AxoGraph X
Data Acquisition Manual

PLEASE NOTE:

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- Alternatively navigate using the table of contents on following pages. Clicking on an item of interest will jump to the relevant page of the main text.

Chapters

Contents

1 Introduction

1.1 Explore the Acquisition Software Without a Digitizer

The AxoGraph data acquisition package can run in demo mode and generate simulated electrical signals. This permits the various acquisition programs and options to be explored.

The installer named 'Install AxoGraph 4.9 Demo' installs and preloads the acquisition software package. The acquisition programs can only run in demo mode.

The installer named 'Install AxoGraph 4.9' does not preload the acquisition software package. It needs to be loaded manually, as follows…

Open the AxoGraph 4.9 folder, then the Data Acquisition Package folder.
Move the Acquisition Programs Demo folder into the Plug-In Programs folder.
Launch the AxoGraph 4.9 application.

The installer named 'Install AxoGraph 4.9 + Acq' installs and preloads a fully functional acquisition software package. If no digitizer is connected, the acquisition programs revert to demo mode.

Skip to section 2.2 for more detailed information on the acquisition programs.

1.2 Hardware Requirements for Data Acquisition

Data is acquired to memory, so a minimum of 32 MByte RAM is recommended for episodic data acquisition, and 64 MByte for continuous acquisition at a high sampling rate (see Section 4.1 for additional information on memory requirements).

AxoGraph runs on all Power Macintosh computers, and on older Macs with a 680x0 CPU and an FPU. Data acquisition requires a Digidata 1320 series, or an Instrutech ITC-16 or ITC-18 digitizer. The Digidata 1320 consists of an external unit that connects to the Mac via a SCSI cable. Newer PowerMacs that do not have a built-in SCSI bus (G4 and newer) will also require the installation of a SCSI card into a PCI bus slot inside the computer. The Digidata ships with a SCSI card. The ITC-16 and ITC-18 digitizers consist of an external rack-mounting data acquisition unit and a bus interface card which plugs into a slot inside the computer. For the ITC-16, interface cards are available for both NuBus and PCI bus computers. The ITC-18 is only available with a PCI bus card. For more information on digitizer hardware, visit….

Digidata 1320	http://www.axon.com/CN_Digidata1320.html
ITC-16 and ITC-18	http://www.instrutech.com/

Summary of hardware requirements

Machine	Digitizer	Interface Card
68K Mac	ITC-16	NuBus
PowerMac prior to G4*	ITC-16, ITC-18	PCI Bus
	Digidata 1320 series	not required – uses built-in SCSI
Power Mac G4 and newer	ITC-16, ITC-18	PCI Bus
	Digidata 1320 series	SCSI card (shipped with Digidata)

* Very early PowerMacs prior to the 7100 model had a NuBus, not a PCI bus. Check the bus type of any PowerMac 6000 or 7000 series computer before purchasing an Instrutech digitizer interface card.

Memory Recommendations
32 MB RAM or more for episodic acquisition
64 MB RAM or more for continuous acquisition
Virtual memory is not recommended when running data acquisition package

1.3 Install Acquisition Hardware and Software

This section describes five steps required to set up a computer for data acquisition…

(1) Connect a digitizer to the computer
(2) Load the data acquisition software
(3) Test the software installation
(4) Connect electronic equipment to the digitizer
(5) Describe the connections

(1) Connect a digitizer to the computer

Always shut down the computer before connecting a digitizer.

To connect an Instrutech ITC-16 or ITC-18 digitizer:
An interface card and connecting cable are supplied with the Instrutech digitizer
Open the computer case and install the interface card into an internal slot
(usually a PCI bus slot, but could be a NuBus slot in an old 680x0 Mac)
Close the case and connect the cable between the interface card and the digitizer
Plug in and switch on the digitizer
Power up the computer

To connect an Axon Digidata 1320 series digitizer:
An ‘AdvanSys’ PCI to SCSI card and a SCSI cable are supplied with the Digidata
Open the computer case and install the SCSI card into an internal PCI slot
Close the case and connect the supplied SCSI cable from the card to the Digidata
Plug in and switch on the Digidata
Power up the computer
Insert the AdvanSys CD, and run the installation software

Alternative connection strategy for Axon Digidata 1320 series digitizer
All Mac models prior to the G4 have a built-in SCSI port at the rear of the computer. It is possible to connect a Digidata 1320 directly to this port, or to daisy-chain it with an external hard drive. However, the cable supplied with the Digidata will not connect to the Mac’s built-in port, because it uses a

different physical format. Cables and adapters for connecting between the three main SCSI port formats are available at computer shops.

(2) Load the data acquisition software

There are two different installers for AxoGraph 4.9.

• 'Install AxoGraph 4.9 + Acq' installs a fully functional acquisition package and preloads the package. The acquisition programs are automatically loaded into toolbars and the Program menu.

If an item named Acquisition appears under the Program menu, then the acquisition package is preloaded. The acquisition programs can be run by clicking toolbar buttons at the bottom of the screen, or from submenus of the Program menu.

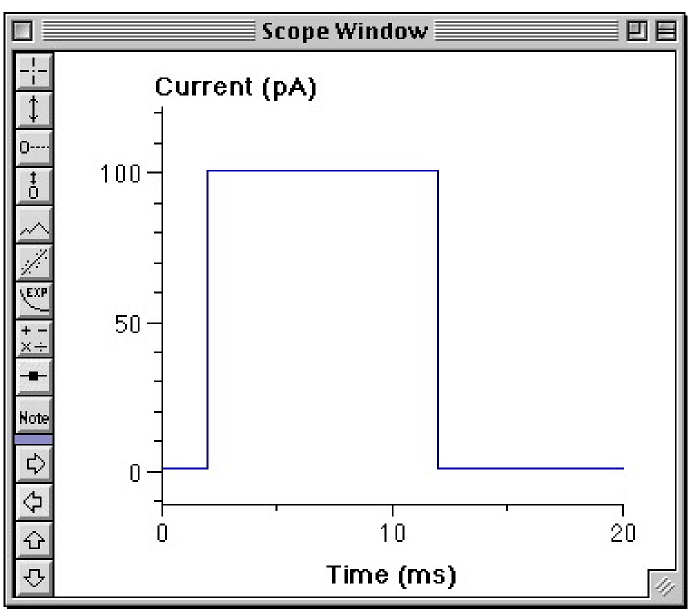
• 'Install AxoGraph 4.9' installs only a demo version of the acquisition software and does not preload the acquisition package. The acquisition package will need to be loaded manually, as described below.

If the acquisition package is not preloaded, it can be manually loaded as follows.
Open the AxoGraph 4.9 folder, then the Data Acquisition Package folder.
Move the Acquisition Programs Demo folder into the Plug-In Programs folder.
Launch the AxoGraph 4.9 application.

(3) Test the software installation

This section assumes that the AxoGraph data acquisition software has just been loaded for the first time. If a digitizer has not been installed, skip to step (c).

(a) Make sure the digitizer is plugged in and the power is on.
(b) Connect a cable from **Analog Input 0** to **Analog Output 0**.
(c) Launch AxoGraph. A toolbar will appear at the bottom of the screen.
(d) Change the toolbar popup menu setting to Scope.
(e) Click on the Run Scope button in the toolbar.



A **Scope Window** will appear with a live, updating trace containing a square pulse.

If AxoGraph can not communicate with the digitizer, or if initialization of the digitizer hardware fails, an alert will appear asking you to check that a digitizer is connected to the computer, and that it is switched on. When the dialog is dismissed demo mode will be initiated, and a **Scope Window** with a simulated trace will appear.

If the acquisition software is running in demo mode, skip to section 2.2.

(4) Connect electronic equipment to the digitizer

After the digitizer has been installed and tested, connect cables from the electronic equipment to the digitizer. The digitizer has BNC connectors that are grouped and labeled as follows.

Connectors for…	Digidata 1320 Label	ITC-16 and ITC-18 Label
Analog input signals	ANALOG IN	ADC INPUT
Analog output signals	ANALOG OUT	DAC OUTPUT
Digital input signals	DIGITAL IN	TTL INPUT
Digital output signals	DIGITAL OUT	TTL OUTPUT
Triggering acquisition	TRIGGER IN	TRIG IN

This manual will describe these connectors using the generic terms **Analog Input**, **Analog Output**, **Digital Input**, **Digital Output** and **Trigger Input**.

Connect cables carrying voltage signals that are to be displayed and recorded to the **Analog Input** connectors. Use the **Analog Output** connectors to deliver command voltages that will drive or trigger the electronic equipment. The **Digital Input** connectors can be used to receive 'logic' (false / true) signals which are either 0 or 5 V. The **Digital Output** connectors can be used to deliver logic signals which step between 0 and 5 V. **Digital Outputs** are useful for triggering an instrument, turning a signal on and off, or for delivering timing signals to synchronize the equipment.

(5) Describe the connections

AxoGraph requires information about the connections made to the input and output channels of the digitizer. Specifically, it requires a signal name for each connection, and the signal's units and gain for each analog connection. To enter this information…

(a) Change the toolbar popup menu setting to Configuration.
(b) Click on the Full Configuration button in the toolbar.

A series of dialogs will appear. Each of these dialogs is described in greater detail in Chapter 2. Please skip to Chapter 2 if the following outlines are too terse.

- The first series of dialogs ask which input and output channels on the digitizer are being used. Turn on the check box next to each group of channels then each individual channel that has a cable connected.

- The next dialog is only relevant to electrophysiologists using a patch clamp or microelectrode amplifier from Axon Instruments, Dagan or Warner. AxoGraph can read and interpret the telegraph signals (gain, filter frequency, etc.) that are output by these amplifiers. If one or more amplifiers is connected, then several additional dialogs will appear asking which digitizer channels the telegraph signals are connected to.

- The next few dialogs request signal names and units for the input and output channels that have cables connected. The names should clearly distinguish between the various channels, but should not be too long, because they will be used to label the axes of the data window. If the same name is given to two different channels, AxoGraph will report an error and repeat the series of dialogs. The signal units should be given in the most convenient form. For example, if an instrument is measuring a small current then the most convenient units for the corresponding **Analog Input** channel may be 'nA' (nanoAmps). If an **Analog Output** is controlling a fine mechanical positioner, then most convenient units for this channel may be 'µm' (micrometers).

- The next two dialogs request the gains for the **Analog Input** and **Analog Output** channels. The gains can be specified in either of two different ways. The user can choose the most convenient format. The options are…
  (a) milliVolts at the digitizer per unit at the electronic equipment, or
  (b) units at the electronic equipment per Volt at the digitizer.

  For example...

  An amplifier generates an output signal of 10 mV per nA.
  The gain will be either,
  10 mV at the **Analog Input** per nA at the amplifier, or
  100 nA at the amplifier per Volt at the **Analog Input**.

  A mechanical translator generates a movement of 0.4 µm per mV of command signal.
  The gain will be either,
  2.5 mV at the **Analog Output** per µm at the translator, or
  400 µm at the translator per Volt at the **Analog Output**.

- The next dialog requests the holding levels for the **Analog Output** channels. Each **Analog Output** will be set to the holding level when AxoGraph is launched, and returned to the holding level between periods of data acquisition. In general, all holding levels should be set to zero.
- The next dialog asks whether the user will ever switch a patch clamp amplifier between voltage-clamp and current-clamp modes. If the answer is 'yes' then a series of dialogs is present that simplifies the task of switching the amplifier clamp mode. If the answer is 'no', then the configuration procedure is complete.

The connections to the digitizer are now fully described. Some additional configuration may be required before running the data acquisition programs (sampling rate, trigger mode, etc.). Chapters 3 to 9 contain a detailed description and guide to all the data acquisition programs. Section 1.5 provides a brief overview of the features available in these programs.

1.4 Trouble-Shoot Hardware and Software Installation

This section presents some common problems and possible solution…

Problem: At start-up AxoGraph reports that it can not find the digitizer
Most probable reason: digitizer has not been correctly installed and connected.
Shut down the computer.
Check that the cable between the digitizer and computer is securely plugged in at both ends.
Check that the digitizer power cable is securely connected.
Make sure the digitizer is switched on, and its front power light is on.
Reboot the computer.
If a Digidata 1320 series digitizer is connected:
  Launch the SCSI monitoring application 'SCSIProbe' that was installed from the AdvanSys CD.
  At the top of the SCSIProbe window there is a popup menu labeled 'SCSI Buses'
  Click on this popup menu. In the list there should be an item labeled 'AdvanSys…'
    If this item does not appear, then the AdvanSys PCI -> SCSI card
    has not been installed correctly, or is not working correctly.
  Select the item labeled 'AdvanSys…'
  A 'Digidata 1320' item should now appear in the list labeled 'SCSI Devices'
    If this item does not appear, then there is a problem with the connection
    to the Digidata, or with the Digidata hardware.
  If the 'Digidata 1320' item does appear then all the hardware is correctly installed and connected.

