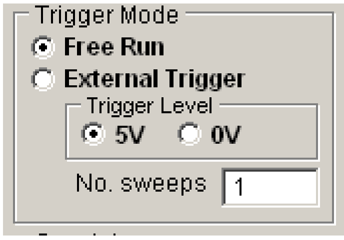
6) Set the trigger mode to **Free Run** if the recording is to start immediately or **External Trigger** if it is to be triggered by an external signal (see 6.1 below for details).
7) Start recording, by clicking the **Record** button.
8) If you want to stop recording before the preset duration has been acquired, click the **Stop** button.

6.1. Trigger modes

There are two trigger modes

- Free Run
- 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 is not possible or required.



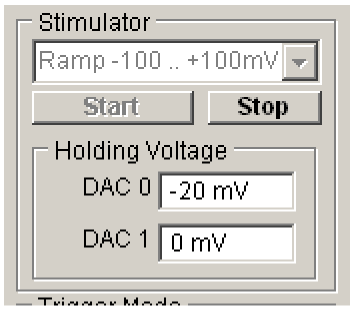
When the trigger mode is set to **External Trigger**, recording only starts after a trigger pulse is received on the External Trigger input of the laboratory interface. External Trigger mode is used when it is necessary to synchronise recording with an external event such as the pulse from a stimulator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

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

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.)

6.2. Stimulator

A wide variety of stimulus voltage waveforms can be generated on DAC output channels DAC0 & DAC1 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 DAC 0 and DAC 1 output voltage to the holding levels.

The holding voltages applied to DAC 0 (patch clamp command voltage) and DAC 1 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.)

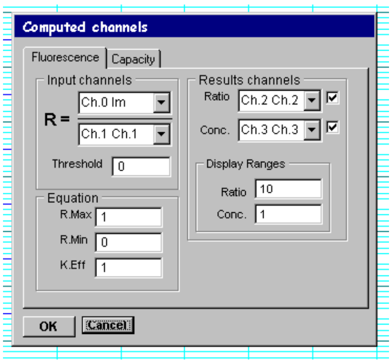
Stimulus protocols can consist of a series of one or more pulses, incremented in amplitude or duration to create a family of pulses. Complex stimulus waveforms can be produced, including series of rectangular steps, ramps, and digitised analogue signals. Individual protocols can also be linked together to automatically apply a series of different protocols during an experiment. Protocols are created using the Stimulus Protocol Editor and stored as protocol files (*.sti files). (See section 7)

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

6.3. Special features

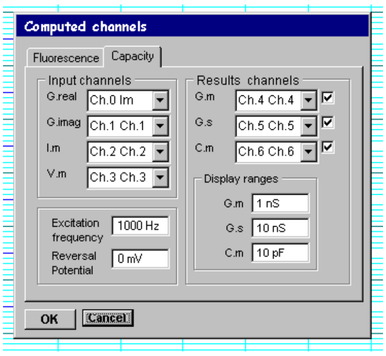
WinEDR has a number of special features for processing of FURA-2 fluorescence, cell capacity and real-time event frequency. In these modes of operation certain input channels are computed on-line from the others. To define the input channels and computed results channels click the **Config** button to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

6.3.1. Cell fluorescence mode



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.

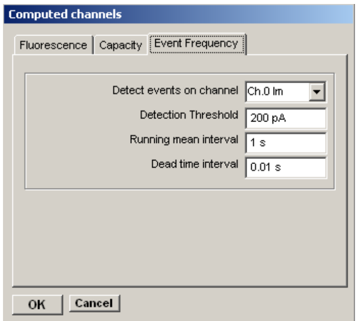
6.3.2. Cell capacity mode



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.

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).

6.3.3. Event Frequency display



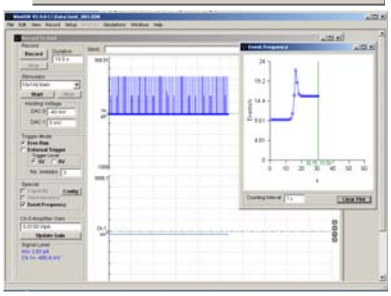
Selecting the **Event Frequency** special option opens a display window showing the frequency of occurrence of signals from a selected input channel. Signals are detected using a threshold-based event detector which is configured on the Event Frequency page of the Computed Channels dialog box. To configure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

a) Select the input channel on which the signals are to be detected from the **Detect events in channel** list.

b) Set the threshold which the signal must exceed to be detected in the **Detection threshold** box

c) Set the averaging interval for the threshold baseline level in the **Running mean baseline** box.

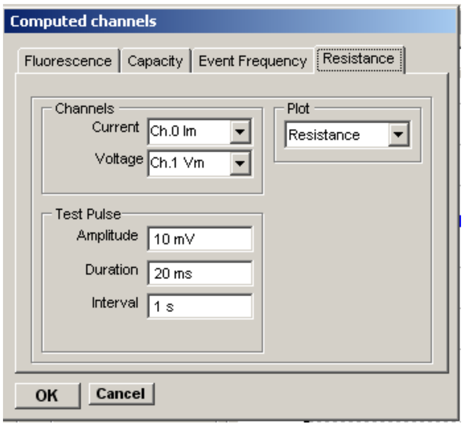
d) Set the time after detection of an event within which detection is disabled in the **Dead time interval** box to exceed the duration of a signals to be detected.



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.

6.3.4. Cell Resistance Display

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. To configure the resistance plot:

a) Select the input channel on which the current signals are to be detected from the **Current** channel list.

b) Select the input channel on which the voltage signals are to be detected from the **Voltage** channel list.

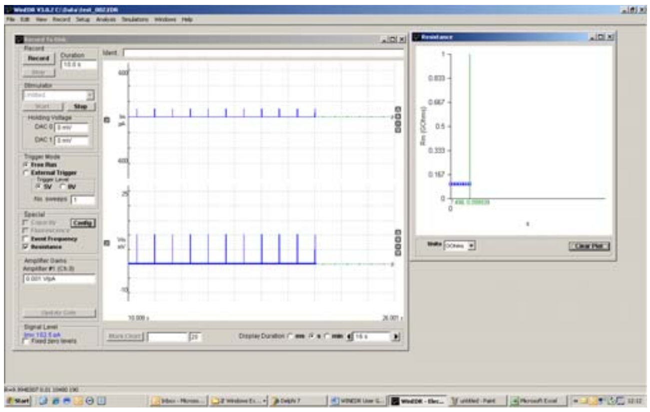
c) Set the amplitude of the test pulse in the **Amplitude** box.

d) Set the duration of the test pulse in the **Duration** box.

e) Set the interval between pulses in the **Interval** box.

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

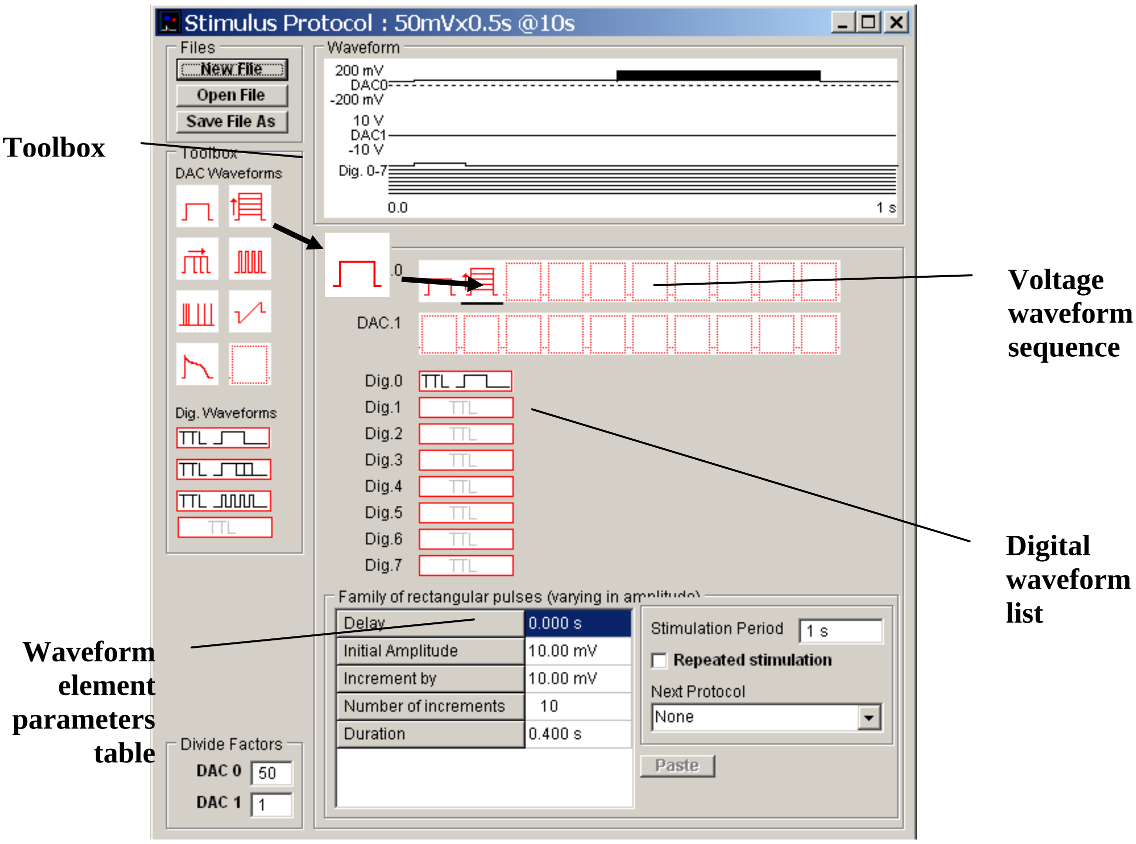


7. Creating stimulus protocols

To create a new (or edit an existing) stimulus protocol file, select from the WinEDR main menu

Setup
Stimulus Protocol Editor

to open the stimulus protocol editor. A diagram of the output waveforms appears in the **Waveform** display box. To create a stimulus protocol, click the **New File** button to create a blank protocol.



7.1. Creating voltage waveform stimuli

Voltage waveforms are constructed by dragging waveform elements from the **Toolbox** and dropping them into the selected **voltage waveform sequence**. (**DAC 0** or **DAC 1**). A voltage waveform can consist of a sequence of up 10 separate waveform elements. The amplitude and duration for each element is defined in its **parameters table** which can be made to appear by clicking the element in the waveform sequence.

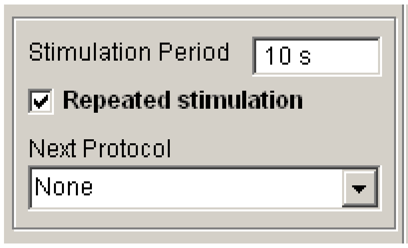
Seven different waveform elements are available (rectangular pulse; family of pulses incrementing in amplitude; family of pulses incrementing in duration; train of pulses; noise; ramp; user-defined waveform) Details of each waveform shape and its parameters are defined in Table 7.1

7.2. Creating digital stimulus waveforms

To create a digital stimulus waveform, drag a digital stimulus element from the Toolbox and drop it into the selected digital output channel. Each digital output channel controls the on (5V) / off (0V) state of a digital output line. The duration and inverted/non-inverted signal polarity for each protocol is defined in its parameters table which can be made to appear by clicking on the element in the protocol list. Details of each digital stimulus waveform and its parameters are defined in Table 7.2

7.3. Repeated and linked protocols

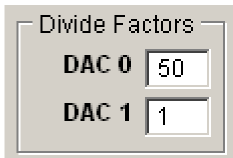
Waveform protocols can be made to repeat by ticking the **Repeated Stimulation** option and entering a repetition time in **the Stimulation Period** box.



Multiple stimulus protocols can also be linked together by selecting the name of another protocol in the **Next Protocol** list (When linked protocols are in use, the time set in Stimulation Period determines the time interval between protocols).

7.4. Command voltage divide factors

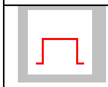
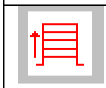
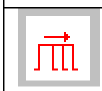
Most voltage and patch clamp amplifiers divide down their command voltage input signals by some factor. Enter the scaling factor into the **Divide Factor** box. This factor is used to scale the stimulus voltage output to the voltage output channel to obtain the correct voltage at the cell. (**NOTE**. The voltage divide factor for DAC 0 is set automatically when an amplifier supported by WinEDR has been selected. See To create a stimulus protocol, first click the **New File** button to create a blank protocol.

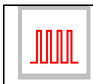
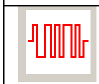
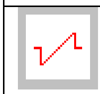


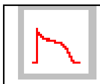
7.5. Saving a stimulus protocol

On completion, a stimulus protocol can be saved for use, by clicking the **Save File As** button and providing a name for the protocol file. Protocol files stored in the folder **c:\winedr\\stim** (the default location) appear in the stimulus protocols list in the recording window.

Existing protocol files can be loaded into the editor for modification by clicking the **Open File** button and selecting a file.

	Table 7.1 Voltage waveform Elements
	**Rectangular voltage pulse of fixed size** This is a simple pulse, which does not vary in amplitude and duration between records. It has 3 parameters. **Initial Delay:** the delay period before the pulse begins. **Amplitude:** the pulse amplitude (mV). **Duration:** the duration of the pulse. This element can be used to provide series of stimuli of fixed size or, in combination with other elements, to provide fixed pre-conditioning pulses.
	Family of rectangular pulses varying in amplitude This is a rectangular voltage pulse whose amplitude is automatically incremented between recording sweeps. It has 5 parameters. **Initial delay:** the delay period before the pulse begins. **Start at Amplitude:** the amplitude of the first pulse in the protocol sequence. **Increment by:** the increment to be added to the pulse amplitude between records. **Number of increments:** the number of steps in the sequence. **Pulse duration:** the duration of the pulse. This element is typically used to explore the voltage-sensitivity of ionic conductances, by generating records containing the whole-cell membrane currents evoked in response to a series of voltage steps to different membrane potentials.
	**Family of rectangular voltage pulses varying in duration** This is a rectangular voltage pulse whose duration can be automatically incremented between recording sweeps. It has 5 parameters. **Initial delay:** the delay period before the pulse begins. **Amplitude:** the amplitude of the pulse. **Pulse duration:** the duration of the pulse. **Increment by:** the increment to be added to the pulse duration between records. **Number of increments:** the number of steps in the sequence. This element is most commonly used as a variable duration preconditioning pulse in 2 or 3 step protocols for investigating inactivation kinetics of Hodgkin-Huxley type conductances.

	**Train of rectangular voltage pulses** This is a train of rectangular voltage pulses of fixed size. It is defined by 5 parameters: **Initial delay:** the delay period before the series of pulses begin. **Amplitude:** the amplitude of each pulse in the series. **Duration:** the duration of each pulse. **Pulse interval:** (within train) determines the time interval between pulses. **Number of pulses:** defines the number of pulses in the train. This element can be used to produce a series of stimuli to observe the effect of repeated application of a stimulus at a high rate. It can also be used to produce a train of pre-conditioning stimuli for a subsequent test waveform.
	**Noise** This element generates a pseudo-random binary sequence of pulses which randomly step a fixed amplitude +/- the holding level. It is defined by 3 parameters: **Initial delay:** the delay period before the series of pulses begin. **Amplitude:** the amplitude of each pulse in the series. **Duration:** the duration of each pulse. PRBS noise provides a source of white noise (containing equal amounts of all frequencies within Nyquist limit) which can be used to investigate the impedance characteristics of cells.
	**Voltage ramp** This element produces a linear voltage ramp between two voltage levels. It is defined by 4 parameters **Initial delay:** the delay period before the series of pulses begin. **Start at amplitude:** the voltage level at the start of the ramp. **End at amplitude:** the voltage level at the end of the ramp. **Ramp duration:** the time taken for the voltage to slew between the start and end amplitudes. Voltage ramps provide a means of rapidly generating the steady state current-voltage relationship for an ionic conductance. (Note that, the ramp generated by the computer is not truly linear, but consists of a staircase of fine steps. These steps can be smoothed out, by low-pass filtering the voltage stimulus signal before it is fed into the patch clamp.)



Digitised analogue waveform

Digitised analogue waveforms which have been previously acquired by WinWCP (or synthesised by another program) can be used as a waveform element. Only one digitised waveform element is permitted per protocol.

To insert a digitised waveform:

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

b) Waveforms can be inserted into the protocol by clicking the

button to copy a waveform stored in the Windows clipboard, or by clicking the

To load a waveform stored in a data file.

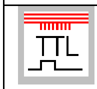
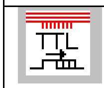
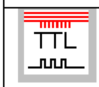
The parameters table consists of:

Initial delay: the delay period before the waveform begins.

DAC update interval: the time interval between waveform points

A list of the first 1000 data points of the analogue waveform. The waveform can be altered by modifying this list.

The waveform in the clipboard or the data file can be formatted either as a single column of voltage values or as a pair of time (ms or s), voltage values. The DAC update interval is determined from the time column when one is available.

	Table 7.2 Digital waveform elements
	**Digital pulse (fixed duration)** This produced a digital pulse on the selected output line of fixed duration. It is defined by 4 parameters. **Initial delay** defines the delay before the start of the pulse. **Duration** defines the duration of the digital pulse. **Invert Signal** defines whether the digital pulse is an OFF-ON or an ON-OFF pulse. If set to No, the digital line is initially OFF (0V) and switches to ON (5V) during the pulse. If set to Yes, the digital line is initially ON (5V) and switches to OFF (0V) during the pulse. The digital pulse element can be used to switch open or close valves controlling the flow of solutions over a cell. Multiple digital outputs can be used to simultaneously open one valve while another is closed.
	**Family of digital pulse (varying in duration)** This produced a digital pulse on the selected output line, with a duration which is incrementable between records. It is defined by 5 parameters. **Initial delay** defines the delay before the start of the pulse. **Starting duration** defines the duration of the first pulse in the protocol. **Increment by** defines the amount that the duration is incremented between records. **Number of increments** defines the number of increments in the protocol. (Note that, if there are any voltage waveform elements in use within the protocol, the number of increments defined here must be the same.) **Invert Signal** defines whether the digital pulse is an OFF-ON or an ON-OFF pulse. If set to No, the digital line is initially OFF (0V) and switches to ON (5V) during the pulse. If set to Yes, the digital line is initially ON (5V) and switches to OFF (0V) during the pulse.
	**Train of digital pulses** This produces a series of digital pulses of fixed intervals and of fixed duration. It is defined by 5 parameters. **Initial delay** defines the delay before the start of the first pulse in the series. **Pulse duration** defines the duration of the each pulse in the series. **Inter-pulse interval** defines the time interval between pulses in the series. **Number of pulses** defines the number of pulses in the series. **Invert Signal** defines whether the digital pulse is an OFF-ON or an ON-OFF pulse. If set to No, the digital line is initially OFF (0V) and switches to ON (5V) during the pulse. If set to Yes, the digital line is initially ON (5V) and switches to OFF (0V) during the pulse. This element can be used to apply a rapid train of stimuli to a cell.

8. Viewing recordings stored on file.

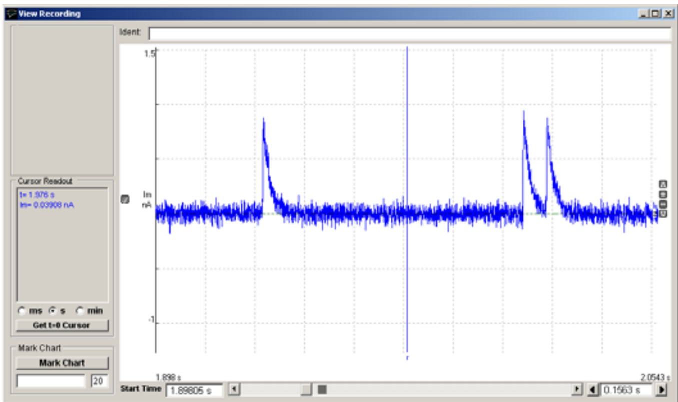
To review a recording after it has been acquired and stored in a data file, select one of the signal viewing options from the **View** menu. The **Compressed View** option displays the complete recording in compressed into a single window, with the facility to zoom into selected regions. The **Page View**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

8.1. Compressed View

To display the recorded signal in the compressed view, select

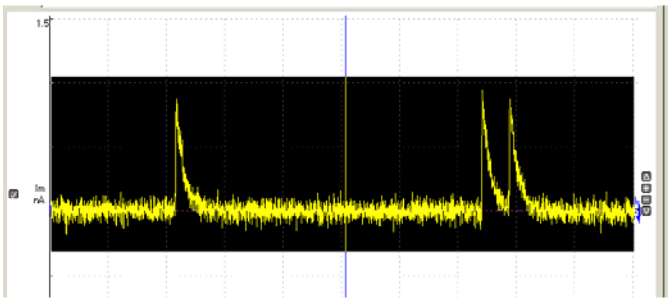
View
Compressed View

to open the **Compressed View** module shown here. The whole recording contained within the data file is displayed in a compressed form on the screen. (**Note**. For large data files this can incur a delay before initial display.) Measurements can be made of the signal levels within each channel using a movable cursor. The displayed recording can be printed out.



8.2. Magnifying a section of the recording

The vertical magnification of each plot can be expanded to a selected region by moving the mouse to the upper limit of the region, pressing the left mouse button, drawing a rectangle to indicate the region and releasing the mouse button.



The vertical magnification and location 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.

You can set all channels back to minimum magnification by selecting

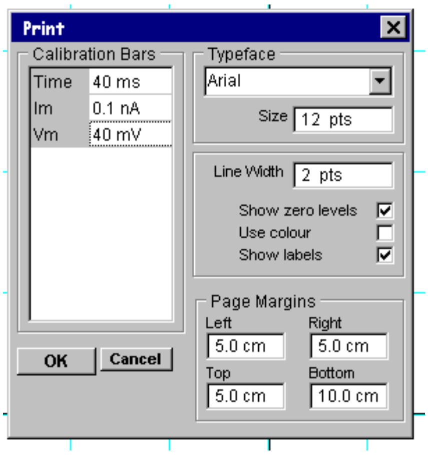
View
Zoom Out (All)

8.3. Printing records

To print the recording displayed on the screen, select

File
Print

To open the **Print** 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 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.

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**.

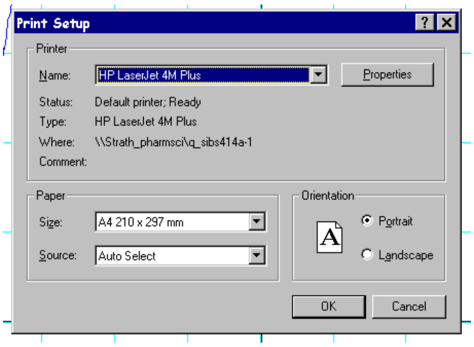
When all plot parameters have been set, click the **OK** button to initiate printing.

8.4. Choosing a printer and output format.

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

File
Print Setup

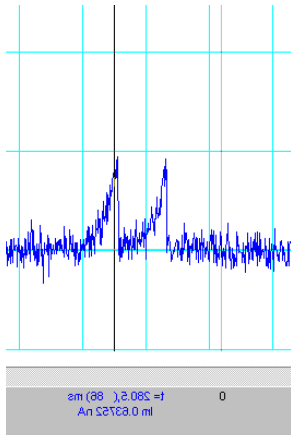
to open the **Print Setup** dialog box.



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**.

8.5. Cursor measurement of 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 ← or → 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 the window, below the cursor. Time measurements are made relative to the start of recording and (in brackets) relative to the location of the t=0 cursor. Signal levels are measured relative to each channel's horizontal zero level cursor which can be dragged up or down by the user.

8.6. Copying recordings to the Windows clipboard

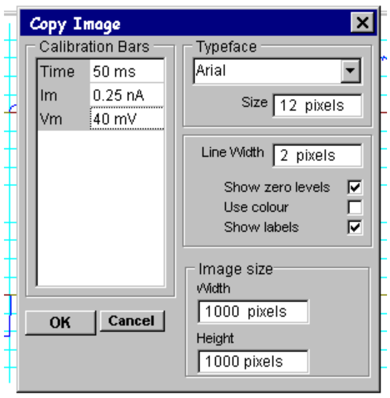
A recording consists of an array of A/D converter sample values. A table of data values for the active display record can be copied to the clipboard by selecting

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.)

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

Edit
Copy Image



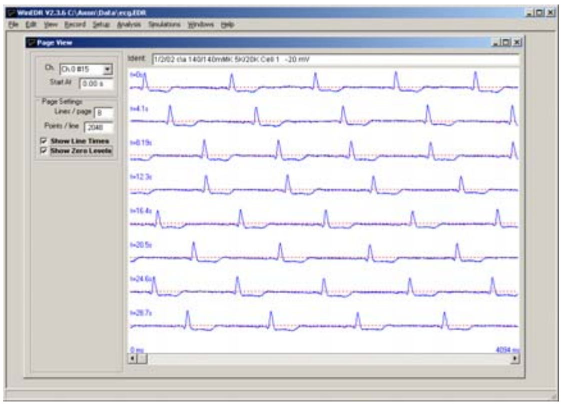
to open the Copy Image dialog box.

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.

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

8.7. Page View

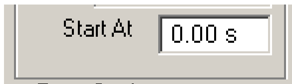
To view a section of a selected channel within the digitised recording as a series of sequential records on a page, select



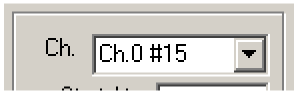
View
Page View

to open the **Page View** module. One channel of the digitised recording is displayed (uncompressed) as a sequence of lines within the display window.

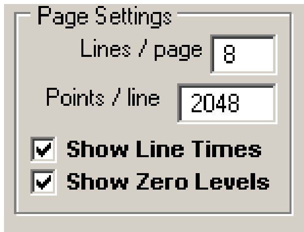
Dragging the scroll bar at the bottom of the display shifts the display forwards and backwards throughout the recording, one line at a time.



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.

8.8. Magnifying the displayed lines

The signals displayed on each line can be magnified by double-clicking on the displayed signal to switch to “zoom mode” and following the same procedure as for the compressed view (see 7.2). Only the vertical magnification can be changed, and changes to the display magnification settings for any one are automatically applied to all lines on the page.