Problem: AxoGraph data acquisition features are not available under the Program menu
Most probable reason: the AxoGraph data acquisition package is not loaded.
Open the AxoGraph 4.9 folder.
Open the folder named Plug-In Programs and confirm that the Acquisition Programs folder is not present.
Search for this folder, then move it into the Plug-In Programs folder
If the Acquisition Programs folder is already present, then there may be more than one copy of AxoGraph installed on the computer.
Delete the other copies of AxoGraph.
Alternatively, make sure that all AxoGraph aliases point to the latest version.
Launch AxoGraph 4.9
The acquisition programs should now appear under the Program menu.

Problem: AxoGraph data acquisition programs only run in demo mode
Most probable reason: either the digitizer has not been correctly installed and connected (see above), or the demo version of the data acquisition package is loaded.
To check whether the demo acquisition package is loaded, open the AxoGraph 4.9 folder.
Open the folder named Plug-In Programs and confirm that the Acquisition Programs Demo folder is present.
Drag this folder out of the Plug-In Programs folder.
Search for the folder named Acquisition Programs, then move it into the Plug-In Programs folder.
Launch AxoGraph 4.9
A text box will appear saying that the programs are loading.
If no dialog box appears as the programs are loading, then the acquisition package has found and initialized the digitizer.

Problem: data acquisition programs run, but do not display the correct signal
Most probable reason: the digitizer is not correctly connected to the electronic equipment, or the acquisition package is not correctly configured, or the digitizer is not working correctly.

It is important that the electronic equipment and the digitizer reference their electrical signals to the same ground. This is not usually a problem as most equipment uses the BNC cable outer shield as the signal ground. However, some amplifiers output a signal that is not referenced to the BNC cable shield, but to a separate signal ground. They have an external connector (usually labeled ‘signal ground’) that must be connected to the same ground as the digitizer. Instrutech digitizers have an external connector labeled ‘signal ground’ for this purpose. The Axon Digidata uses the BNC cable earth as its signal ground.

To check the configuration of the AxoGraph data acquisition package …
Select the Configuration popup menu in the toolbar.
Click on the Full Configuration button.
Describe each of the cables that are connected from the electronic equipment to the digitizer.
Make sure that all these cables are actually connected as described.

To check that the digitizer is working correctly…
Disconnect all BNC cables from the digitizer
Connect a cable directly between **Analog Output 0** and **Analog Input 0**
Launch AxoGraph and switch the toolbar popup menu to Scope.
Click on the Run Scope button, then the space-bar to halt the Scope.
Click on Channels button.
Acquire data from **Analog Input 0** and direct the test pulse to **Analog Output 0**.
Click on the Pulse button and enter a large pulse amplitude.
Select Relative time and specify an Onset of 10% and Width of 50%.
Click on the Run Scope button.
Hit the “a” key to autoscale the sweeps. A square pulse should be visible.

If a square pulse is NOT displayed, this would indicate a serious problem with the digitizer hardware.
If a square pulse is displayed, this would indicate that everything is working correctly with the AxoGraph acquisition package and the digitizer hardware. The problem must lie with the electronic equipment, or its connection to the digitizer.

1.5 Optionally Remove the Electrophysiology Features

Several of the features of the data acquisition package were developed specifically for electrophysiology research. These include the patch-clamp test pulse, and support for telegraph signals from patch-clamp amplifiers. If these features are not required, they can be removed to simplify the user interface. Open the Acquisition Programs folder (in the Plug-In Programs folder). Drag the Electrophysiology folder out of the Plug -In Programs folder. Quit and re-launch AxoGraph to reset the Program menu and toolbars.

1.6 Summary of Features

• Full support of Axon Digidata 1320 series digitizers and Instrutech ITC-16 and ITC-18 digitizers

Axon Digidata 1320 series
- 16 analog and 4 digital inputs
- 2 analog and 8 digital outputs
- 4 dedicated telegraph inputs
- maximum sample rate of 500 kHz (Digidata 1321A) or 250 kHz (Digidata 1320)

Instrutech ITC series
- 8 analog and 4 digital inputs
- gain control on analog inputs (ITC-18 only)
- 4 analog and 4 digital outputs
- maximum sample rate of 200 kHz

All digitizers
- external trigger can initiate acquisition
- all 16 rear-panel digital input and output channels can be accessed between episodes using an online analysis command

• Digital storage scope
- measure and display peak amplitude, rise-time, half-width
- ‘hot-keys’ for changing the display range settings on the fly
- AC couple (via baseline subtraction)
- average multiple sweeps
- sweep capture and overlay
- optional output of a test pulse and a trigger pulse during each sweep
- custom analysis program can be applied to every sweep

- scope sweeps can be triggered in several different ways...
  - at regular user-defined intervals
  - locked to the line frequency
  - from an external logic signal
  - from a signal on an analog input channel
  - trigger on amplitude or first-derivative threshold crossings
- display a pre-trigger period

• Digital chart recorder
- scrolling display
- mark events with tags and comments
- optional output of a regular timer or test pulse
- measure and display event frequency
- custom analysis applied during continuous acquisition

• Protocol driven data acquisition
- regular, external or keyboard trigger for episodic data acquisition
- unlimited episode width
- deliver complex waveforms to the analog and digital outputs
  - square pulses, trains of pulses and ramps
  - incrementing pulse amplitudes and onset times
  - arbitrary series of pulse amplitudes from a table
  - arbitrary waveform output
  - enter a function (exponential, sine wave, etc.)
  - convert a recorded signal to an output waveform
- ‘hot-keys’ for initiating predefined protocols
- real-time data analysis during acquisition
  - monitor event amplitude or rate versus time
  - real-time analysis programs can be customized or extended
- all 16 rear-panel digital input and output channels can be accessed between episodes using an online analysis command

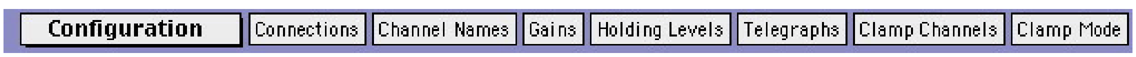
• Patch-clamp test pulse for electrophysiology research
- measure seal resistance during patch recordings
- measure electrode series resistance, membrane capacitance and membrane resistance during whole-cell recordings

2 Configuration

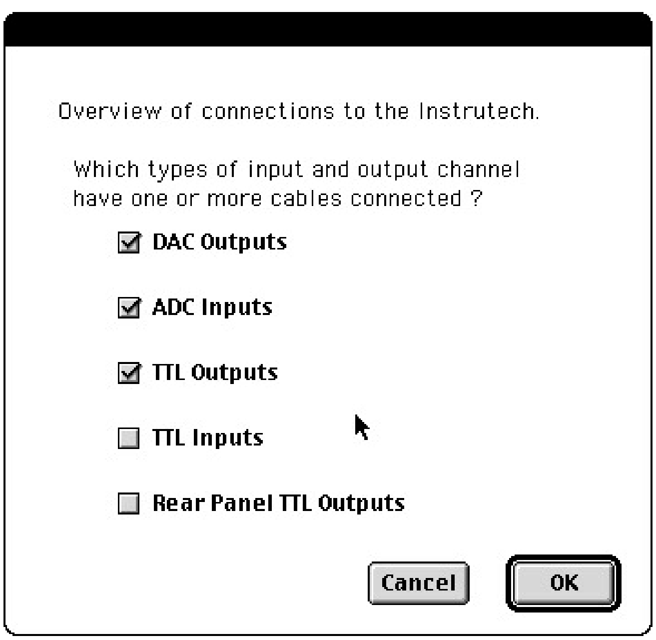
2.1 Introduction

AxoGraph needs to know which input and output channels of the digitizer receive connections. It also needs to know the gain (amplification) and the units for the signal carried by each connection. AxoGraph uses this information to scale the input and output signals and to label the axes in the data display windows. There are five separate programs that can be used to describe the connections and signals: Connections, Channel Names, Gains, Holding Levels and Telegraphs. The program, Full Configuration, runs each of these five programs, one after the other. Running all five programs fully describes the connections to the digitizer. If the connections and the signal gains remain constant, configuration will only need to be performed once when the data acquisition software is first installed. Signal amplification is the configuration parameter that is most likely to be altered. When ever the gain of a signal is adjusted, run the Gains program to inform AxoGraph of the change.

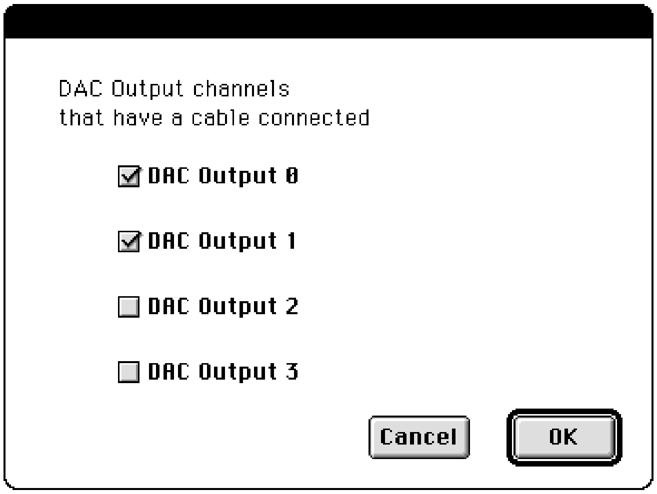
AxoGraph presents a pop-up menu in the toolbar at the bottom-left of the screen. When the Configuration item is selected in this pop-up menu, a new row of buttons appears in the toolbar. The following sections describe the function of each of the buttons in the Configuration toolbar.



2.2 Connections



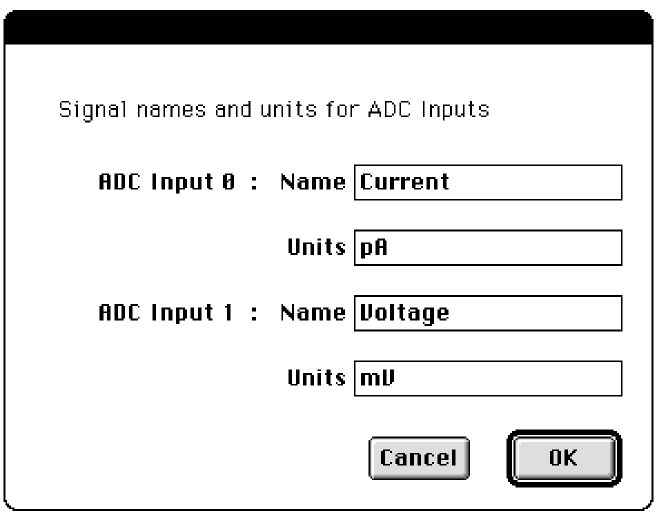
The Connections button initiates a series of dialogs that ask which digitizer channels have a cable connected. The first dialog (shown at left) requests an overview of the connections. This helps to simplify the subsequent series of dialogs.



Next, a series of dialogs request the connections for the **Analog Input** channels, the **Analog Output** channels, etc. Each of these dialogs has the same layout as shown at left for the **Analog Outputs** dialog.

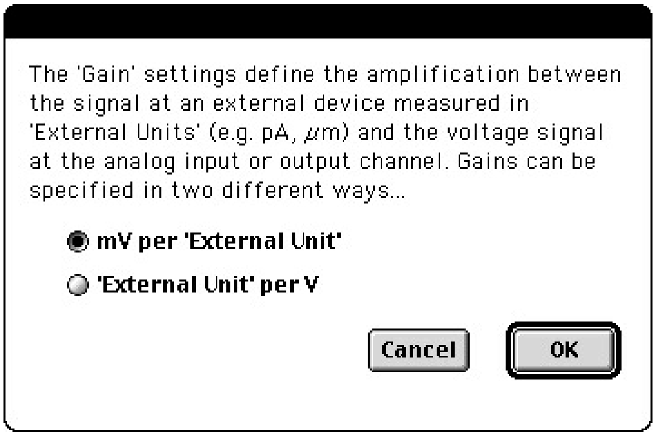
2.3 Channel Names

The Channel Names button initiates a series of dialogs that request signal names for the input and output channels that have a cable connected. The names should clearly distinguish between the various channels, because all subsequent dialogs request channels by name, not number. Also, the names should not be too long because they will be used to label the axes in the data display window. Dialogs are only presented for channels that have a cable connected (see Section 2.2.). The series of dialogs request signal names for the **Analog Input** channels, the **Analog Output** channels, the **Digital Input** channels and the **Digital Output** channels. A dialog will not appear if there are no cables connected to a group of channels.



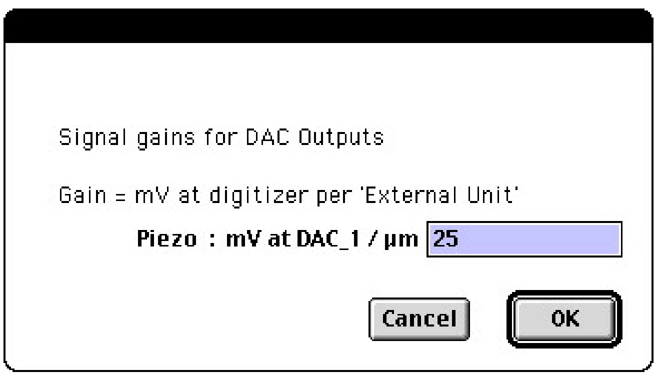
The dialogs for the **Analog Input** and **Analog Output** channels also request signal units. Units should be given in the most convenient format. For example, if an instrument is measuring a small current then the most convenient units for the corresponding **Analog Input** signal may be ‘nA’. If an **Analog Output** signal is controlling a fine mechanical positioner, then most convenient units may be ‘µm’. All four dialogs have the same layout as shown at left for the **Analog Inputs** dialog.

2.4 Signal Gains



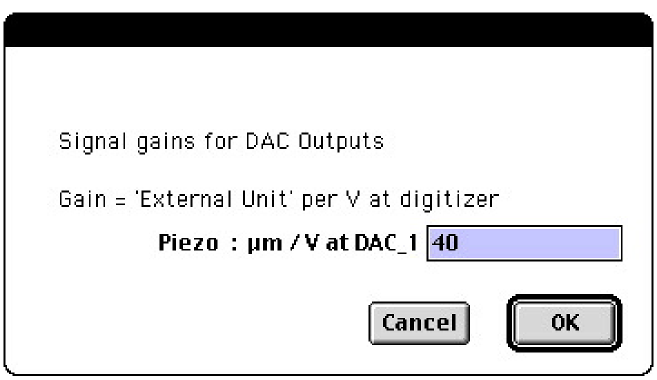
The Gains button brings up a series of dialogs that asks about the signal amplification or conversion factor for each of the electronic devices connected to the digitizer. The first dialog ask how the gains are to be specified in the subsequent dialogs. A choice is offered simply for convenience, because different devices specify their gains in different ways.

The two subsequent dialogs request the signal gain (amplification and conversion factor) for each **Analog Input** and **Analog Output** channel that has a cable connected. If the gain of an **Analog Input** or an **Analog Output** signal is specified via a telegraph, then that channel will not appear in the gain dialog (see Section 2.7). Two examples follow…

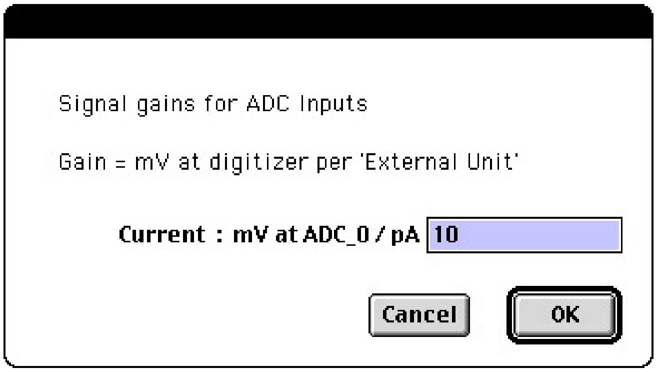


Analog Output Gain
A piezoelectric mechanical positioner generates a movement of 40 µm per Volt. The positioner's command input is connected to **Analog Output 0**, and the signal name and units for this channel are 'Piezo' and 'µm' (see Section 2.3).

Depending on the option chosen in the first dialog, the gain will be specified either as 25 mV per µm,

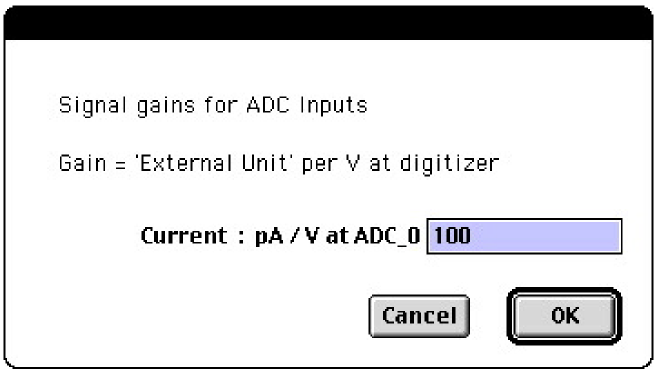


or as 40 µm per V.



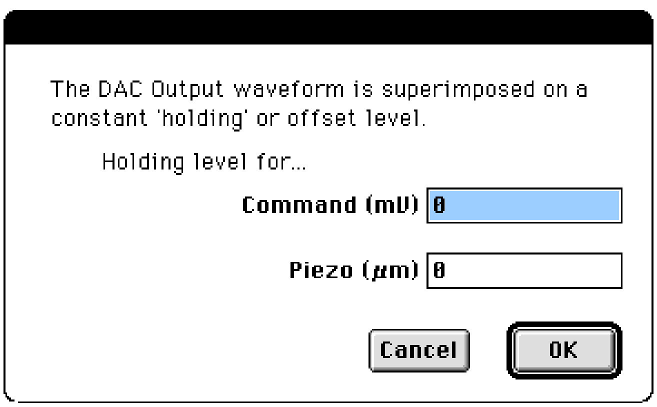
Analog Input Gain
A voltage-clamp amplifier generates a signal of 10 mV per pA. The amplifier's output is connected to the digitizer at **Analog Input 0**, and the signal name and units for this channel are 'Current' and 'pA' (see Section 2.3).

Depending on the option chosen in the first dialog, the gain will be specified either as 10 mV per pA,



or as 100 pA per Volt.

2.5 Holding Levels



The Holding Levels button brings up a dialog that requests the level at which to hold the signal on each of the active **Analog Output** channels (those that have a cable connected). Any command signals sent to an **Analog Output** will be superimposed on the holding level. For most purposes, the holding levels will be set to zero as shown at left.

The signals on the active **Analog Output** channels will be set to the requested holding levels immediately after clicking OK in this dialog. All output channels will be set to their holding levels when AxoGraph is launched, and at the termination of an acquisition protocol (see Chapter 6, 'Protocol Driven Data Acquisition'). Scope and Chart test pulses will be superimposed on the holding level of the pulse output channel.

2.6 Save and Load Configuration

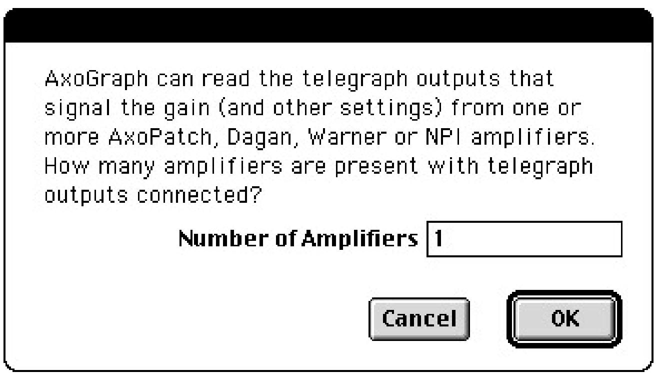
A mechanism is provided for switching quickly between different configurations of the digitizer connections. This feature is useful when the instruments connected to the digitizer are changed between two or more standard configurations on a regular basis. A set of connection parameters can be saved to a file by selecting, Program ➔ Acquisition Options ➔ Save Configuration. The file is saved in the same folder as the AxoGraph application. A set of parameters can be loaded by selecting, Program ➔ Acquisition Options ➔ Load Configuration.

2.7 Telegraphs

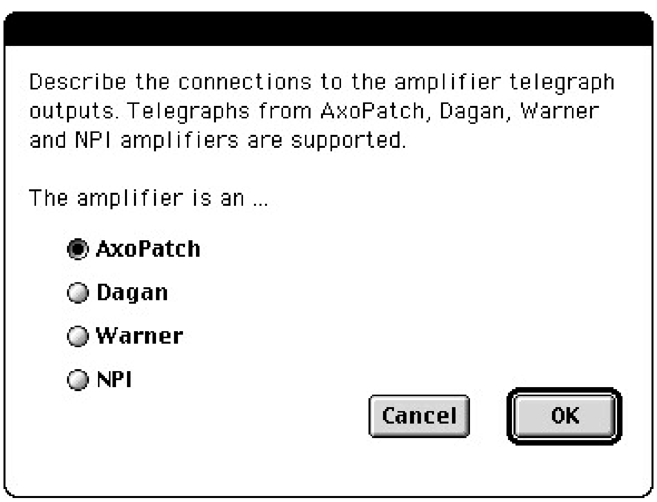
The telegraphs feature is relevant to electrophysiologists using a patch clamp amplifier manufactured by Axon Instruments, Dagan, Warner or NPI.

If a patch clamp amplifier is not being used then review Section 1.5, 'Removing the Electrophysiology Features', before skipping forward to Chapter 3.

AxoGraph can read and interpret the telegraph signals (gain setting, etc.) that are output by most patch clamp amplifiers. Most importantly, it can adjust the gain of the specified input signal according to the voltage on the gain telegraph channel. Other supported signals include the AxoPatch voltage-clamp mode telegraph, filter frequency telegraph and whole-cell capacitance compensation telegraph.

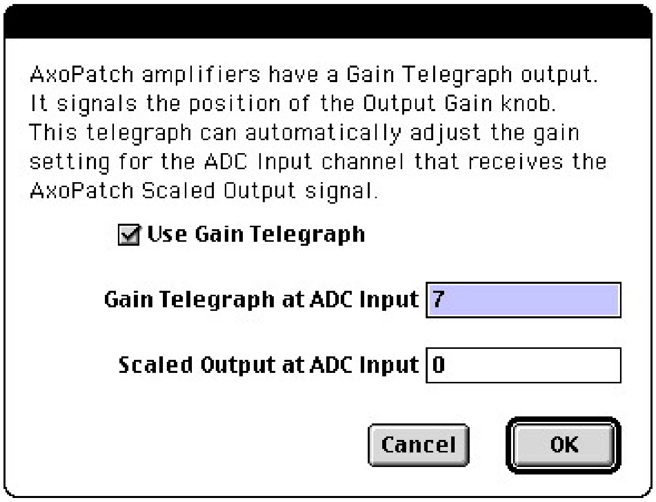


For experiments involving simultaneous presynaptic and postsynaptic recording, there may be two or more amplifiers connected to the digitizer. AxoGraph can support telegraphs from up to four amplifiers simultaneously. The Telegraphs button brings up the dialog shown at left.



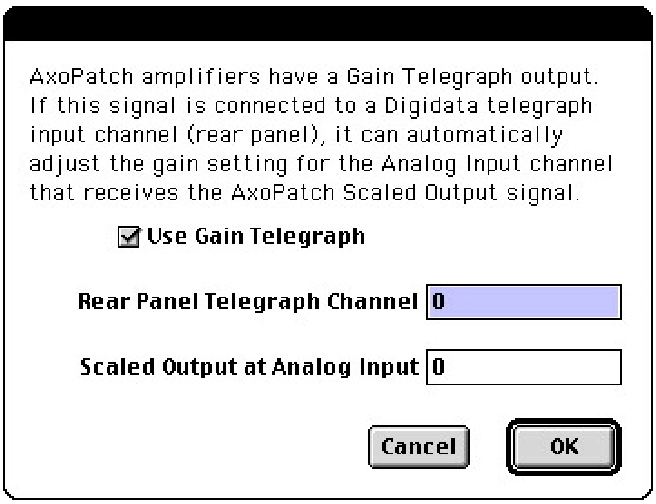
The next two dialogs request the manufacturer of the patch clamp amplifier (shown at left) and the amplifier's model number (not shown).

Gain Telegraph



Next, one or more dialogs appear asking for the channel that each of the telegraph signals is connected to. The first dialog asks about the gain telegraph connection. For Instrutech digitizers, the telegraph signal must be connected to an ADC input channel (as shown at left).