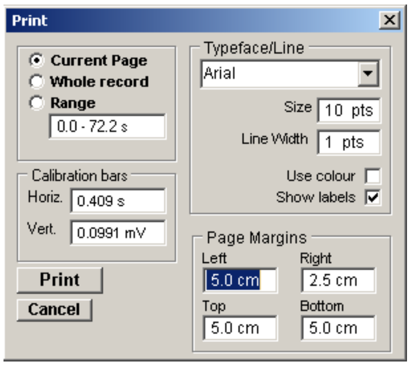
8.9. Printing records in page view

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

File
Print

to open the **Print** dialog box. There are 3 printing options

- Current Page
- Whole record
- Range



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

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.)

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.

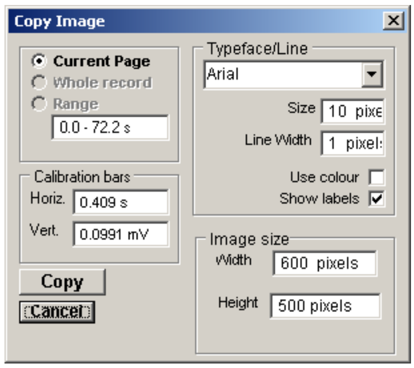
You can set the size of the plotted record on the printed page, by adjusting the size of the **page margins**.

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

8.10. Copying the page view display to the Windows clipboard

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

Edit
Copy Image



to open the **Copy Image** 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

9. Detecting transient signals within a recording

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

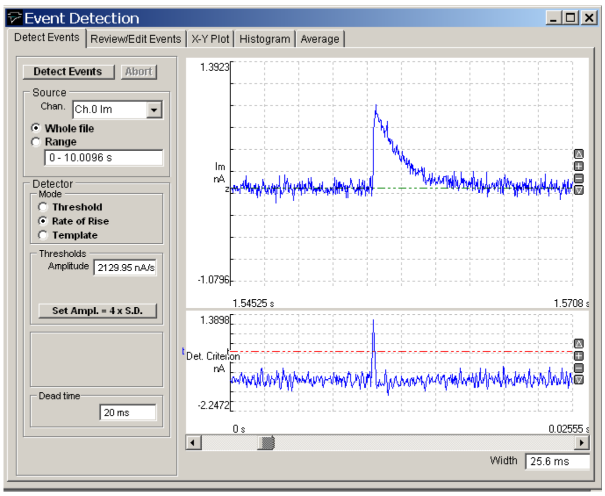
to open the Detect Events module. The module is split into 3 pages

- Detect Events
- Review/Edit Events
- X/Y Plot

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.

9.1. 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.



The displayed traces can be magnified vertically by double-clicking the panel to switch into the display "zoom" mode, and adjusting the upper and lower limits of the displayed region box.

Three different types of event detection method are supported:

- Amplitude threshold
- Rate of rise
- Template matching

The **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.

The **rate of rise** method uses a signal detection criterion based upon the signal rate of change computed using a 5-point Savitsky-Golay differentation algorithm.

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.

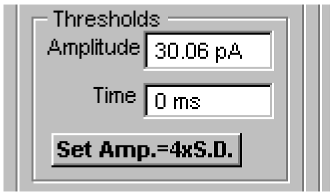
To scan a section of the continuous digitised recording for event :-

1) 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) If there is more one channel within the recording, select the channel to be scanned for signals, from the **Channel** list.

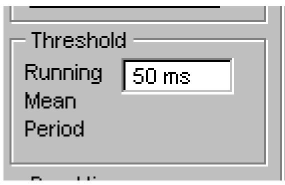
3) Select the event detection method (**Amplitude**, **Rate of rise** or **Template**).

4) Set the detection **Thresholds**. Set the level which the signal detection criterion has to cross for an event to be detected in the **Amplitude** box. (The position of the amplitude threshold is indicated by a horizontal cursor on the Det Criterion display window. The amplitude threshold can also be adjusted by dragging this cursor up or down.) Set the period of time which the signal has to remain above the amplitude threshold before detection is accepted in the **Time** box. (The **Set Amp.=4xS.D.** button can be used to set the amplitude threshold to 4x the standard deviation computed from the displayed signal).

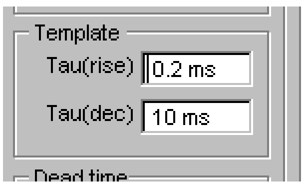


5) To avoid repeated detection of the same signal, set the time period before another signal can be detected in the **Dead Time** box. (Usually set equal to the expected duration of the signals being detected.)

6) [Amplitude threshold method] Amplitude threshold measurements are made relative to a moving average baseline level which tracks slow changes the signal level. Set the period of time over which the baseline average is computed in the **Running Mean Period** box. (The smaller the time period, the faster the tracking rate of the baseline.).



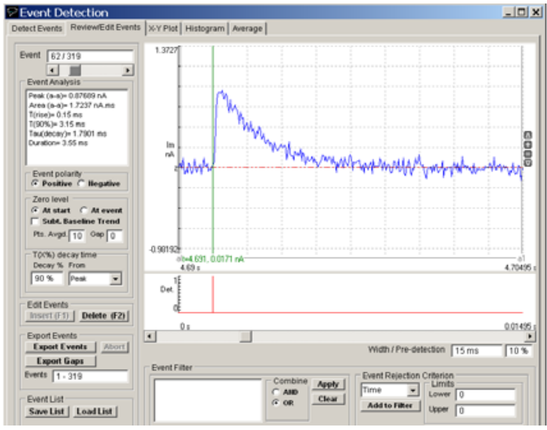
[Template method] Enter the rising (τ_{rise}) and decaying (τ_{decay}) time constants which define the shape and duration of the template waveform into the **Tau(rise)** and **Tau(decay)** boxes. (Suitable values can be obtained by detecting a typical signals using one of the other detection methods, exporting the record to WinWCP and fitting an MEPC curve to it.).



7) Click **Detect Events** to begin the event detection process.

9.2. 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.

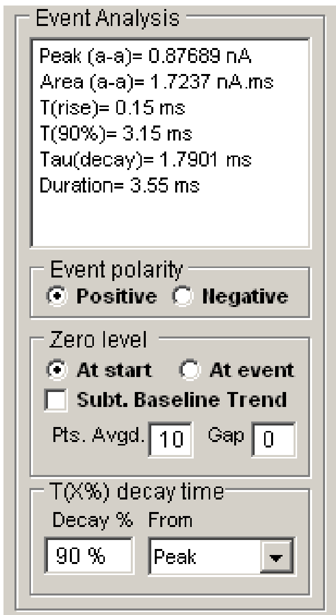
Note. When measurements are being made, the display width should be adjusted to display one event only.

9.2.1. Manually adding/removing events

The current selected 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 event and clicking the **Insert Event** button (or pressing the F1 key).

9.2.2. Event Analysis

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

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 block 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.

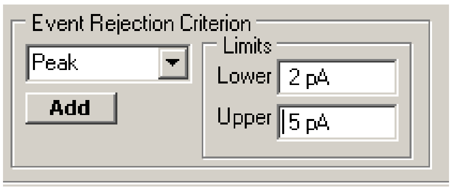
Peak Detection Window: Define the peak detection region using the pair of "**a0-a1**" cursors. (This option can be used to exclude stimulus artefacts or other signals feature from the waveform analysis)

Decay Time: Enter the required T(X%) 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.

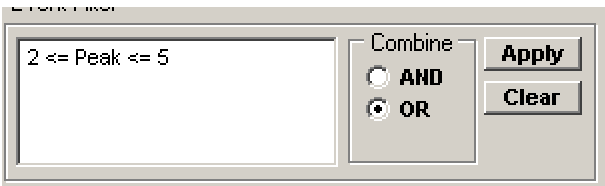
9.2.3. Event filtering

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.
2) 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 >=2pA and <=5pA will be rejected.)
3) Click the **Add** button to add the criterion to rejection list.



4) If additional criteria are required, repeat (1)-(3), 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.
5) Click the **Apply** button to remove events matching the defined criteria.

(**Note**. Events are permanently deleted from the event list by the filter. It is prudent to save the original detected event list (see **Save List** below) before applying the filter. so it can be restored if necessary (using **Load List**).

9.3. Saving/Loading the detected event times list

The list of event detection times can be saved as an ASCII text by clicking the **Save List** button and entering the name and location of a file into the Save Event List dialog box.

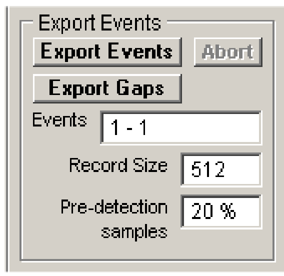
A list of event times can be re-loaded (replacing the existing list) by clicking the **Load List** button and entering the name of a file in the Load Event List dialog box.

9.4. Exporting events for analysis by WinWCP

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.

To export detected events :

1) Enter the range of events to be exported in the **Events** box.



2) Set the size of the event waveform record in the **Record Size** box. (A multiple of 256 samples, up to 32768).

3) Set percentage of the waveform record to be taken before the detection point, in the **% Pre-detection samples** box.

4) Click the **Export Events** button and select the name of a WCP data file to hold the data.

9.5. Extracting 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 the gaps between events, following

To extract and save the gaps between events:

1) Enter the range of events to be exported in the **Events** box.

2) Set the size of the region around the event detection point which will not be copied to the gap record in the **Record Size** box. (A multiple of 256 samples, up to 32768).

3) Set the proportion of the record before the detection point, in the **% Pre-detection samples** box.

4) Click the **Export Gaps** button and select the name of a new EDR data file to hold the data.

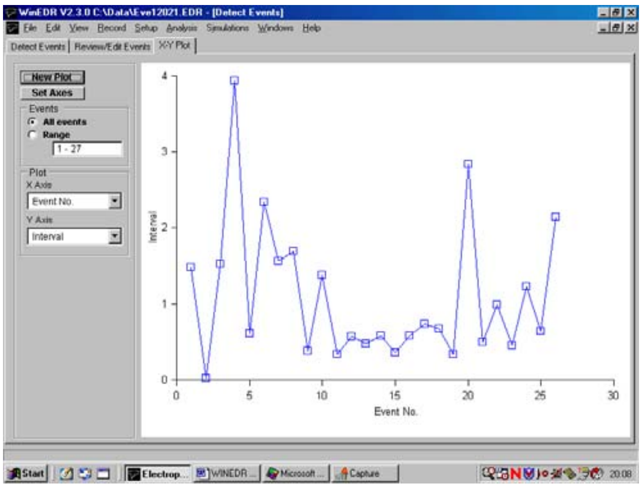
9.6. Plotting Event Analysis measurements

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

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**)

To create an X-Y plot :

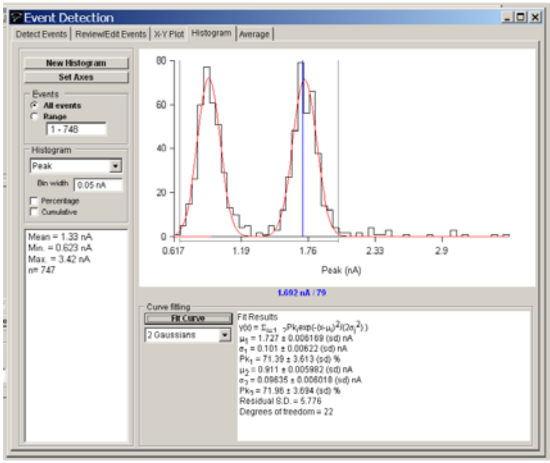


1) Select the variable to be plotted on the horizontal axis from the **X Axis** variable list. (If Rate has been selected enter the interval over which the event frequency is to be calculated.)
2) Select the variable to be plotted on the vertical axis from the **Y Axis** variable list.
3) Select the **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.

9.7. Plotting histograms of Event Analysis measurements

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



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 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 **Percentag**e 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.

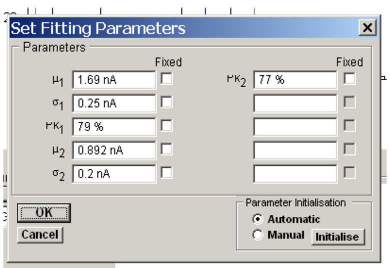
9.7.1. Fitting gaussian curves to histograms

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 '|' region of interest cursors.

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

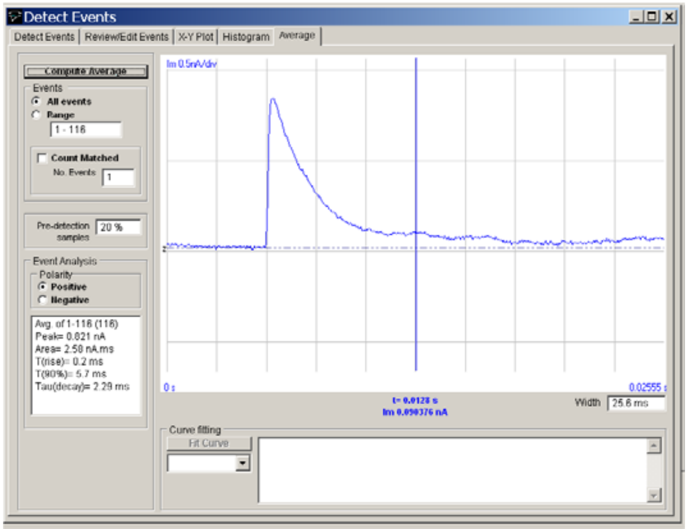
3) Click the **Fit Curve** button and enter an appropriate set of initial guesses for the gaussian function parameters (μ_i, σ_i, Pk) (Note. A set of initial guesses are computed automatically, but it is often necessary to adjust these to better match the location and size of the observed histogram peaks). 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.