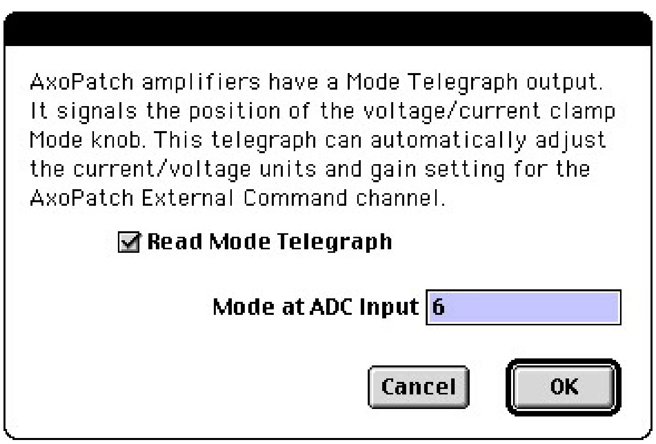
Before each trace is acquired the signal on **Analog Input** 7 will be sampled, and the voltage will be used to adjust the gain for the signal on **Analog Input** 0. This is the **Scaled Output** signal from the patch clamp amplifier.



The gain telegraph dialog has a slightly different format when the digitizer is a Digidata 1320 series (as shown at left). This is because the Digidata has four dedicated telegraph input channels on the rear panel. Telegraph signals must be connected to these channels.

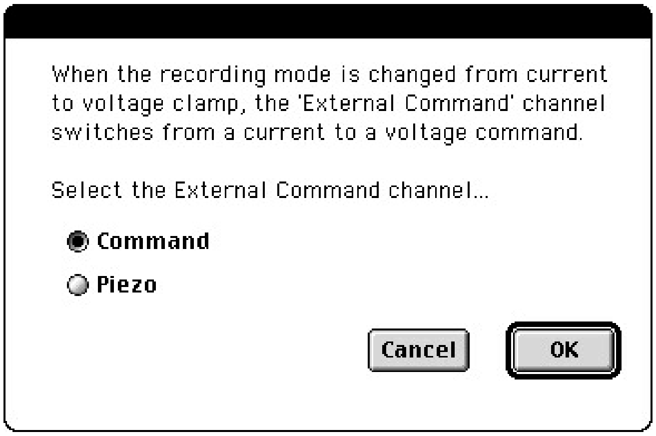
Before each trace is acquired the signal on **Rear Panel Telegraph** 0 will be sampled, and the voltage will be used to adjust the gain for the signal on **Analog Input** 0. This is the **Scaled Output** signal from the patch clamp amplifier.

Mode Telegraph



The next dialog only appears if the amplifier is an AxoPatch 200. This amplifier supports a Mode telegraph which signals whether the AxoPatch is in current-clamp or voltage-clamp mode. Both the **Scaled Output** and the **External Command** connections on the AxoPatch change their behaviour depending on the mode.

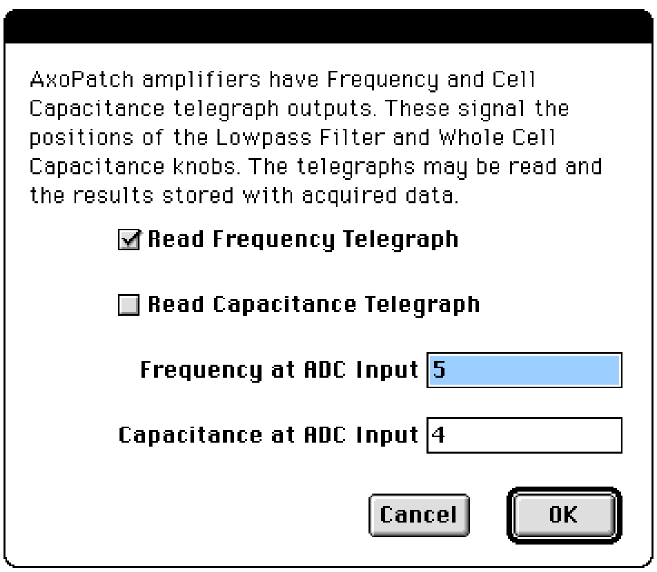
Before each trace is acquired the signal on **Analog Input** 6 will be sampled, and the voltage will be used to determine the AxoPatch clamp mode.



The units and gain setting for the AxoPatch **External Command** connection will be interpreted as either a current command or a voltage command depending on the AxoPatch recording mode. The next dialog asks which **Analog Output** channel is connected to the **External Command** signal. The signal units will be adjusted to ‘pA’ for current-clamp, or ‘mV’ for voltage-clamp.

The Mode Telegraph also adjusts the units for the signal connected to the AxoPatch **Scaled Output**. If the gain telegraph is active, then the **Scaled Output** channel has already been specified (see Gain Telegraph dialog above), so no dialog is presented. However, if the gain telegraph is not active, then dialog appears asking which **Analog Input** channel is connected to the **Scaled Output** signal. The units of the specified channel will be adjusted to ‘mV’ for current-clamp, or to ‘pA’ for voltage-clamp.

Frequency and Capacitance Telegraphs



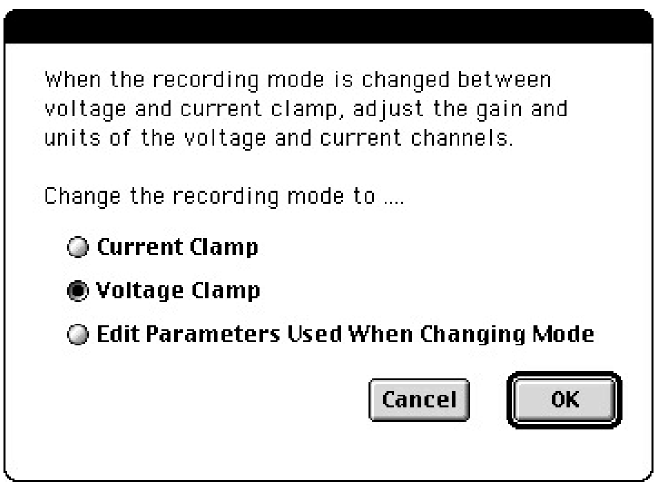
The next dialog only appears if filter frequency or cell capacitance telegraphs are supported by the patch clamp amplifier.

Before each trace is acquired the signal on **Analog Input** 5 will be sampled, and the voltage will be used to determine the amplifier's filter frequency setting. This setting will be stored in the **Notes** section of the acquired data file.

2.8 Clamp Mode

The Clamp Mode feature is only relevant to electrophysiologists using a voltage-clamp amplifier (other than an AxoPatch 200B) that is occasionally switched between current- and voltage-clamp modes. The AxoPatch 200B has a mode telegraph output that signals a change of clamp mode. When this telegraph is used, the Clamp Mode program is not needed, and is removed from the Configuration toolbar. Most other voltage-clamp amplifiers have only a single connection for an **External Command** signal. The signal is interpreted as a voltage command when the amplifier is in voltage-clamp mode, and as a current command when the amplifier is in current-clamp mode. The gain and unit settings for the **Analog Output** channel that generates the **External Command** signal, will change when the clamp mode is changed. In addition, some amplifiers have a **Scaled Output** signal that reports the holding current when the amplifier is in voltage-clamp mode, and the membrane potential when the amplifier is in current-clamp mode. The gain and unit settings for the **Analog Input** channel that receives the **Scaled Output** signal, will change when the clamp mode is changed.

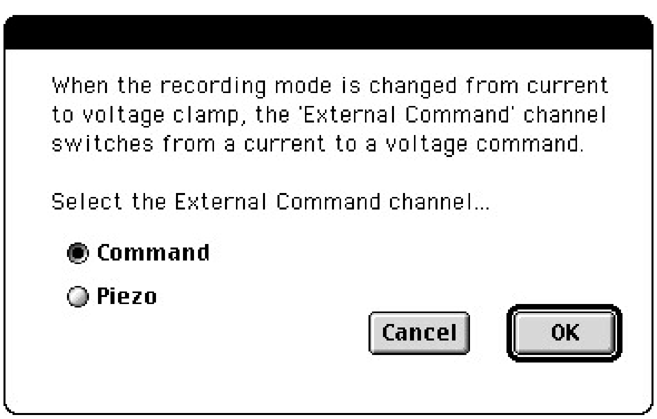
The Clamp Mode button can be used to inform the acquisition program that the clamp mode has changed. AxoGraph then makes all the necessary changes to the configuration settings for the **External Command** and **Scaled Output** channels.



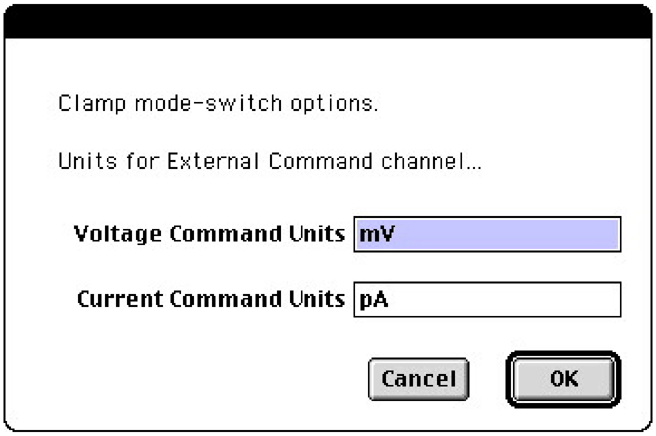
The dialog shown at left indicates that the amplifier is now operating in voltage-clamp mode.

The Clamp Mode program will only work if the configuration programs Connections and Channel Names have already been run, and the **External Command** signal has been described.

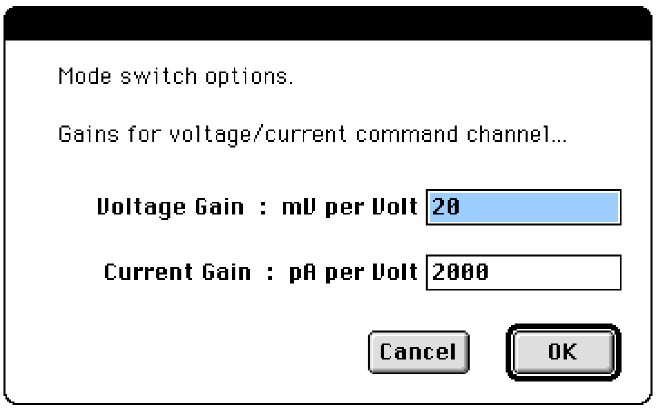
The first time the Clamp Mode program is run, it is necessary to enter the gain and unit settings for the External Command signal in current- and voltage-clamp modes. Selecting the Edit Clamp Mode Parameters option in the previous dialog then clicking **OK**, brings up a series of dialogs that request the mode switch parameters.



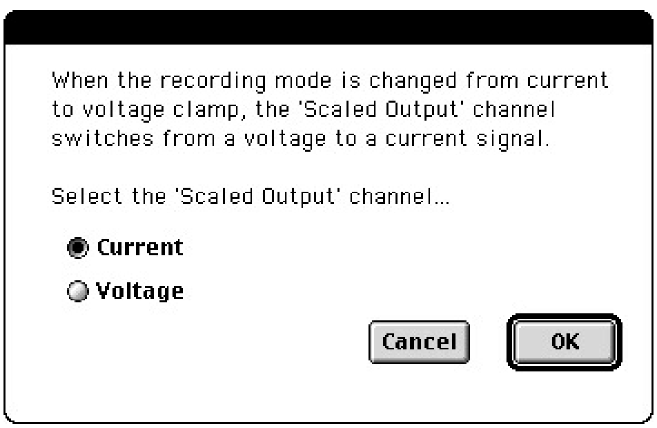
The first dialog asks which **Analog Output** channel is connected to the **External Command** of the voltage-clamp amplifier. The channels are listed by name.



The second dialog requests the units for the voltage and current clamp commands. These will generally be ‘mV’ for the voltage command and either ‘pA’ or ‘nA’ for the current command.

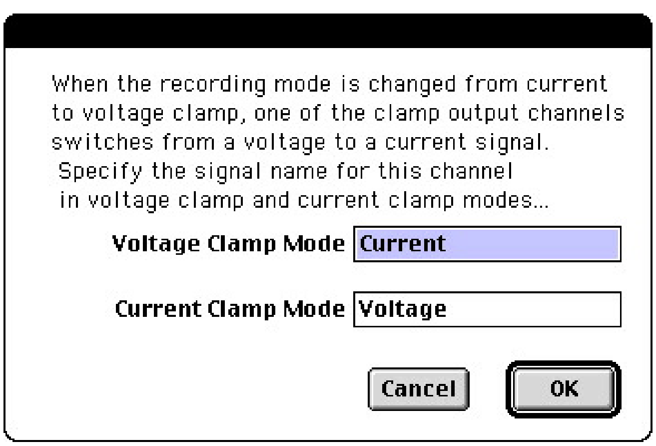


The third dialog requests the gains for the voltage and current clamp commands. The gains are given in mV of voltage-clamp command per Volt at the **Analog Output**, and in pA of current-clamp command per Volt at the **Analog Output**.



The fourth dialog asks which **Analog Input** receives the amplifier’s **Scaled Output** signal. This signal will report current when in voltage-clamp mode, and membrane potential when in current-clamp mode.

If a gain telegraph is active, then the **Scaled Output** channel has already been specified (see Gain Telegraph dialog above), so this dialog will not be presented.



The fifth dialog asks for the signal names to be used for the **Scaled Output** channel when in voltage-clamp mode and current-clamp mode.

3 Digital Oscilloscope

3.1 Introduction

The Scope program implements all the main features of a storage oscilloscope. It is designed for monitoring an intermittent electrical signal. Each time the scope is triggered, an electrical signal is plotted versus time in the scope window. The displayed waveform is termed a ‘sweep’. Successive sweeps can be overlaid (storage mode), and erased manually. Alternatively, the window can be erased automatically before displaying each new sweep. Sweeps can be triggered from an external pulse, or they can be triggered at regular intervals by the computer. There is a ‘line trigger’ mode that synchronizes the start of each sweep with the power line frequency (50 or 60 Hz). A single sweep can be ‘captured’ and overlaid on later sweeps. Signals on up to 8 channels can be displayed simultaneously.

Signal waveforms are displayed, but are not saved to disk. See Chapter 4, ‘Digital Chart Recorder’, or Chapter 5, ‘Protocol Driven Data Acquisition’ for programs that can save the acquired signals to disk.

In addition to the standard storage scope features outlined above, AxoGraph's digital scope can average a signal over multiple sweeps and display the running average. It can send a synch pulse to a **Digital Output** at the beginning of each sweep, and a test pulse to an **Analog Output** during each sweep. It can also measure and display the peak amplitude, rise-time and width of an event in each sweep.

AxoGraph presents a pop-up menu in the toolbar at the bottom-left of the screen. When the Scope item is selected in this pop-up menu, a new window titled Scope Window will appear. The following sections describe the function of each of the buttons in the Scope toolbar.

3.2 Run Scope

The Run Scope button starts the digital scope. A series of sweeps will be displayed in the scope window. Hitting the space-bar on the keyboard halts the scope.

The rate at which the traces appear will depend on how the sweeps are triggered. The various trigger options are described in Section 3.7, below.

3.3 Hot Keys

The Hot Keys button opens a documentation window which describes keyboard shortcuts for controlling the digital oscilloscope. Here is the list of the scope hot keys, and their actions.

space-bar : halts the scope

a : Automatically adjust Y axis range to the size of the signal
f : Adjust Y axis to display the full **Analog Input** range
c : Capture a trace for comparison with subsequent traces
e : Erase the scope
(useful when the Erase between sweeps option is turned off)

up-arrow : increase Y-axis range (zoom out)
down-arrow : decrease Y-axis range (zoom in)
right-arrow : increase sweep width
left-arrow : decrease sweep width

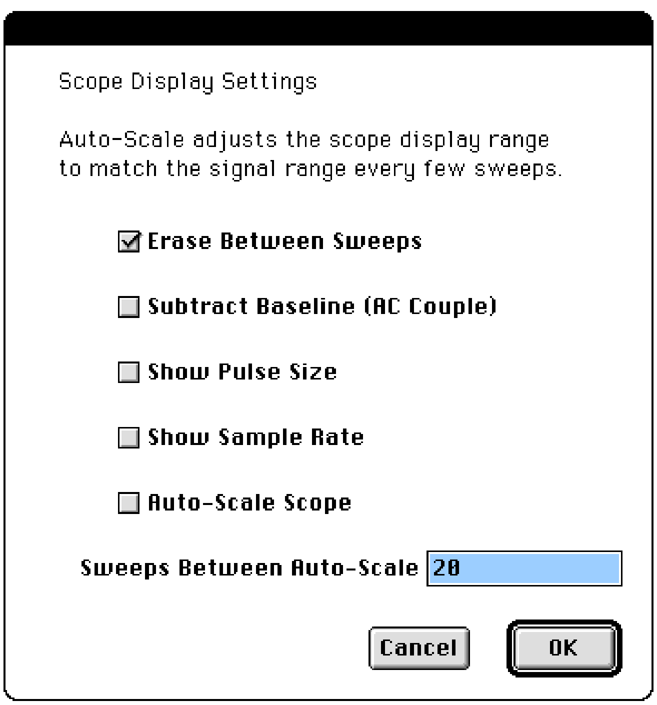
The following keys modify the Scope test pulse.

+ : increase pulse amplitude
- : decrease pulse amplitude
i : Invert pulse

The following keys are active when using an internal trigger.
(The test pulse in inactivated in this trigger mode.)

+ : increase trigger level
- : decrease trigger level

3.4 Display



The Display button in the Scope toolbar brings up the Scope Display Settings dialog. This asks whether to display information about the acquisition parameters in the scope window. It also asks how sweeps are to be displayed.

Erase Between Sweeps
When this check box is turned **off**, each new sweep is overlaid on preceding sweeps. This emulates the behavior of a storage oscilloscope. Typing the letter "e" while the scope program is running erases the scope window. When this check box is turned **on**, the scope is erased before each new trace is displayed. This emulates the behavior of a standard oscilloscope.

Subtract Baseline (AC Couple)
When this check box is turned on, the signal amplitude is calculated over a short interval

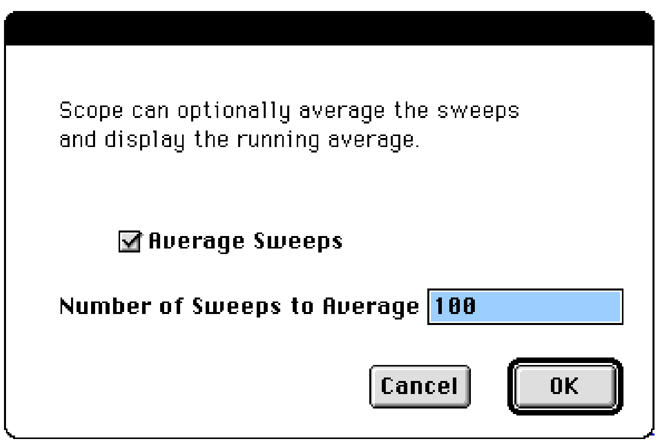
at the start of each sweep. This 'baseline' amplitude is subtracted from the signal before it is displayed. This procedure emulates the 'AC couple' option available on electronic oscilloscopes. It is useful in situations where the signal of interest is superimposed on a larger drifting signal. It stabilizes the signal at the center of the display range.

Show Pulse Size
When this check box is turned **on**, the amplitude of the test pulse is displayed in the scope window.

Show Sample Rate
When this check box is turned **on**, the acquisition sample rate is displayed in the scope window.

Auto-Scale Scope
When this check box is turned **on**, the Y axis display range of the scope window is automatically adjusted to display the full amplitude of each signal (auto-scale). The auto-scale function is performed periodically. The interval between each auto-scale can be entered in the Sweeps Between Auto-Scale field. When the Auto-Scale Scope check box is turned **off**, the auto-scale function can be performed at any time by typing the letter "a" while the scope program is running (see Section 3.3, 'Hot-Keys').

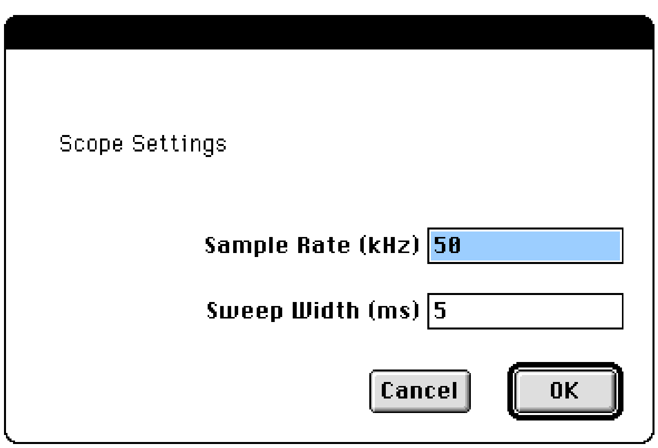
3.5 Average



The Average button brings up the dialog shown at left.

When the Average Sweeps check box is **on**, a running average of successive sweeps is calculated and displayed in the scope window. This is useful for improving the signal-to-noise characteristics of the displayed signal.

3.6 Timebase

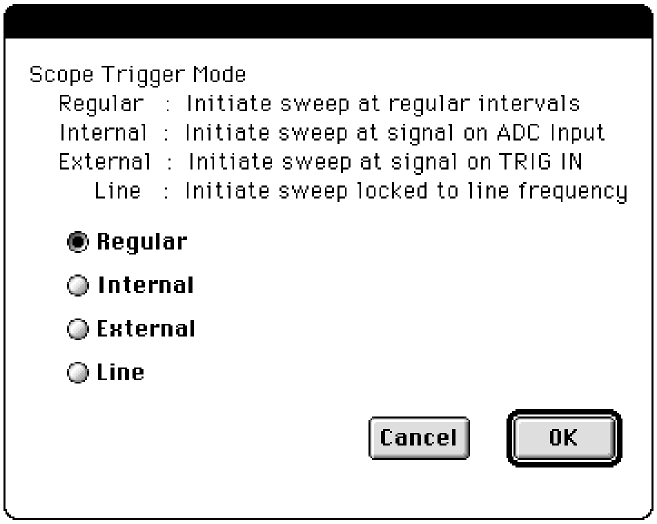


The Timebase button in the Scope toolbar brings up the dialog shown at left. This dialog controls the sample rate used by the Scope program to acquire signals. When several input channels are active, the signal on each channel will be sampled at the requested rate. This dialog also controls the width of each sweep in milliseconds.

The sample rate should be at least twice the highest frequency of interest in the signal. The best results are obtained when the signal is low-pass filtered at a frequency equal to half the sample rate.

Because of limitations in the digitizer hardware, it is not always possible to sample data points exactly at the requested sample rate. See Section 11.2, ‘Hardware Limitations on the Sampling Rate’ for a discussion of these limitations.

3.7 Trigger



The Trigger button in the Scope toolbar brings up the Scope Trigger Mode dialog. This dialog controls how sweeps are initiated (triggered). The are four options....

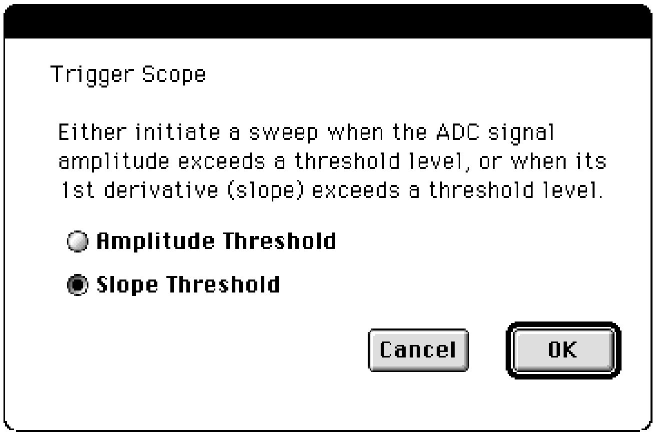
Regular
When this mode is selected, sweeps are initiated at a regular interval that is specified in a subsequent dialog (not shown). If the sweep start-to-start time is set to zero, sweeps will be initiated as rapidly as possible. If Scope can not keep up with the requested sweep start-to-start time, a warning dialog will appear when the program is halted.

Internal
When this mode is selected, sweeps are initiated whenever a signal on one of the **Analog Input** channels crosses a specified threshold. The procedure for setting the threshold is described below.

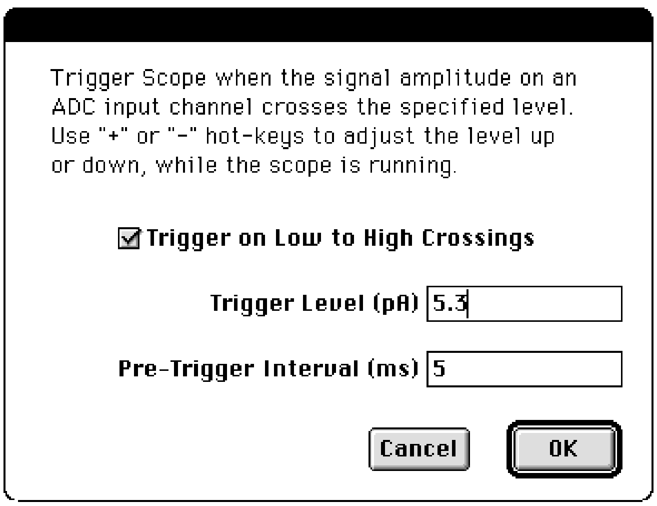
External
When this mode is selected, sweeps are initiated whenever a 5 Volt signal appears on the digitizer connector labeled **Trigger Input**. This permits the start of the scope sweep to by synchronized with an external event.