9.8. Calculating and displaying event averages

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.



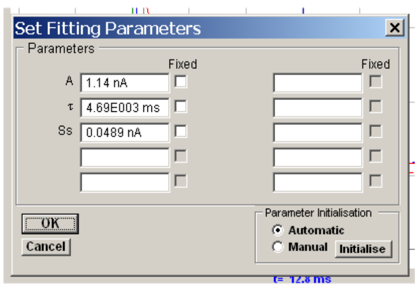
To compute the event average waveform:

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

9.8.1. Fitting curves to averages

To fit one or more exponential functions to the average:

4) Define the segment of the average waveform be fitted using the pair of 'a' analysis region cursors.
5) Define the starting point (time = 0) of the exponential function to be fitted, using the t=0 cursor
6) Select the number of exponential functions to be fitted from the **Curve Fitting** list.
7) 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.

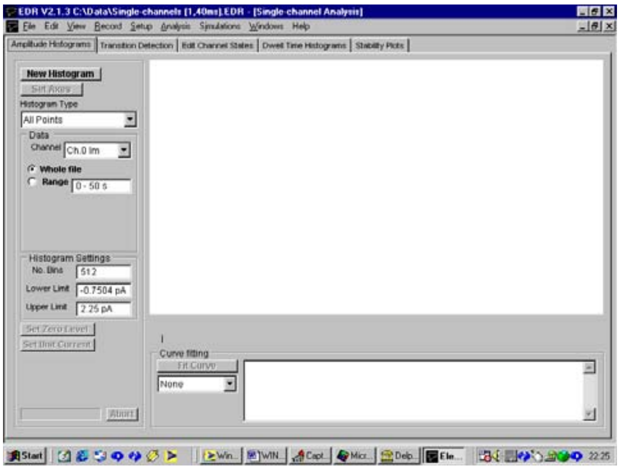


The best fit curve us superimposed in red over the fitted region of the waveform. The parameters of the best fitting equation are displayed in the Curve Fitting results area.

10. Analysis of Single-channel Currents

To analyse a single-channel current recording, select

Analysis
Single-channel Analysis



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

- Amplitude Histograms
- Transition Detection,
- Edit Channel States,
- Dwell Time Histograms
- Stability Plots

The **Amplitude Histograms** page provides tools for compiling and analysing the ion channel current amplitude distribution. 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 **Edit Channel States** page provides an editor for visually inspecting each detected state, and rejecting artefacts from the analysis. 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. The **Stability Plots** page 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 :-

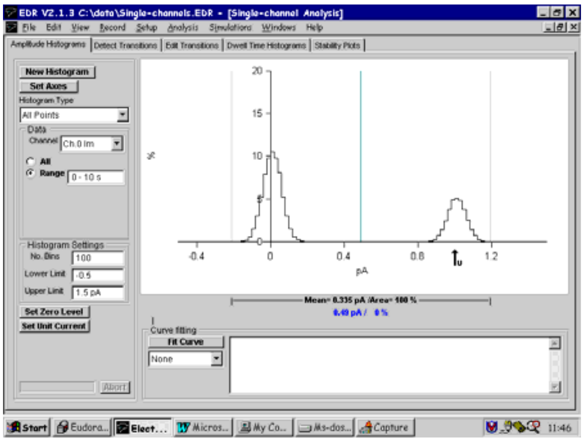
a) Current amplitude histograms are computed to determine the single-channel current/conductance, number of channels in the patch, and the steady-state probability of a channel being open.

b) 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.

c) 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).

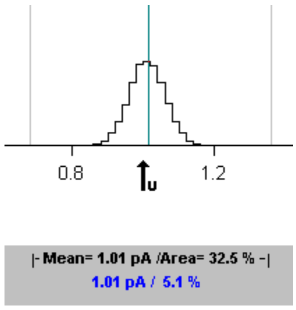
10.1. Analysis of current amplitudes



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

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 blue 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.

10.1.1. Defining zero and single-channel current levels

The location of the peaks within the current amplitude histogram can be used to define the zero and single-channel current levels for the recording. The zero level is set by moving the readout cursor to the mid-point of the peak associated with the closed channel state. and clicking the **Set Zero Level** button. Current measurements are made relative to this point and the X axis range of the histogram is adjusted to account for this.

The single-channel current level (indicated on the histogram by a ↑u symbol) is set similarly, by moving the cursor to the mid-point of the peak associated with the open channel state and clicking the **Set Unit Current** button. (**Note**. The zero and single-channel current levels set by this procedure are used in the transition detection procedure)

10.1.2. 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_{open}.

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 :-

a) Select **All Points** from the **Histogram Type** list.

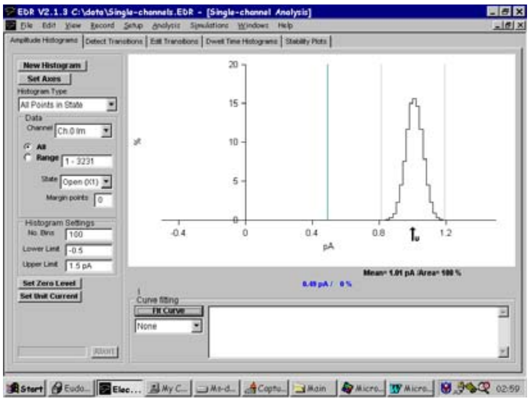
b) If more than one signal channel is available, select the channel to be used from the **Channel** list.

c) 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.

d) Enter the width of the histogram bin in the **Bin Width** box.

e) Click the **New Histogram** button to compile and display the histogram.

10.1.3. All Points in State Amplitude 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 <u>after</u> transition detection has been completed.

To compute the histogram :-

a) Select **All Points In State** from the **Histogram Type** list.

b) If more than one signal channel is available, select the channel to be used from the **Channel** list.

c) 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.

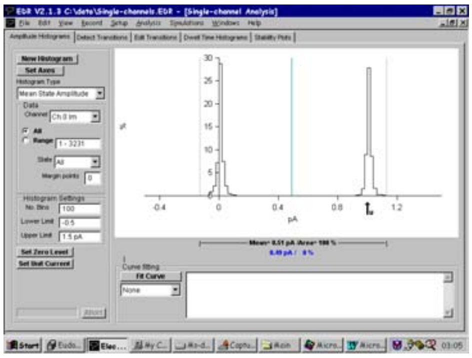
d) Select the channel state(s) (**Close**, **Open(X1)** or **All**) to be included in the histogram from the **State** list.

e) 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.

f) Enter the width of the histogram bin in the **Bin Width** box.

g) Click the **New Histogram** button to compile and display the histogram.

10.1.4. 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 :-

h) Select **Mean State Amplitude** from the **Histogram Type** list.

i) If more than one signal channel is available, select the channel to be used from the **Channel** list.

j) 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.

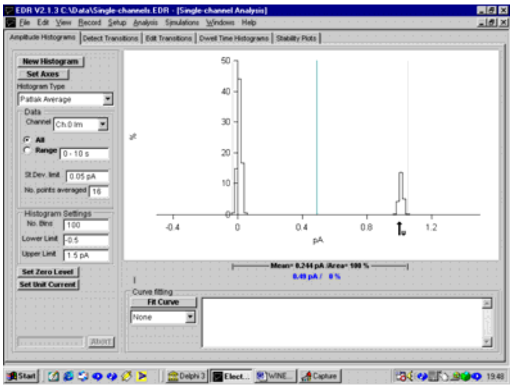
k) Select the channel state(s) (**Close**, **Open(X1)** or **All**) to be included in the histogram from the **State** list.

l) Enter the width of the histogram bin in the **Bin Width** box.

m) 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.

10.1.5. *Patlak running average*



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 exclude from the histogram.

To compute the histogram :-

a) Select **Patlak Average** from the **Histogram Type** list.

b) If more than one signal channel is available, select the channel to be used from the **Channel** list.

c) 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.

d) Enter the number of sample points in the running average in the **No points averaged** box.

e) Enter the exclusion limit for the running mean standard deviation in the **St. Dev. Limit** box. (**Note**. A suitable value is the standard deviation (σ) of a gaussian function fitted to the all points current amplitude distribution around the zero or single-channel currents levels.

f) Enter the width of the histogram bin in the **Bin Width** box.

g) 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.

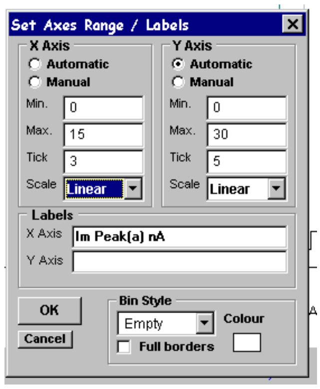
10.1.6. *Cursor Measurements (avg) Histogram*

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

10.1.7. *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.

10.1.8. *Customising histograms*

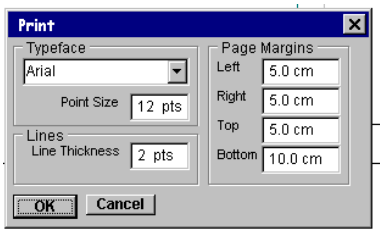


If you want to alter the X or Y axis range, scaling or labels on a histogram, click the **Set Axes** button to open **the Set Axes Range / Labels** dialog box.

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.

10.1.9. *Printing histograms*



To print the displayed histogram, select

File
Print

To open the **Print** dialog box. Click the **OK** button to plot the graph.

10.1.10. *Copying the histogram data points to the Windows clipboard*

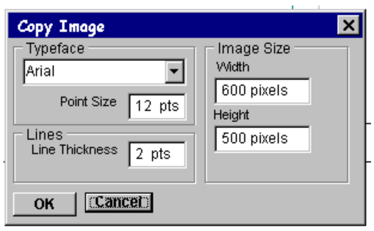
The numerical values of the X,Y data points which generate the histogram can be copied to the clipboard by selecting