Line
When this mode is selected, sweeps will be synchronized with the power line voltage cycle. The Axon Digidata 1320 series incorporates a hardware line trigger. The Instrutech ITC series need to be triggered from software, so a second dialog appears asking whether the power line frequency is 50 or 60 Hz. Sweeps will be initiated at the shortest possible interval that is a multiple of 20 ms (for 50 Hz line) or 16.6666 ms (for 60 Hz line). The ‘line’ trigger mode is useful when testing for contamination of a signal by power line noise. It can be used in conjunction with sweep averaging to improve sensitivity (see Section 3.5 above).

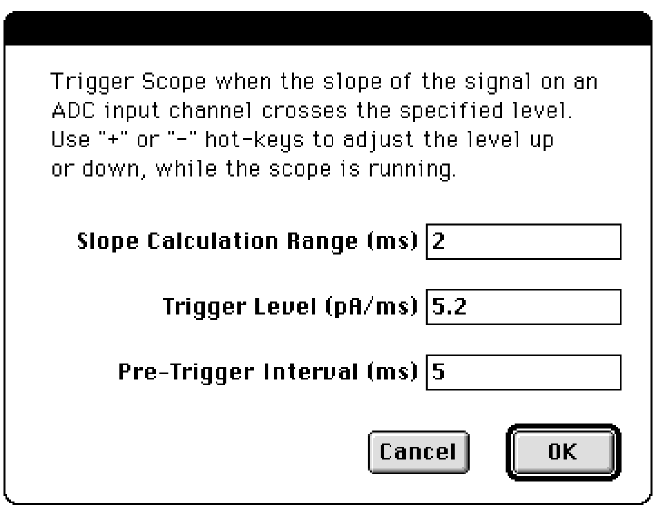
When Internal triggering is selected, a series of three dialogs appears that request additional information about when to initialize each sweep. The first dialog (not shown) asks which **Analog Input** channel carries the signal that will be used to trigger the sweeps. This can be any of the connected signals. Typically, one of the signals being displayed in the scope window will be used as the trigger signal.



The next dialog asks whether to initiate a sweep when the signal amplitude crosses a threshold level, or when the slope of the signal exceeds a threshold level.



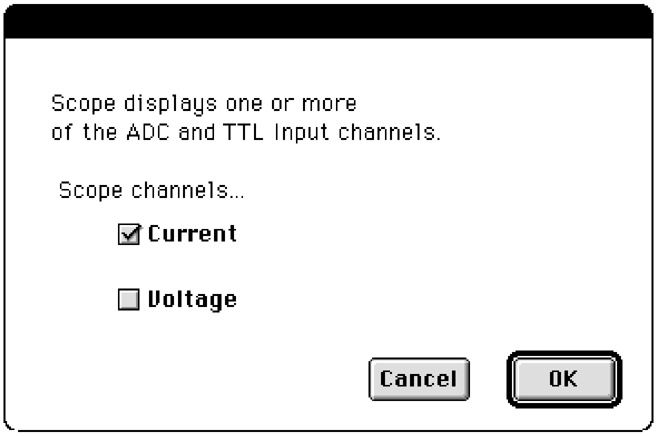
If Amplitude Threshold is selected, the next dialog requests the direction of threshold crossing (low-to-high or high-to-low) that initiates the sweep. It also requests the threshold level that must be crossed.
The signal can optionally be displayed both before and after the threshold crossing event that triggers the sweep. This is achieved by setting the Pre-Trigger Interval to a value greater than zero.



If Slope Threshold is selected, the next dialog requests the range over which to calculate the slope, and the slope threshold level that initiates the sweep.

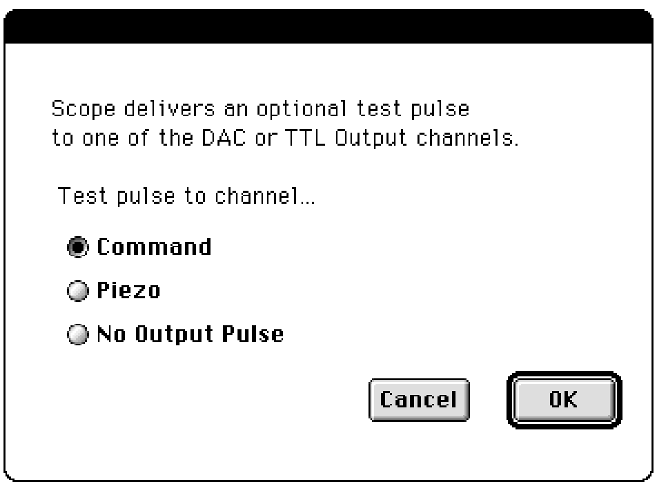
The slope of the signal is calculated by fitting a line over the specified calculation range. This calculation is continuously updated as the signal is acquired, until the slope exceeds the specified threshold. If the Trigger Level is set to a negative value, then a sweep will be triggered when the slope of the signal is more negative than the threshold. This permits triggering on negative-going events.

3.8 Channels



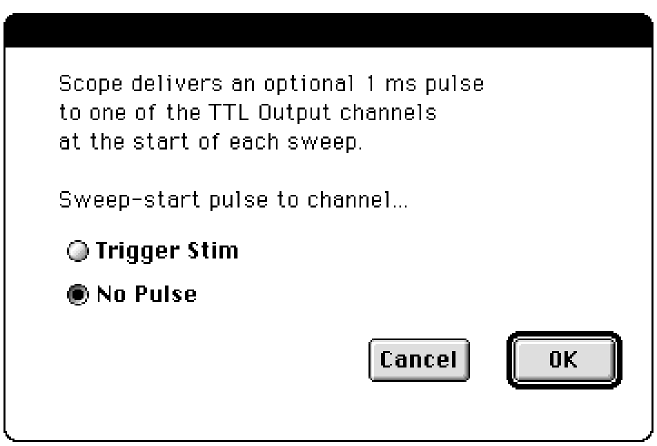
The Channels button in the Scope toolbar initiates a series of three dialogs. The first dialog asks which **Analog Input** and **Digital Input** signals to display in the scope window. Only channels that have a cable connected are listed (see Section 2.2, ‘Connections’). Channels are listed by name, not by number (see Section 2.3, ‘Channel Names’).

When two or more input channels are selected, the signals will be displayed by default in separate groups within the scope window. The signals from multiple channels can be overlaid by selecting either Combine or Group under the Trace menu. Channels should only be combined if their signals have the same units (e.g. mV).



The digital scope can optionally deliver a test pulse to one **Analog Output** or **Digital Output** channel during each sweep. The second dialog asks which channel (if any) is to receive the test pulse.

The duration and amplitude of the test pulse can be edited via the Pulse button in the Scope toolbar (see Section 3.10 below).



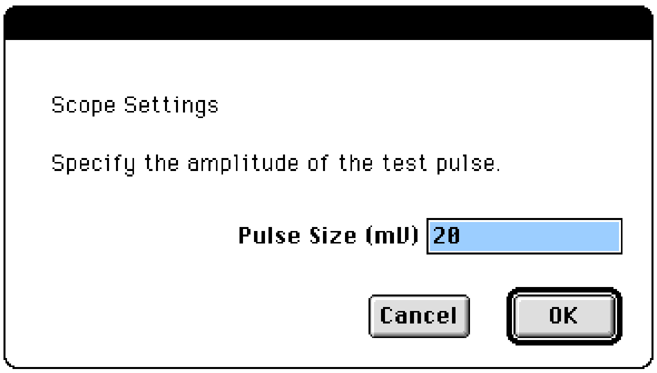
The scope can optionally deliver a brief synchronizing pulse to a **Digital Output** channel every time a sweep is triggered. This pulse could be used to trigger a waveform generator, or an electronic oscilloscope for example. The third dialog asks which channel (if any) is to receive the synch pulse.

3.9 Gains

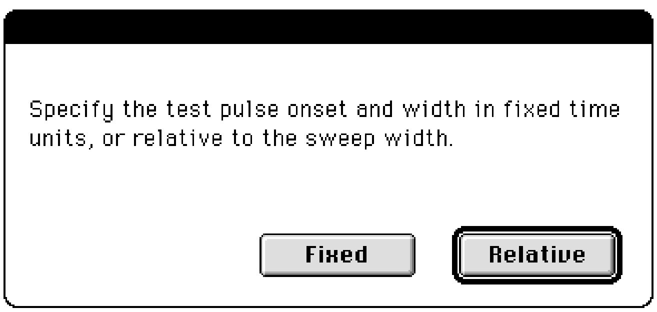
The Gains button in the Scope toolbar brings up two dialogs that request the signal gain (amplification and conversion factor) for each **Analog Input** and **Analog Output** channel. These dialogs are described in detail in Section 2.4, 'Signal Gains'. The Gains button is also found in the Configuration toolbar, but is duplicated in the Scope and Chart toolbars for convenience.

3.10 Test Pulse

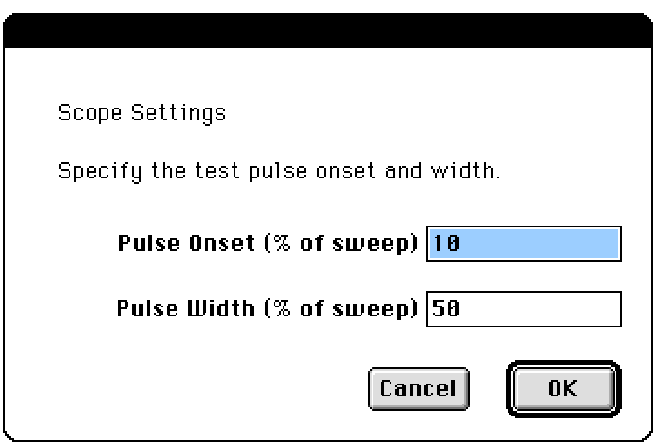
The digital scope can optionally deliver a test pulse to one **Analog Output** or **Digital Output** channel during each sweep (see Section 3.8, 'Channels'). If a test pulse channel has been selected, then the Pulse button in the Scope toolbar brings up a series of three dialogs requesting the amplitude, onset time and duration of the test pulse.



The first dialog only appears if an **Analog Output** channel has been selected. It asks for the pulse amplitude.
The pulse amplitude can also be adjusted using 'Hot Keys' (see Section 3.3).



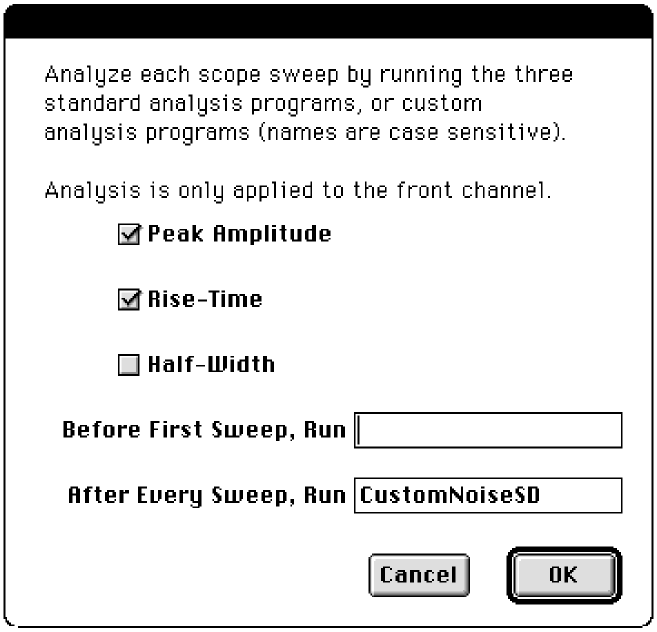
The second dialog asks whether the onset and duration are to be specified in fixed time units (ms), or as a fraction of the sweep width.



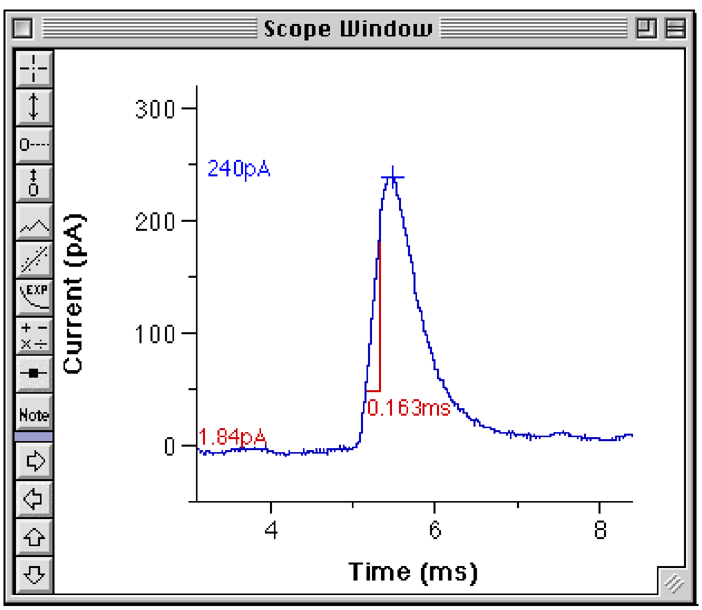
If fixed time units are specified, then the third dialog requests the pulse onset time and pulse width in milliseconds (not shown). The test pulse duration will remain constant even if the scope sampling rate and sweep width settings are changed.

If relative timing is selected, the test pulse will change in duration when the sweep width is changed. The pulse will always occupy the same fraction of the sweep, and the third dialog asks which fraction that will be.

3.11 Analyse



The Analyse button in the Scope toolbar presents the online analysis dialog. The digital scope optionally analyses each sweep immediately after it is acquired, and displays the results overlaid with the sweep. Three standard analysis programs identify the largest event in each episode, and analyse that event. They optionally measure the peak amplitude, the 20-80% rise-time and the half-width of the event. Custom analysis programs can also be run before the first sweep, and after every sweep. For example, the program 'CustomNoiseSD' calculates the standard deviation of the signal in the baseline region before the start of the test pulse. The code for this program can be found in the file named 'Custom Analysis' which is located in the 'Acquisition' sub-folder of the 'Acquisition Programs' folder.



When the digital scope is run with online analysis active, the scope window will display the results superimposed on each sweep. The figure at left shows a single Scope sweep with the peak amplitude, the rise-time, and the noise SD analysis results superimposed. The noise SD was calculated and displayed by a custom analysis program which is described below.

3.12 Custom Analysis

Custom analysis programs can be run before the first sweep, and after every sweep. A program run before the first sweep would typically set up global parameters that control subsequent analysis. For information about writing a programs in AxoGraph, see the chapter on programming in the AxoGraph User Manual.

Two example online Scope analysis programs are supplied with AxoGraph. They are..

CustomNoiseSD: Calculates and displays the standard deviation (SD) of the signal over the first 10% of each sweep. To activate the program, bring up the Analyse dialog and enter the name ‘CustomNoiseSD’ in the ‘After Every Sweep’ field.

ScopeSpectrumSetup,
ScopeSpectrum: calculates the power spectrum of the signal in each sweep and displays the result in a separate window on log-log axes. To activate the program, bring up the Analyse dialog and enter the name ‘ScopeSpectrumSetup’ in the ‘Before First Sweep’ field and ‘ScopeSpectrum’ in the ‘After Every Sweep’ field.

The source code for the ‘CustomNoiseSD’ analysis program is presented on the next page. This code can also be found in the file named ‘Custom Analysis’ which is located in the ‘Acquisition’ sub-folder of the ‘Acquisition Programs’ folder. It should be a useful starting point for writing simple custom analysis programs.

The ‘CustomNoiseSD’ program is automatically loaded when AxoGraph is launched, because it is located in a sub-folder of the Plug-In Programs folder. When a new custom analysis program is written, it can be loaded and tested as follows. First, save the new custom analysis program in the Plug-In Programs folder or sub-folder. Next, select the AxoGraph menu item Program → Reload Plug-Ins. Finally, enter the custom analysis program name in the After Every Sweep field of the online analysis dialog (as shown above).

Source code listing for the program 'CustomNoiseSD'. The first line of the source file must be 'LocalLanguage C' to inform AxoGraph that the following code is written in the C programming language.

```
localLanguage C

/* Custom analysis program calculates and displays
   the standard deviation (SD) of the signal measured
   over the first 10% of each sweep */

void CustomNoiseSD
{
   short window, trace;
   float yArray[0];
   float xMin, xMax;
   float noiseSD, theScale;
   string yUnits;

/* Get the front window and trace number */
   GetFront (window, trace);
   GetXRange (window, xMin, xMax);

/* Get the baseline region before the start of the test pulse */
   xMax = startPulsePnt * sampleInterval;
   yArray = yRange(window, trace, 0, xMax);

/* Make sure we got at least 3 points */
   if (ArraySize(yArray) > 2) {

   /* Calculate the SD */
      noiseSD = SD(yArray);

   /* Get the displayed Y-axis units and scale factor */
      DisplayedYUnits(window, trace, yUnits);
      DisplayedYScale(window, trace, theScale);

   /* Move to a point just below the baseline */
      DrawMove (xMin, Mean(yArray)-2*noiseSD);
      DrawPixelMove (5, 12);
      DrawSetSize (12);

   /* Display the noise SD (in Y-axis units) */
      DrawString (concat(noiseSD*theScale:3,yUnits));
   }
}
```

4 Digital Chart and Tape Recorder

4.1 Introduction

The Chart program implements all the main features of a chart recorder. It can continuously acquire data at audio rates (up to 50 kHz on some computers) so it could also be used as a digital tape recorder. The program is designed for continuously monitoring and recording electrical signals. The signals are plotted versus time in a scrolling chart window. The digital chart recorder can be stopped and restarted many times in a single recording session. The gain and sampling rate can be changed during a recording session. The chart can be annotated at any time during the recording with comments and event markers.

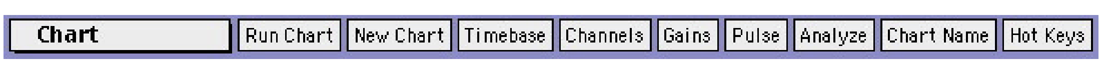
In addition to the standard features of a chart recorder outlined above, AxoGraph's digital chart recorder can send a regular test or stimulus pulse to an **Analog Output** during continuous recording.

Chart data is acquired to memory, and is not automatically written to disk. To write the acquired data to disk, the chart file must be saved manually. This can be done at any time during a recording session. The digital chart recorder will halt with an error message when all available system memory is exhausted.

The maximum chart length can be estimated as follows. Switch to the Finder, and select About This Computer… under the Apple menu. Note the size of the Largest Unused Block of memory. If Chart is recording from N channels, then AxoGraph requires 4 x (N+1) bytes per sample point, and 4 x (N+1) x SampleRate bytes per second. For example, if there is 36 MBytes of memory available, and Chart is recording 2 channels at 1 kHz, then...

Maximum Chart Length = Available Memory / (4 x (N+1) x SampleRate)
= 36,000,000 / (4 x (2+1) x 1,000)
= 3,000 seconds or 50 minutes

AxoGraph presents a pop-up menu in the toolbar at the bottom-left of the screen. When the Chart item is selected in this pop-up menu, a dialog will appear asking for the file name and destination folder for the chart data file. A new chart window will then appear with the selected name. The following sections describe the function of each of the buttons in the Chart toolbar.



4.2 Run Chart

The Run Chart button starts the digital chart recorder. The signal on one or more channels will be displayed in the scrolling chart window. Hitting the space-bar on the keyboard stops the chart. Clicking the Run Chart button restarts the chart.

4.3 New Chart

The New Chart button first closes the old chart window (if one is open), then opens a new chart window. The user is given the option of saving or discarding the old chart data. The new chart file name is generated by incrementing the sequence number at the end of the file name.

4.4 Hot Keys

The Hot Keys button opens a documentation window with information about keyboard shortcuts for controlling the digital chart recorder. Here is a list of the chart hot keys, and their actions.

space-bar : halts the chart recorder

a : Auto-adjust Y axis range to the size of the signal
f : Adjust Y axis to display the full **Analog Input** range
t : Add an event marker (tag) and a comment to the chart

up-arrow : increase Y-axis range (zoom out)
down-arrow : decrease Y-axis range (zoom in)
left-arrow : increase the time range (zoom out)
right-arrow : decrease the time range (zoom in)

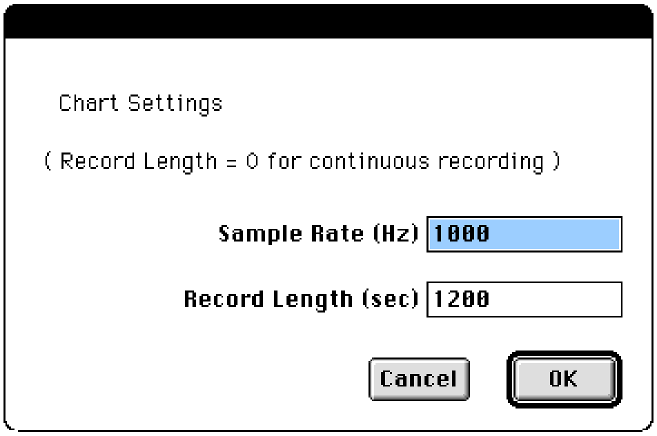
4.5 Event Markers

The action of the event marker (tag) hot key, "t", depends on the sample rate and the size of the internal buffer in the digitizer. If the buffer will not overflow in the next few seconds, then a dialog appears asking for a comment describing the event.

The dialog indicates how long there is before the buffer overflows. If there is insufficient time to enter a comment, then a tag is added without an associated comment.

Tags are displayed as vertical dashed lines in the chart window. Tag comments are stored in the chart window's **Notes**, together with the time that the event occurred. These comments can be accessed by selecting Display → Comments and Notes, or by clicking the **Note** button in the vertical toolbar at the left of the chart window. If the chart is halted and restarted, the stop and start times will be included automatically in the list of event markers.

4.6 Timebase

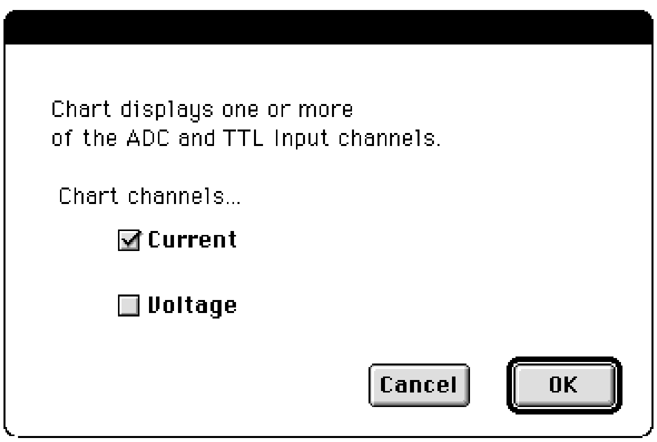


The Timebase button in the Chart toolbar brings up the dialog shown at left. This dialog controls the sample rate used by the Chart program to acquire signals. When several input channels are active, the signal on each channel will be sampled at the requested rate. The sample rate should be at least twice the highest frequency of interest in the signal. The best results are obtained when the signal is low-pass filtered at a frequency equal to half the sample rate.

This dialog also controls the length of the chart record. The chart recorder will halt automatically when the record reaches the length specified in the Record Length field. For example, the above dialog requests a 20 minute recording (20 x 60 = 1200 sec). If the Record Length is set to zero, then recording will continue until all available system memory is full.

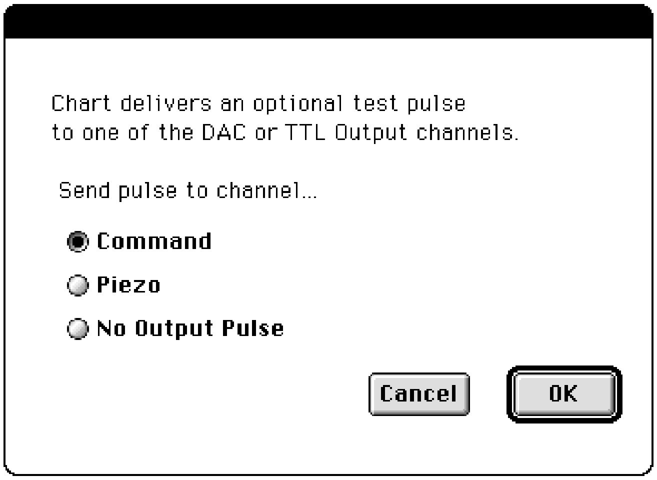
Because of limitations in the digitizer hardware, it is not always possible to sample data points exactly at the requested sampling rate. See Section 11.2, ‘Hardware Limitations on the Sampling Rate’ for a discussion of these limitations.

4.7 Channels



The Channels button in the Chart toolbar brings up two dialogs. The first dialog asks which **Analog Input** and **Digital Input** signals to display in the chart window. Only channels that have a cable connected are listed (see Section 2.2, ‘Connections’). Channels are listed by name, not by number (see Section 2.3, ‘Channel Names’).