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>
```

10.1.11. *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 **copy image** 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.

10.1.12. Gaussian curve fitting

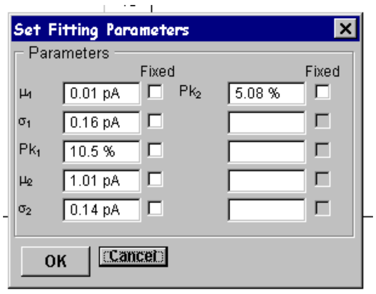
The peaks of the current amplitude histogram can often be fitted by a mixture of one or more normal (or gaussian) probability density functions. The height *y(i)*, of bin *i* with its mid-point at current *I(i)*, is given by

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

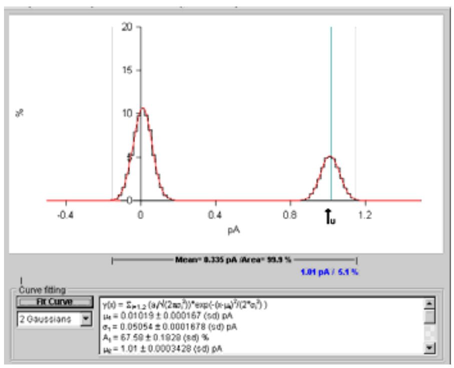
where *w* is the histogram bin width. Each gaussian component is defined by 3 parameters; A_i the percentage of the total area under the component, μ_i the mean current, and σ_i the standard deviation about the mean value. WinEDR uses an iterative least squares curve fitting procedure to find the gaussian 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:

8) Define the range of amplitudes containing the peak(s) to be fitted using the pair of grey '|' region of interest cursors.
9) Select the number of Gaussian functions to be fitted from the **Curve Fitting** list.
10) Click the **Fit Curve** button and enter an appropriate set of initial guesses for the gaussian function parameters (μ_i, σ_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.



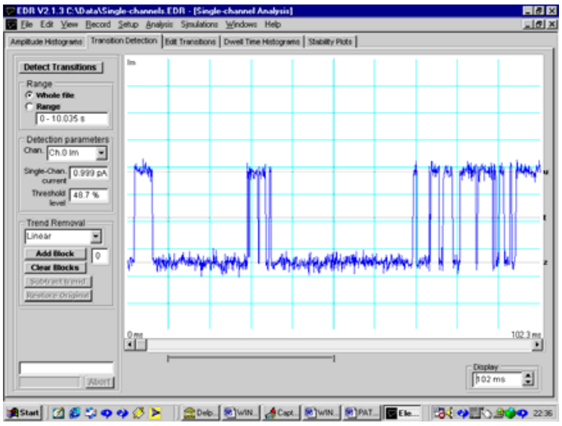
The best fitting gaussian function(s) are superimposed (in red) on the histogram. The values of the function parameters along with their estimated standard errors are displayed in curve fitting results area at the bottom of the display.



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

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.

10.2. Detecting channel open/close transitions



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.

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 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.

10.2.1. Detecting transitions

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

a) If necessary, vertically magnify the displayed signal by double-clicking the display panel to switch into “zoom” mode, and adjusting the upper and lower limits of the displayed region box. The time period on display can also be adjusted by increasing or decreasing the **Display** time value.

b) 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.

c) The position of the zero current level (z), single-channel current level (u) and transition threshold (t) are indicated by horizontal cursors. 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

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

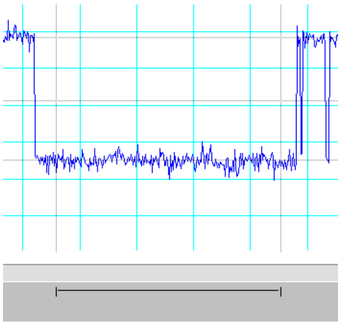
During transition detection an idealised noise-free current signal, representing the detected sequence of open/closed channel states is superimposed (in red) on the recorded current signal. This process can take several minutes with large data files and/or slow computer, progress being indicated by the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

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.

10.2.2. Removal of 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 outlined in 9.2.1. 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 :-



a) Click the **Clear Blocks** button eraser any existing baseline trend line.

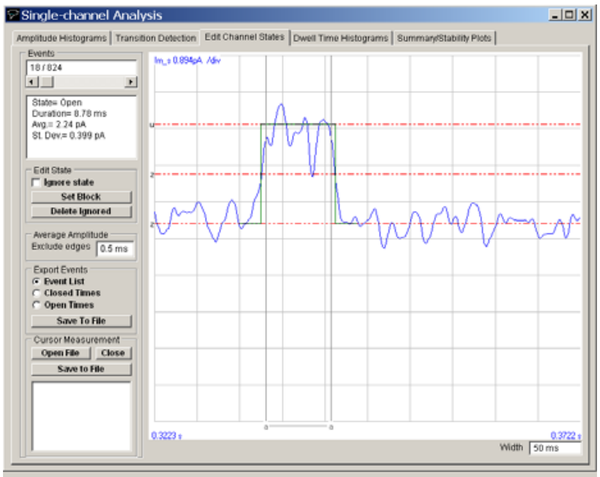
b) Scroll through the displayed recording and locate a section where the channel is closed for an extended period. Select the closed interval for inclusion in the baseline current line, using the '|--|' region selection cursors, and click the **Add Block** button to add the average signal level during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

c) Repeat (b), selecting baseline current periods throughout the recording until an adequate representation of the trend has been obtained.

d) 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.

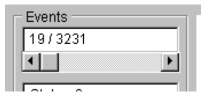
e) Click the **Subtract Trend** button to remove the baseline trend from the digitised signal. (If necessary, to. restore the original signal click the **Restore Original** button.)

10.3. Displaying and 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.

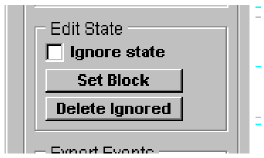
The idealised channel open/closed state is superimposed (in green) on the digitised current signal. The displayed signal can be vertically magnify the displayed signal by double-clicking the display panel to switch into "zoom" mode, and adjusting the upper and lower limits of the displayed region box. The time period on display can also be adjusted by increasing or decreasing the **Display** 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. The region within the display channel state, used to compute the average state current is indicated the pair of vertical **a-a** cursors.



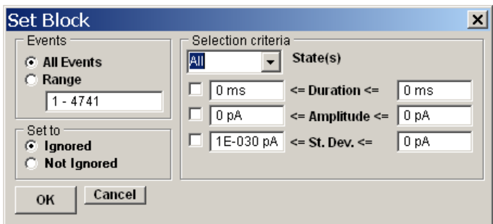
Individual channel states within the state list can be selected for display using the state selection scroll bar or by entering a state number into the box.

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 average to eliminate the parts of the signal which are in the process of transition between states.

10.3.1. Editing the channel event list



Artifactual channel states 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 set the Ignore State for a block of events, click the **Set Block** button to open the Set Block dialog box.

a) Select the type of state to be modified (**Closed**, **Open** or **All** for both types of state) from the **State** list.

b) 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.)

c) Select the **Ignore State** (Ignored, Not Ignored) setting to be applied when an event is found to match the selection criteria.

d) Select the **All Events** option to apply the criteria to all events or select **Range** and enter a specific range of events

e) 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.

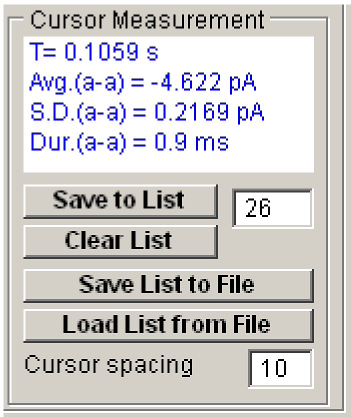
10.3.2. Exporting the list of channel states

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

a) 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).

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

10.3.3. 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.

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.

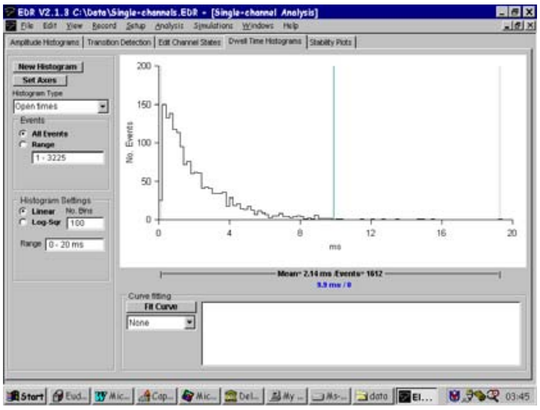
The amplitude, duration and standard deviation measurements stored in this list can be plotted (vs. time) on the Stability Plot page (See 9.5 Stability Plots). A histogram of the distribution of amplitude measurements can be plotted on the Amplitude Histograms page.

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.

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

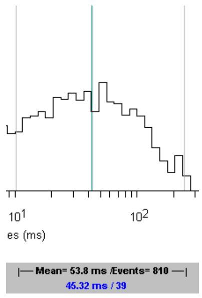


- Open times
- Closed times
- Burst Length
- Single open times
- Opens times within bursts
- Openings per burst
- External file

Open and **Closed times** histograms are simply the distribution of dwell times spent in the channel open and closed states. The **Burst length** histogram plots 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. The **Single open times** histogram plots the dwell times of single openings (ones which do not form part of a burst of openings). Conversely, the **Open times within bursts** histogram plots the dwell times of openings which are part of a burst. Finally, histograms can also be produced from list of dwell times read in from external files. Each type of histogram can be plotted using **linearly** spaced, fixed width, bins, or **logarithmically** increasing variable width bins.

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 blue readout cursor.

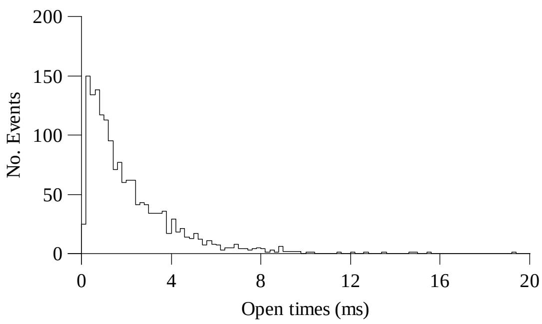
The number of events contained in the bins falling within a selected range of dwell times, and the mean dwell time of that region, can be displayed by placing the pair of grey '|--|' cursors at the limits of the region of interest.



10.4.1. *Linear histograms with fixed width bins*

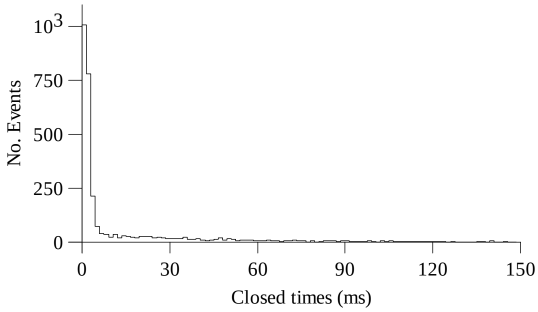
A **Linear** dwell time histogram is compiled by counting the number of state dwell times which fall into a series of equal sized time bins spread over a given range of times. Each dwell time, t_{state}, is assigned a bin number, i_{bin}, according to the formula

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



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.

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.



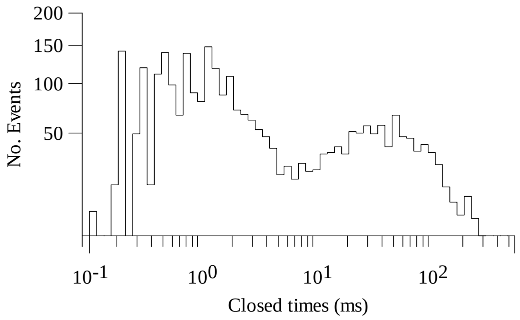
10.4.2. Logarithmic histograms with variable bin widths

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

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

where *bpd* is the number of histogram bins per decade (10-fold) change in dwell time. and n_d 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[1].

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.



[1] The rationale behind this is that standard deviation of the number of counts in a histogram bin is equal to square root of the mean bin count. Plotting using a square root scaling thus equalises bin variability between small and large bins.

10.4.3. Plotting a dwell time histogram

To plot a dwell time histogram :-

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

b) 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.

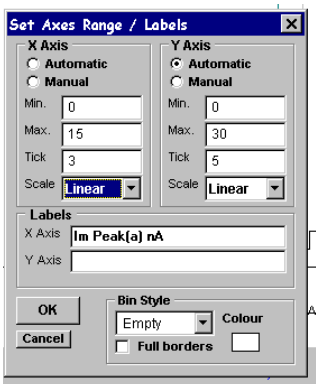
c) If Burst Length, or any of the other burst-related histogram types have been selected, enter the critical closed time value used to distinguish between intra- and inter-burst closed intervals in the **T.critical** box.

d) Enter the number of histogram bins in the **No. Bins** box.

e) Set the type of bin spacing to be used. 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. 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.

f) Click the **New Histogram** button to plot the histogram.

10.4.4. Customising histograms

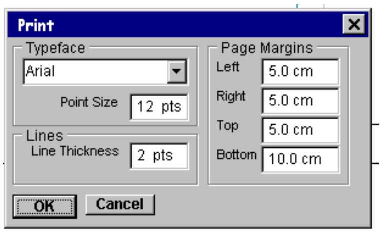


If you want to alter the X or Y axis range, scaling or labels on a histogram, click the **Set Axes** button to open **the Set Axes Range / Labels** dialog box.

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. To define the colour used for the solid fill, click the **Colour** box, and select 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.

10.4.5. Printing histograms



To print the displayed histogram, select

File
Print

To open the **Print** dialog box. Click the **OK** button to plot the graph.

10.4.6. Copying the histogram data points to the Windows clipboard

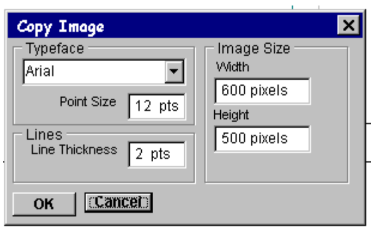
The numerical values of the X,Y data points which generate the histogram can be copied to the clipboard by selecting

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>
```

10.4.7. 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 **copy image** 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.

10.4.8. *Fitting exponential p.d.f.s to dwell time histograms*

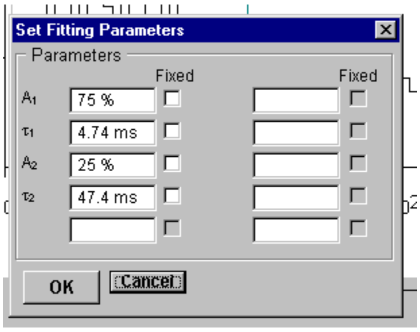
Dwell time distributions can usually be described in terms of a mixture of exponential probability density functions, where the probability *p(t)* of dwell time, *t*, being observed is given by

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

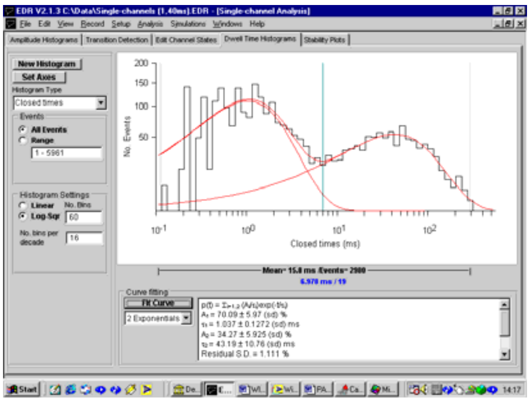
where n_{exp} is the number of exponential components in the mixture, A_i is the fraction of the total number of dwell times associated with component *i*, and τ_i is the mean dwell time. Mixtures of up to 5 exponential p.d.f.s can be to the dwell time histograms, using either an iterative least squares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

To fit an exponential mixture to a histogram :-

11) Define the range of dwell times containing the histogram bins exponential distributions to be fitted using the pair of grey '|--|' region of interest cursors.
12) Select the number of exponential components in the mixture to be fitted from the **Curve Fitting** list.
13) Click the **Fit Curve** button and enter an appropriate set of initial guesses for the exponential function parameters (A_i, τ_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 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.)



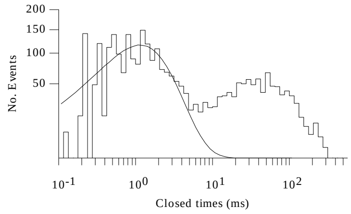
The residual standard deviation (SD_{res}) between the histogram data and the fitted curve provides a measure of the goodness of fit. The smaller the value of SD_{res} the better the fit.

Note. 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.

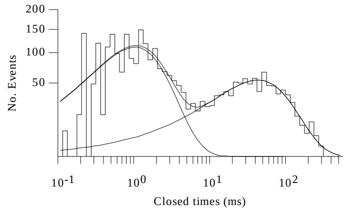
10.4.9. *Determining the required number of exponential components*

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 on the right show the results of fitting mixtures of 1, 2 and 3 exponential components.

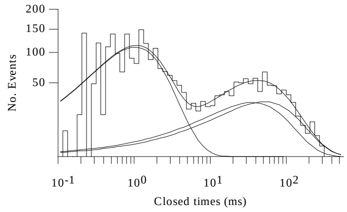
a) One exponential (SD_{res} = 1.4%)



a) Two exponentials (SD_{res} = 1.11%)



a) Three exponentials (SD_{res} = 1.13%)



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

The mixture of two exponentials (b) 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.

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_a 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.

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

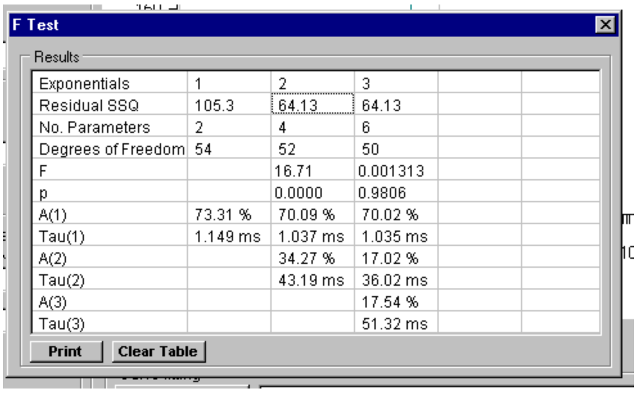
Large positive values of $F_{a,b}$ indicate that extra exponentials in function *b* result in a better fit to the data than function *a*, small positive values indicate that the fit is little improved and negative that it is worse. The significance probability of the observed $F_{a,b}$ value can be determined 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 dialog box.

The residual sum of squares, number of function parameters and degrees of freedom for each function 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 $p \leq 0.05$.



Exponentials	1	2	3		
Residual SSQ	105.3	64.13	64.13		
No. Parameters	2	4	6		
Degrees of Freedom	54	52	50		
F		16.71	0.001313		
p		0.0000	0.9806		
A(1)	73.31 %	70.09 %	70.02 %		
Tau(1)	1.149 ms	1.037 ms	1.035 ms		
A(2)		34.27 %	17.02 %		
Tau(2)		43.19 ms	36.02 ms		
A(3)			17.54 %		
Tau(3)			51.32 ms		

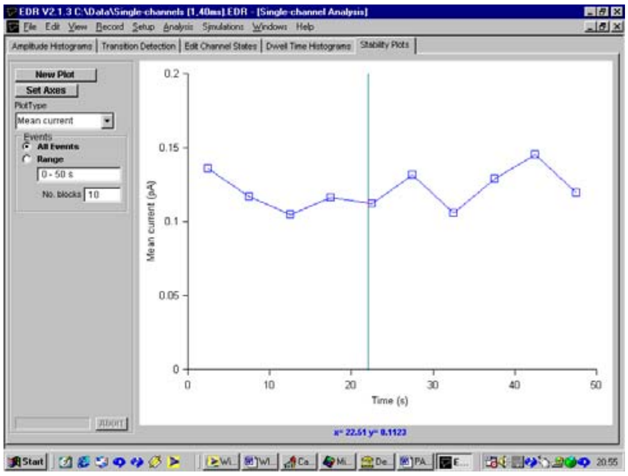
Click the **Print** button to print a hard copy of the F test results table. The table of results can be cleared by clicking the **Clear Table** button.

10.5. 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 **Stability Plots** page provides a set of tools for revealing the presence of trends in the single-channel current amplitudes or dwell times. The following parameters can be plotted

- Mean current
- Open probability
- Closed times
- Open times
- Single-channel currents
- Current vs Open times
- Cursor Meas. (Avg)
- Cursor Meas. (Dur)
- Cursor Meas. (S.D.)



The **Mean current** plot 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. The **Open probability** plot 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).

The **Closed times** and **Open times** plot displays trends in the channel dwell times in the closed and open states. The channel event list is divided into a series of contiguous blocks, the mean closed or open dwell time for each block is computed and plotted vs. the mid-point time for each block.

The **Single-channel currents** plot 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.

The **Current vs Open times** plot 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.

The **Cursor Meas. (Avg), Cursor Meas. (Dur)** and **Cursor Meas. (S.D.)** plots display (vs time) the average current, duration and standard deviation measurements stored in the the manual cursor measurements lists. (See 9.3.3. Cursor Measurements).

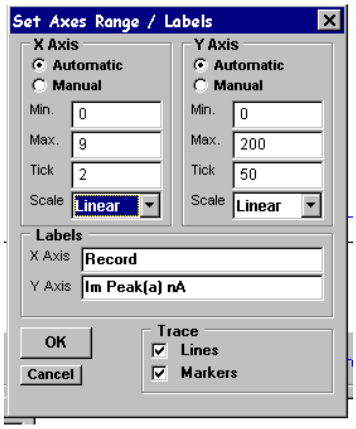
10.5.1. Creating stability plots

To create a stability plot :-

1) Select the type of plot to be created form 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.

10.5.2. Customising the plot



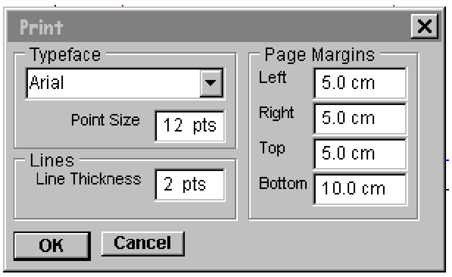
If you want to alter the X or Y axis range, scaling or labels, click the

Set Axes

button to open **the Set Axes Range / Labels** dialog box. Axis limits and tick spacing are initially set to default values based upon the range of the data. You can change the axis limits by entering new values for into **Min, Max**. and **Tick** (spacing) boxes for the X and Y axes.

An axis can be made Linear or Logarithmic by selecting the option from its **Scale** list. Labels for the X and Y axes can be entered into the **Labels** boxes. A type face can be selected for the plot from the **Font** list and its size defined in the **Point Size** box. The graph can be plotted as a line, unconnected markers, or both, by ticking the **Lines**, and/or **Markers** tick boxes.

10.5.3. Printing the plot



To print the displayed graph, select

File
Print

To open the **Print** dialog box. You can set the size of the graph on the page adjusting the **Left, Right, Top** and **Bottom** page margin settings. Click the **OK** button to plot the graph.

10.5.4. Copying the plot data points to the Windows clipboard

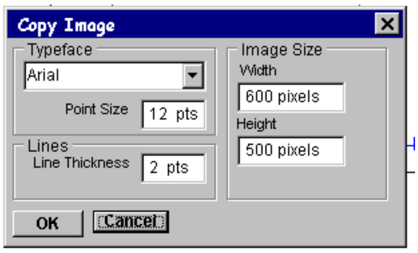
The numerical values of the X,Y data points which generate the graph can be copied to the clipboard by selecting

Edit
Copy Data

The data is placed on the clipboard as a table of X,Y data pairs in tab text format, allowing the data to be copied into programs such as spreadsheets and graph plotting packages, using an Edit/Paste command.

10.5.5. Copying an image of the plot to the Windows clipboard

An image of the graph on display can be copied to the clipboard by selecting



Edit
Copy Image

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

11. Noise analysis

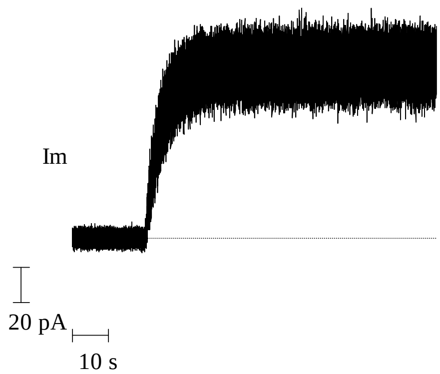
To analyse ionic current noise, select

Analysis
Noise Analysis

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

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 on the right shows a typical recording[2], Recording begins before the agonist is applied in order to obtain a sample of the background noise, the agonist is applied after 20 s and both the mean current and the amplitude of its fluctuations can be seen to increase to a steady state.



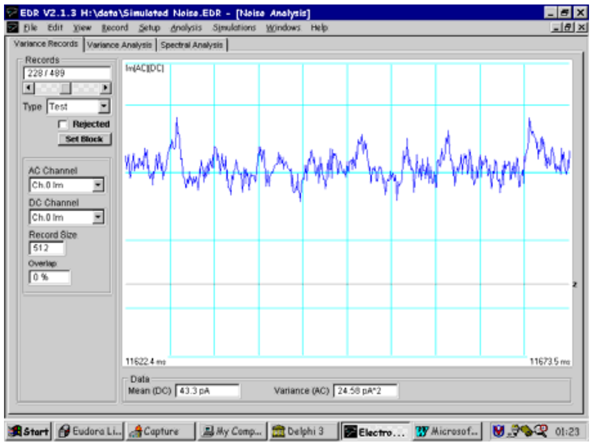
11.1.1. Signal conditioning

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 digitised it in a second input channel (See Dempster 2001).

[2] Created using the Ion channel noise simulation option

11.2. Selection and 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 displayed traces can be magnified vertically by double-clicking the panel to switch into the display "zoom" mode, and adjusting the upper and lower limits of the displayed region 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 :-

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

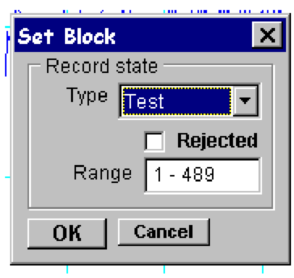
b) If the current noise has been acquired in an additional high-gain, AC-coupled channel, separate from the DC-coupled channel, select this channel with the **AC Channel** list.

c) 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.

d) [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.

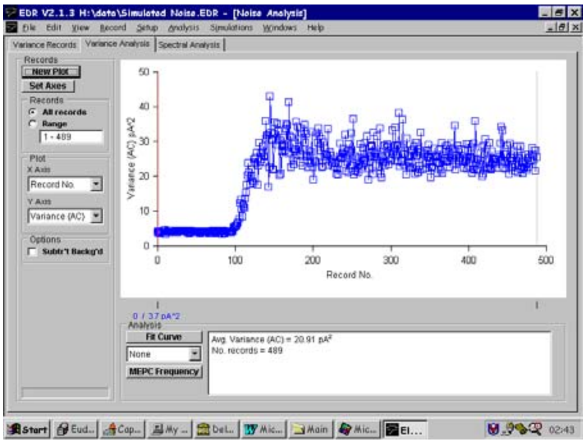
e) Inspect each individual variance record and reject those containing artefacts from analysis by ticking the **Rejected** tick box.

f) 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 processes 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.



g) 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.

11.3. 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.

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, σ^2, computed using the formula

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

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

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

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

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.)

To select and plot a pair of variables :-

a) Define the variable to be plotted on the X axis, by selecting it from the **X Axis** variable list.

b) Define the variable to be plotted on the Y axis, by selecting it from **Y Axis** list.

c) 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.)

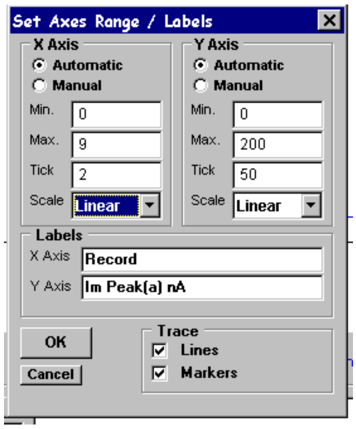
d) Select the **Subtr't Backg'd** option to subtract the average variable value within the Background records from the plotted data

e) Click the **New Plot** button to plot the graph.

11.3.1. Customising the plot

If you want to alter the X or Y axis range, scaling or labels, click the

Set Axes



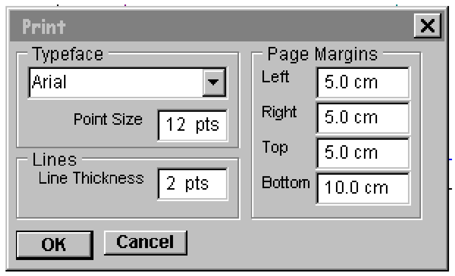
button to open **the Set Axes Range / Labels** dialog box. Axis limits and tick spacing are initially set to default values based upon the range of the data. You can change the axis limits by entering new values for into **Min, Max**. and **Tick** (spacing) boxes for the X and Y axes.

An axis can be made Linear or Logarithmic by selecting the option from its **Scale** list. Labels for the X and Y axes can be entered into the **Labels** boxes. A type face can be selected for the plot from the **Font** list and its size defined in the **Point Size** box. The graph can be plotted as a line, unconnected markers, or both, by ticking the **Lines**, and/or **Markers** tick boxes.

11.3.2. Printing the plot

To print the displayed graph, select

File
Print



To open the **Print** dialog box. You can set the size of the graph on the page adjusting the **Left, Right, Top** and **Bottom** page margin settings. Click the **OK** button to plot the graph.

11.3.3. Copying the plot data points to the Windows clipboard

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

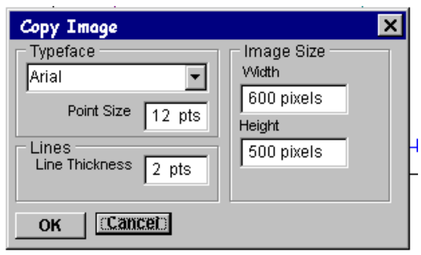
Edit
Copy Data

The data is placed on the clipboard as a table of X,Y data pairs in tab text format, allowing the data to be copied into programs such as spreadsheets and graph plotting packages, using an Edit/Paste command.

11.3.4. Copying an image of the plot to the Windows clipboard

An image of the graph on display can be copied to the clipboard by selecting

Edit
Copy Image



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

11.3.5. Variance-mean current plots

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

$$I_m = I_u \cdot n_c \cdot p \qquad 10.1$$

The variance of the current fluctuations is

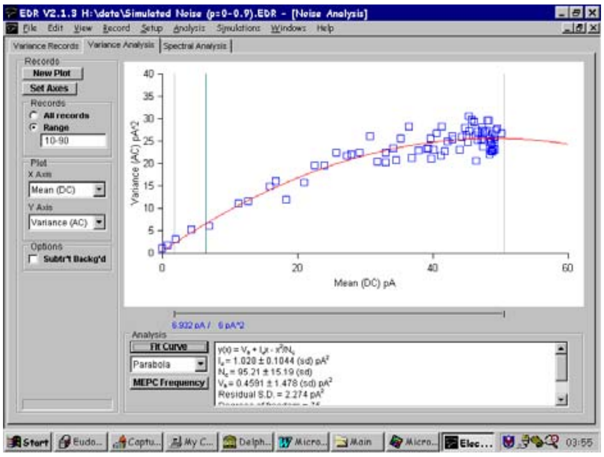
$$\sigma^2 = I_u{}^2 \cdot n_c \cdot p \cdot (1 - p) \qquad 10.2$$

These two equations can be combined to provide a relationship between σ^2 and I_m,

$$\sigma^2(I_m) = I_u \cdot I_m - \frac{I_m{}^2}{n_c} \qquad 10.3$$

Estimates of I_u and n_c can thus be obtained from plots of current variance vs. mean current by fitting eqn. 10.3 using non-linear curve fitting.

To plot a variance-mean current curve and fit eqn. 10.3 :-



a) Select a range of variance records from a period of the recording where the mean current is changing.

b) Select **Mean Current (DC)** from the **X Axis** list and **Variance (AC)** from the **Y Axis** list.

c) Click the **New Plot** button to plot the variance-mean graph.

d) Select the region of the graph to fitted, using the |--| analysis region cursors.

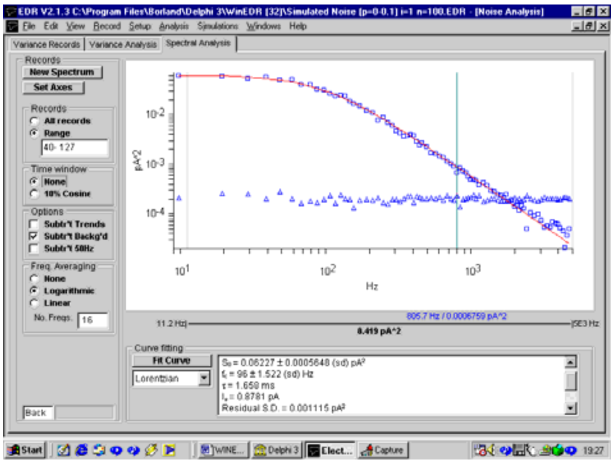
e) Select **Parabola** from the Fit Curve list to fit eqn. 10.3 to the plot.

f) 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.

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).

11.4. Averaged Power Spectra



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

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

To plot a power spectrum :-

a) 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.)

b) 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.

c) 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.

d) 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).)

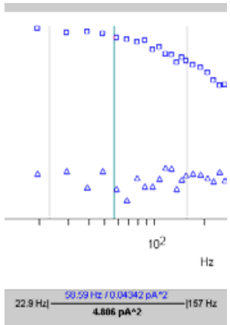
e) Tick the **Subtr't Trends** option to remove any linear trends from the data points within each variance record before computation of its power spectrum.

f) Tick the **Subtr't 50 Hz** option to delete frequencies around 50 Hz from the spectrum to remove 50 Hz interference.

g) 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.



11.4.1. *Fitting Lorentzian functions to power spectra*

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

$$S(f) = \frac{S_0}{1 + (f / f_c)^2} \qquad 10.4$$

At low frequencies, the function tends to a constant value, S_0 (units I^2/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, τ, and the mean open and closed times, t_{open}, t_{closed}, by the formula,

$$\tau = \frac{1}{2\pi \cdot f_c} = \frac{1}{1/t_{open} + 1/t_{closed}} \qquad 10.5$$

If $t_{closed} >> t_{open}$ 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. $p \le 0.1$), the single-channel current and conductance can also be computed from the power spectrum. For small *p* values the second term in the eqn. 10.3 can be neglected leading to a direct relationship between current variance and mean current

$$\sigma^2 = I_u \cdot I_m \qquad 10.6$$

Since the spectrum is the frequency distribution of the variance, σ^2 can be calculated from the integral of the Lorentzian function.

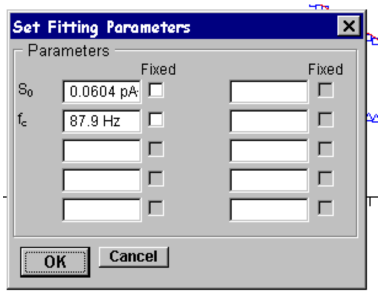
$$\sigma^2 = \frac{f_c \cdot S_0}{2} \qquad 10.7$$

By combining eqns. 10.6 and 10.7 the single-channel conductance can be calculated from the Lorentzian curve fitted to the ionic current power spectrum.

$$I_u = \frac{f_c \cdot S_0}{2 \cdot I_m} \qquad 10.8$$

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

To fit a Lorentzian function to a power spectrum :-



a) Select the region of the spectrum to fitted, using the |--| region of interest cursors.

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

c) 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, τ, computed from eqns. 10.5 and 10.8, are also displayed.

11.4.2. Miniature endplate current noise

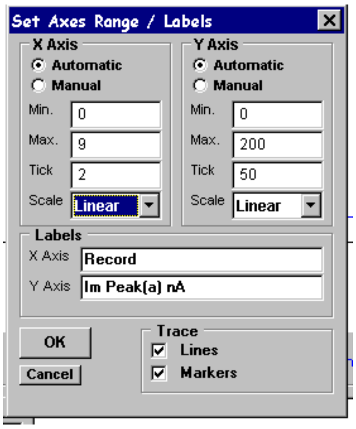
It is also possible to analyse the current fluctuations associated with the miniature endplate currents under conditions of the high frequency release evoked by increasing the K ion concentration of the bathing solution. The MEPC noise spectrum can be modelled with the three parameter (F_r, F_d , A)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

$$S(f) = \frac{A}{\left(1/F_d + 1/F_r\right) f^2 + f^4 / \left(F_r^2 \cdot F_d^2\right)} \qquad 10.8$$

Eqn. 10.8 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, τ_r and τ_d, can be computed from these.

After the τ_r and τ_d, time constants have been established by fitting eqn. 10.8, 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).

11.4.3. *Customising the spectrum*



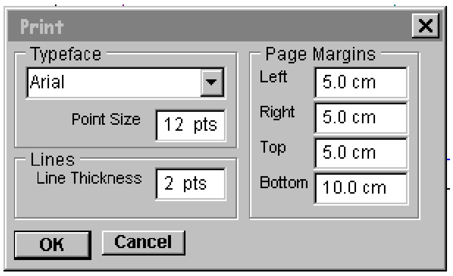
If you want to alter the X or Y axis range, scaling or labels, click the

Set Axes

button to open **the Set Axes Range / Labels** dialog box. Axis limits and tick spacing are initially set to default values based upon the range of the data. You can change the axis limits by entering new values for into **Min, Max**. and **Tick** (spacing) boxes for the X and Y axes.

An axis can be made Linear or Logarithmic by selecting the option from its **Scale** list. Labels for the X and Y axes can be entered into the **Labels** boxes. A type face can be selected for the plot from the **Font** list and its size defined in the **Point Size** box. The graph can be plotted as a line, unconnected markers, or both, by ticking the **Lines**, and/or **Markers** tick boxes.

11.4.4. *Printing the spectrum*



To print the displayed graph, select

File
Print

To open the **Print** dialog box. You can set the size of the graph on the page adjusting the **Left, Right, Top** and **Bottom** page margin settings. Click the **OK** button to plot the graph.

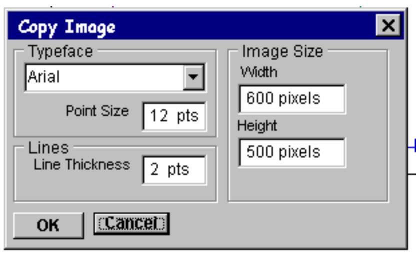
11.4.5. *Copying the spectral data points to the Windows clipboard*

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

Edit
Copy Data

The data is placed on the clipboard as a table of X,Y data pairs in tab text format, allowing the data to be copied into programs such as spreadsheets and graph plotting packages, using an Edit/Paste command.

11.4.6. *Copying an image of the spectrum to the Windows clipboard*



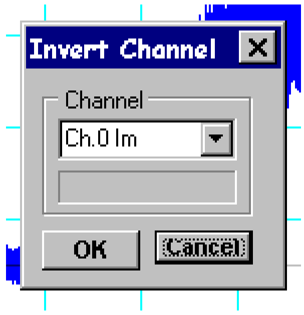
An image of the graph on display can be copied to the clipboard by selecting

Edit
Copy Image

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

12. Signal Processing

12.1. Inverting a signal channel.



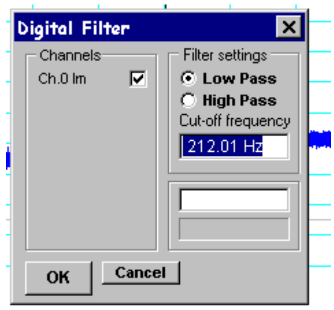
To invert the sign of the digitised data within a signal channel, select

Analysis
Invert Channel

to open the Invert Channel dialog box.

Select the channel to be inverted from the **Channel** list then click the **OK** button.

12.2. Digital low- and high-pass filtering



To apply digital low- or high-pass filtering to one or more channels of the digitised recording, select

Analysis
Digital Filter

to open the Digital Filter dialog box.

a) Select which channels are to be filtered by ticking/unticking each channel's tick box in the **Channels** box.

b) Select the **Low Pass** option to smooth the digitised signal(s) by applying a gaussian low pass filter.

OR

Select the **High Pass** option to remove low-frequency drift by applying a high pass filter.

c) Enter the filter cut-frequency in the **Cut-off frequency** box.

d) Click the **OK** button to apply the filter to the selected digitised signal channels.

Note. The digital filter algorithm is based upon the gaussian filter described in Colquhoun & Sigworth (1995).

12.3. Restoring the original digitised signal

When one of the above signal processing operations is applied to a digitised signal, a copy of the original recorded signal is stored in a back up file (same name as data file, but with a ".bak" file extension.

To restore the original data file, select

File
Restore Original Data

13. Data files

WinEDR uses its own custom data file format for storing digitised signal records. These files are identified by the file extension ".EDR" Data can also be imported from and exported to files in the Axon Binary Format (used by Axon Instruments' pClamp program) and the Cambridge Electronic Design CFS (CED Filing System) formats. Data can also be imported and exported in the form of ASCII text.

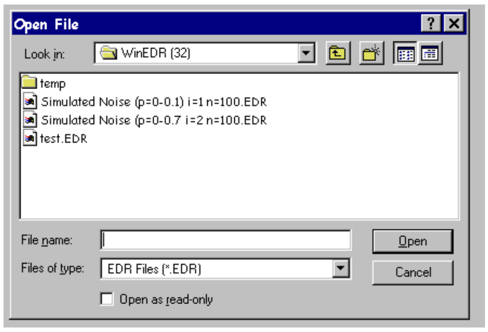
13.1. Opening a existing EDR data file

To load a previously created EDR data file, select

File
Open

to display the **Open File** 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.

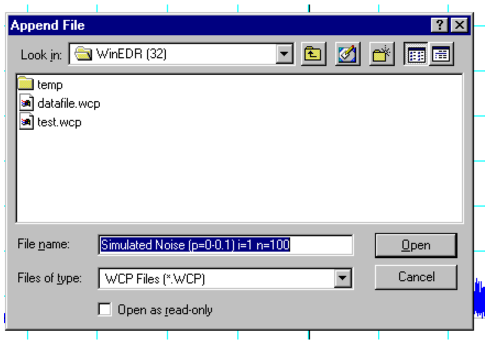


13.2. Appending a WCP data file

To append a WCP data on to the end of the currently open file, select

File
Append

To display the **Append File** dialog box. Select a file (as above) for appending. (**Note.** You can only append files, which have compatible numbers of channels and sampling intervals.)



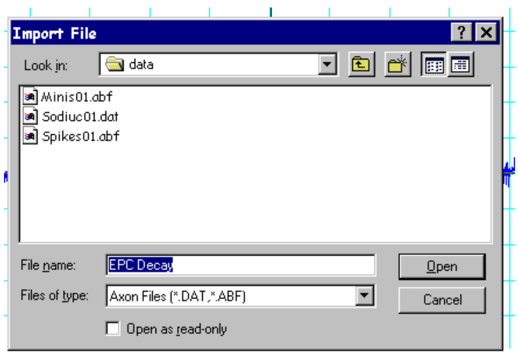
13.3. Importing from foreign data file formats

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

File
Import

To display the **Import File** 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 .EDR 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").

13.3.1. Axon Instruments.

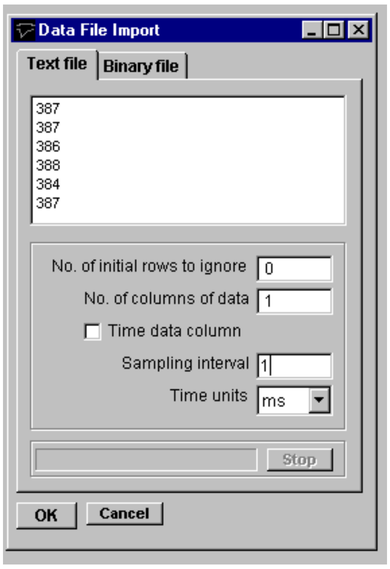
Data files produced by Axon Instruments' pClamp V5 and V6 programs. The files should contain episodic data records, such as created by the CLAMPEX program. Axon data files have a .DAT file extension.

13.3.2. Cambridge Electronic Design

Data files produced by CED's Voltage & Patch Clamp program, in the CFS (CED Filing System) format. CFS files have a .DAT file extension.

13.3.3. ASCII text files.

Files with the sample values stored as ASCII text in tab text format. The **Data File Import** dialog box allows you to view the format of the ASCII data to be imported and to specify how it should be imported.



The data must consist of rows of samples (each row terminated by a carriage return + line feed pair of characters (<cr> <lf>). Channel sample values within each row are separated by <tab> characters.

The number of signal channels in the record to be created is determined from the number of columns in the table, with one of the columns (usually the first) assumed to contain the time.

The first data row(s) in ASCII 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 initial rows to ignore**.

By default, column 1 is assumed to contain the time that each block of samples was acquired. If there is no time data at all, untick **Time data in column 1** option.

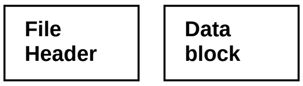
The sampling interval is derived from the times of adjacent rows. If there is no time data (or if the displayed interval is wrong) enter the correct value in the **Sampling interval** box.

Select the units that the time data is expressed in from the **Time units** list.

Click the **OK** button, to import the data when the import settings are complete.

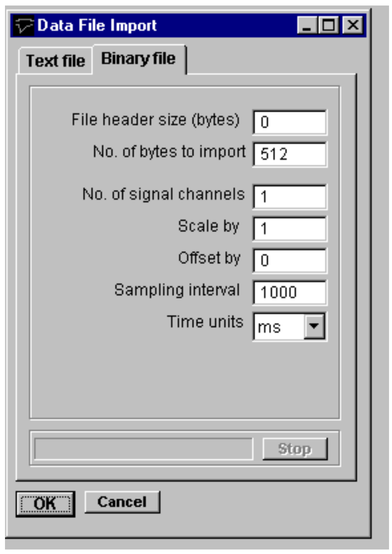
13.3.4. Binary data files

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.)



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

Enter the size of the file header in the **File header** box. (If no file exists, set this value to zero.)

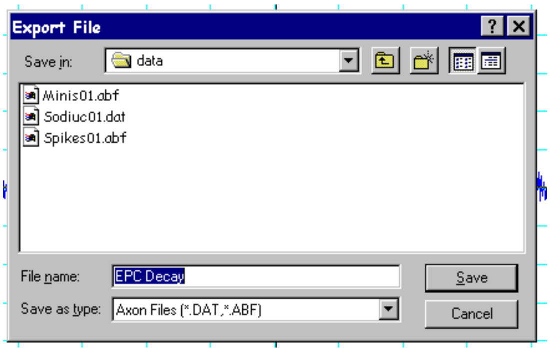
Enter the number of input channel in **the No. of signal channels** box.

The WinEDR data file format, stores sample values as, 12 or 16 bit binary integer numbers. It may be necessary to scale or add an offset to each sample to transform it to be compatible with the WinEDR format. To scale the signal, enter the scaling factor into the **Scale by** box. To add an offset to the signal, enter it into the **Offset by** box.

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.

Click the **OK** button, to import the data when the import settings are complete.

13.4. Exporting to foreign data files



EDR data files can also be exported a number of data file formats. To export the currently open data file, select

File
Export

To open the **Export file** dialog box.

Select the disk drive and folder where the exported file is to be stored from the **Look In** list. The export file name is initially set to the same name as the WCP file, but with a .DAT file extension.

Files can currently be exported in Axon Instruments data file formats.

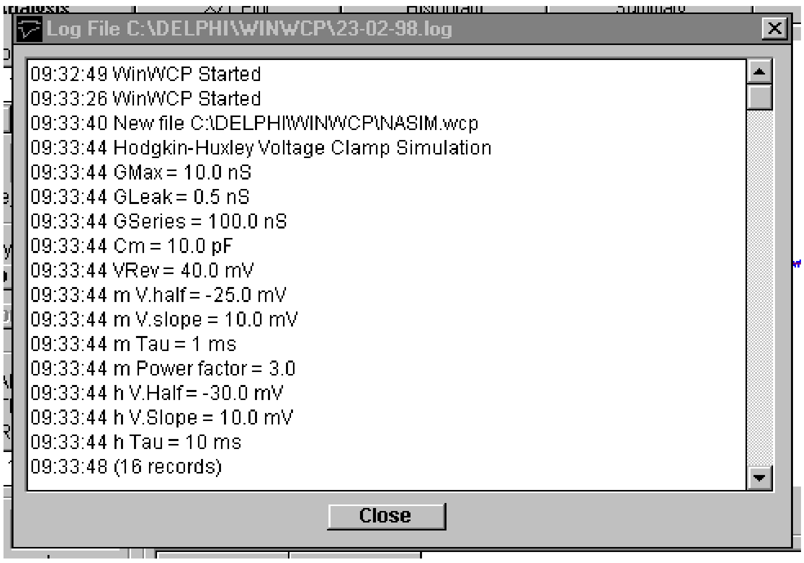
13.5. Experiment Log file

WinEDR maintains a log file of the operations initiated by the user 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.

To display the experimental log, select

File
Inspect Log File



14. Simulations

The simulation modules can be used to generate data files containing simulated waveforms, with known characteristics, which can be used to test the operation of the measurement and analysis modules. Three kinds of waveform can be simulated: miniature post-synaptic currents, ionic current noise and single-channel currents.

14.1. 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,

$$C_o \Leftrightarrow O \Leftrightarrow C_1$$

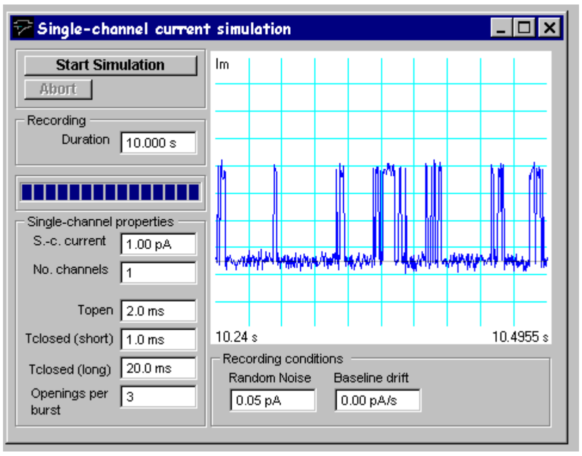
Channel openings occur in bursts with the kinetics of the channels determined by the mean dwell times in each of the 3 states.

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

a) Create a new data file to hold the currents, by selecting
File
New
and entering the name of a new data file.

b) Open the simulation module, by selecting

Simulations
Single-channel currents



c) Enter the single-channel current amplitude in the **S-c current** box.

d) Enter the number of active ion channels in the patch in the **No Channels** box.

e) Enter the mean channel open time in the **Topen** box.

f) Enter the mean number of channel opening per burst of openings in **the Openings per burst** box.

g) Enter the mean intra-burst closed time in the **Tclosed (short)** box and the mean inter-burst closed time in the **Tclosed (long)** box.

h) Enter the standard deviation of the background noise in the **Random Noise** box.

i) [Optional] To apply a linear trend to the signal baseline level enter a non-zero value into the **Baseline drift** box.

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

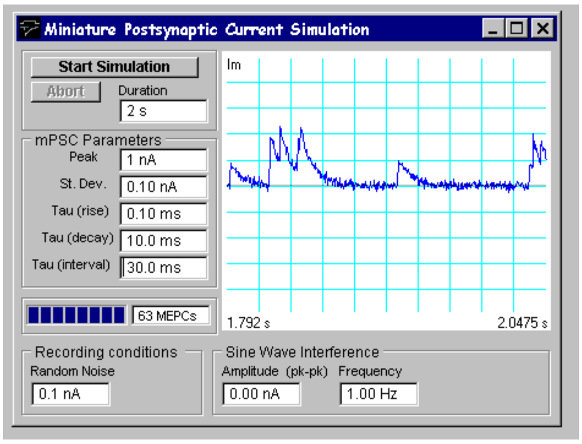
14.2. Miniature post-synaptic currents simulation

The miniature post-synaptic current module generates series of randomly occurring miniature postsynaptic currents (mPSCs) which can be used to test WinEDR's signal detection module (See ?) and the MEPC frequency estimation procedure in the noise analysis module (See ?). The mPSCs are modelled as signals with a rapid exponential rise and slower exponential decay, occurring at random times 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 :-

a) Create a new data file to hold the records, by selecting
File
New
and entering the name of a new data file.

b) Open the simulation module, by selecting
Simulations
Miniature post-synaptic currents



c) Enter time period over which mPSCs are to be generated in the **Duration** box.

d) Enter the mPSC peak amplitude in the **Peak** box.

e) Enter the standard deviation of the inter-event variation in mPSC peak amplitude in the **St. Dev**.

f) Enter the time constant of the mPSC rising phase in the **Tau (rise)** box.

g) Enter the time constant of the mPSC decay phase in the **Tau (decay)** box.

h) Enter the mean interval between the mPSCs in the **Tau (interval)** box.

i) Enter the standard deviation of the signal background noise in the **Random Noise** box.

j) [Optional] To low-pass filter the signals is to be applied to the mEPSC, select the **Low-p filter On** option and enter the cut-off frequency in the box.

k) [Optional] 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.

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

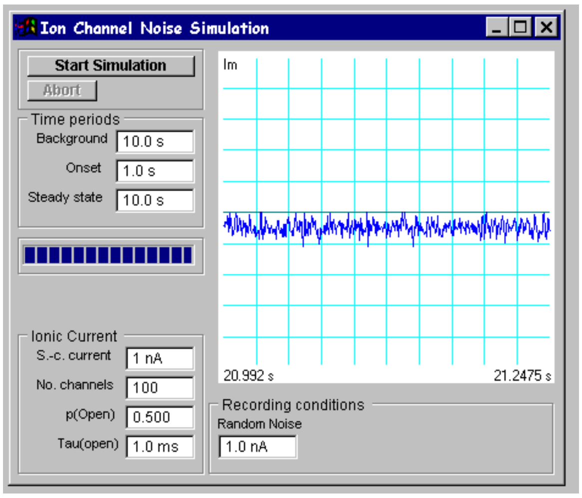
14.3. Ion channel noise simulation

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 :-

a) Create a new data file to hold the records, by selecting
File
New
and entering the name of a new data file.

b) Open the simulation module, by selecting
Simulations
Ion Channel Noise



c) Enter the duration of the initial period before the application of agonist, during which background noise is recorded in the **Background** box

d) Enter the onset time during which agonist concentration is rising in the **Onset** box.

e) Enter the period of time that the agonist concentration is in a steady-state in the **Steady-state** box.

f) Enter the single-channel current amplitude in the **S-c current** box.

g) Enter the number of ion channels in the cell in the **No Channels** box.

h) Enter the steady-state probability of a channel begin open in the **P(Open)** box.

i) Enter the mean channel open time in the **Tau (open)** box.

j) Enter the standard deviation of the signal background noise in the **Random Noise** box.

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

15. 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")
```

15.1. 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)

15.2. 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 (Ω) measurement.

A file (WinEDR VBSCRIPT Example.vbs)containing VBSCRIPT example code can be found in the c:\winedr folder.

16. References

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17. Appendix: EDR data file structure.

This appendix provides a specification of the internal structure of the EDR data file. 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.

A **header block** at the beginning of the file is 2048 bytes in length and 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)
YO0=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)
YO1=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

`DETCH=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)
`DETDD=5-E2 <cr><lf>`	Event detector dead time (s)
`DETBA=5E-2 <cr><lf>`	Event detector.baseline averaging time (s)
`DETPT=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

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

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

$$Y0_1\ Y1_1\ Y0_2\ Y1_2\ .....\ Y0_{nsamples}\ Y1_{nsamples} \qquad \text{A.3}$$

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,

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

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.