When two or more input channels are selected, the signals will be displayed by default in separate groups within the chart window. The signals from multiple channels can be overlaid by selecting either Combine or Group under the Trace menu. In general, channels should only be combined if their signals have the same units (e.g. mV).



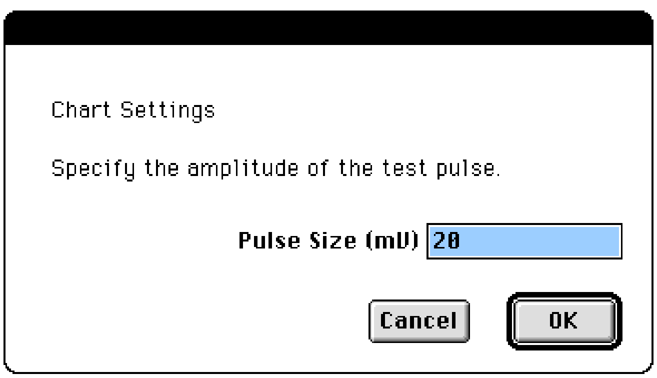
The digital chart recorder can optionally deliver a regular test pulse to one **Analog Output** or **Digital Output** channel. The second dialog asks which channel (if any) is to receive the regular test pulse.

The frequency, duration and amplitude of the test pulse can be edited via the Pulse button in the Chart toolbar (see Section 4.9 below).

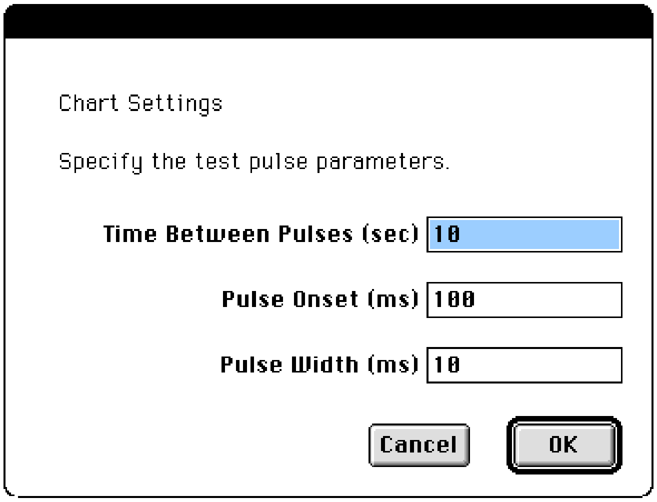
4.8 Gains

The Gains button in the Chart toolbar brings up two dialogs that request the signal gain (amplification and conversion factor) for each **Analog Input** and **Analog Output** channel. These dialogs are described in detail in Section 2.4, 'Signal Gains'. The Gains button is also found in the Configuration toolbar, but is duplicated in the Scope and Chart toolbars for convenience.

4.9 Test Pulse

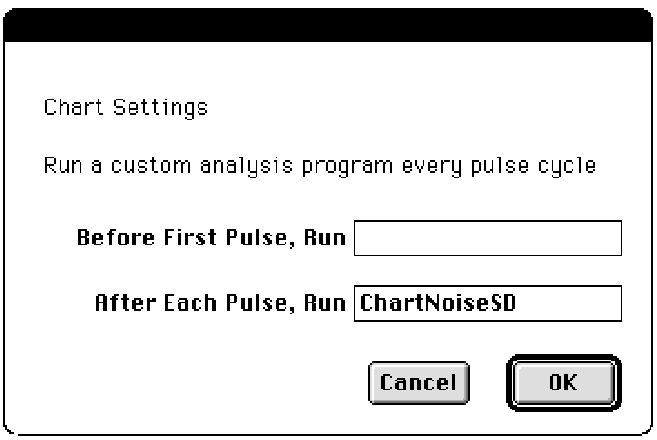


The digital chart recorder can optionally deliver a regular test pulse to one **Analog Output** or **Digital Output** channel during continuous recording (see Section 4.7, 'Channels'). If a test pulse channel has been selected, then the Pulse button in the Scope toolbar brings up a series of three dialogs requesting the time between test pulses, and their amplitude and duration. The first dialog only appears if an **Analog Output** channel has been selected. It asks for the pulse amplitude.



The second dialog asks for the onset time to the first pulse, the time between subsequent pulses, and the duration (width) of each pulse.

4.10 Analyse



The Analyse button in the Chart toolbar brings up the online analysis dialog. The chart recorder optionally runs a custom analysis program after each test pulse cycle (see Section 4.9, 'Test Pulse'). A custom analysis programs can also be run before the first pulse, and would typically set up global parameters that control subsequent analysis. For information about writing a custom analysis program, see the chapter on programming in the AxoGraph Online Manual.

4.11 Custom Analysis

The source code for the 'ChartNoiseSD' online analysis program follows. The code for this program can also be found in the file named 'Custom Analysis' which is located in the 'Acquisition' sub-folder of the 'Acquisition Programs' folder.

The 'ChartNoiseSD' program is automatically loaded when AxoGraph is launched, because it is located in a sub-folder of the Plug-In Programs folder. When a new custom analysis program is written, it can be loaded and tested as follows. First, save the new custom analysis program in the Plug-In Programs folder, or sub-folder. Next, select the AxoGraph menu item Program → Reload Plug-Ins. Finally, enter the custom analysis program name in the After Each Pulse field of the online analysis dialog (as shown above).

Source code listing for the program 'ChartNoiseSD'. The first line of the source file must be 'LocalLanguage C' to inform AxoGraph that the following code is written in the C programming language.

```
LocalLanguage C

/* Custom analysis program calculates and displays
   the standard deviation (SD) of the signal measured
   over the last 10% of each test pulse cycle */

void ChartNoiseSD
{
   short window, trace;
   float yArray[0];
   float xMin, xMax;
   float noiseSD, theScale;
   string yUnits;

/* Get the last 10% of the most recent test pulse cycle */
   GetFront (window, trace);
   xMax = ChartCumulativePoints * sampleInterval;
   xMin = xMax - 0.1 * PulseCycleTime;
   yArray = yRange(window, trace, xMin, xMax);

/* Calculate the SD */
   noiseSD = SD(yArray);

/* Get the displayed Y-axis units and scale factor */
   DisplayedYUnits(window, trace, yUnits);
   DisplayedYScale(window, trace, theScale);

/* Display the noise SD using the Y-axis units */
   DrawMove (xMax, Mean(yArray)+6*noiseSD);
   DrawSetSize (12);
   DrawString (concat(noiseSD*theScale:3,yUnits));
}
```

5 Electrophysiology Test Pulse

5.1 Introduction

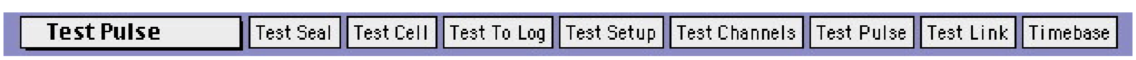
The Test Seal and Test Cell programs are designed to help electrophysiologists set up and perform patch-clamp or voltage-clamp experiments.

If the data acquisition software will not be used for electrophysiology experiments, then review Section 1.5, 'Removing the Electrophysiology Features', before skipping forward to Chapter 6.

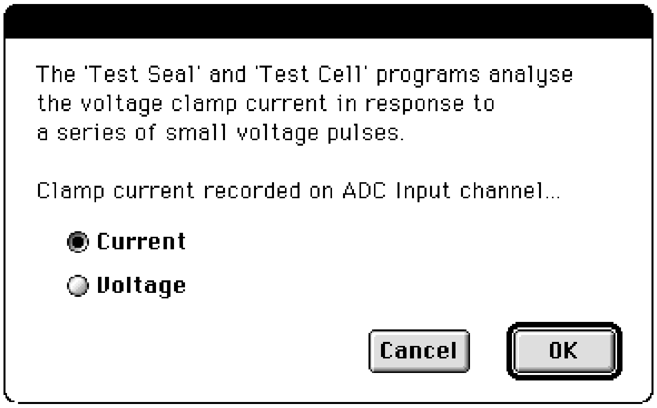
The Test Seal program monitors the input resistance of a patch recording electrode. This is useful for checking the electrode is OK when it is first lowered into extracellular solution, and for monitoring the formation of a GOhm seal. The program delivers a series of small voltage-clamp pulses (typically 1-10 mV), and monitors the clamp current in a scope window. The electrode input resistance is calculated and displayed in the scope window, overlaid on the trace. The input resistance can also be directed to the log window.

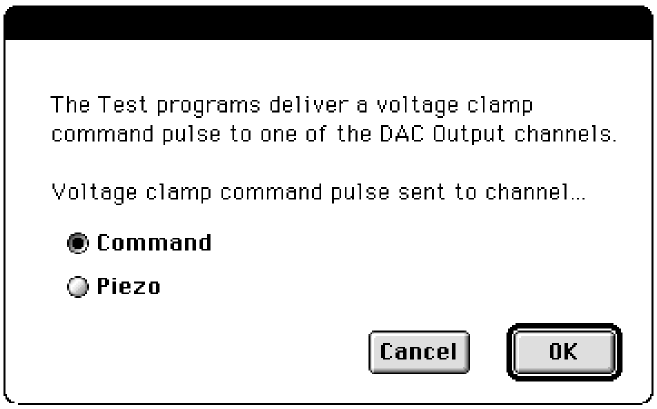
The Test Cell program monitors three electrical parameters when recording in whole-cell voltage-clamp configuration. These are the membrane capacitance and input resistance of the cell, and the series resistance of the electrode. The program delivers a series of small voltage-clamp pulses, and monitors the current in a scope window. The three parameters are calculated from the shape of the response, and displayed in the scope window, overlaid on the trace. The value of the three parameters can also be directed to the log window.

AxoGraph presents a pop-up menu in the toolbar at the bottom-left of the screen. When the Test Pulse item is selected in this pop-up menu, a new window titled Test Pulse Window will appear. The following sections describe the function of each of the buttons in the Test Pulse toolbar.



5.2 Test Channels





The Test Channels button brings up two successive dialogs that define which **Analog Input** channel is connected to the voltage-clamp current signal, and which and **Analog Output** channel is connected to the voltage-clamp command signals. This must be done before using Test Seal or Test Cell.

5.3 Test Pulse Hot Keys

Hot keys can be used to modify the scope display and the test pulse amplitude while the Test Seal or Test Cell program is running. They are a subset of the scope hot keys, described in Section 3.3. To display hot-key information, switch to the Scope toolbar and click on the Hot Key button.

space-bar : halts the test program

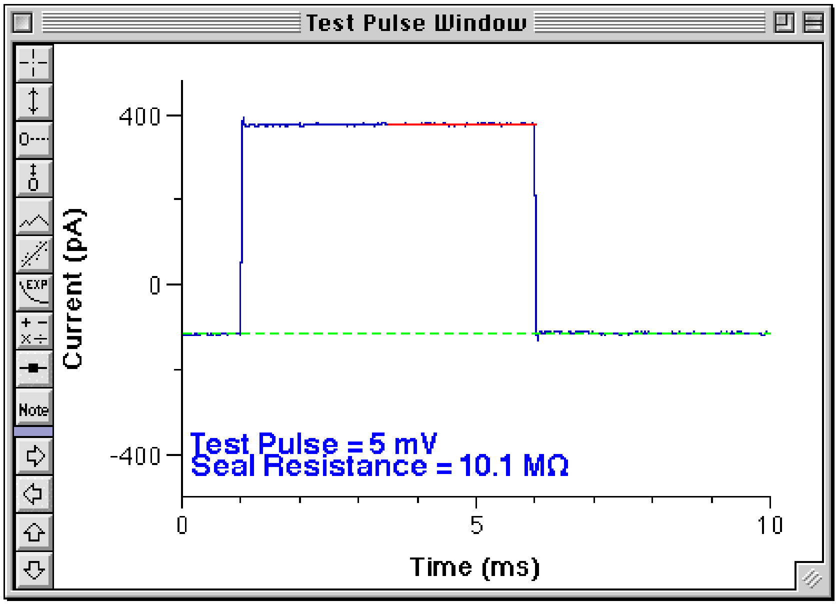
a : Automatically adjust Y axis range to the size of the signal
f : Adjust Y axis to display the full **Analog Input** range

up-arrow : increase Y-axis range (zoom out)
down-arrow : decrease Y-axis range (zoom in)
right-arrow : increase sweep width
left-arrow : decrease sweep width

The following keys modify the test pulse size.

+ : increase pulse amplitude
- : decrease pulse amplitude
i : Invert pulse

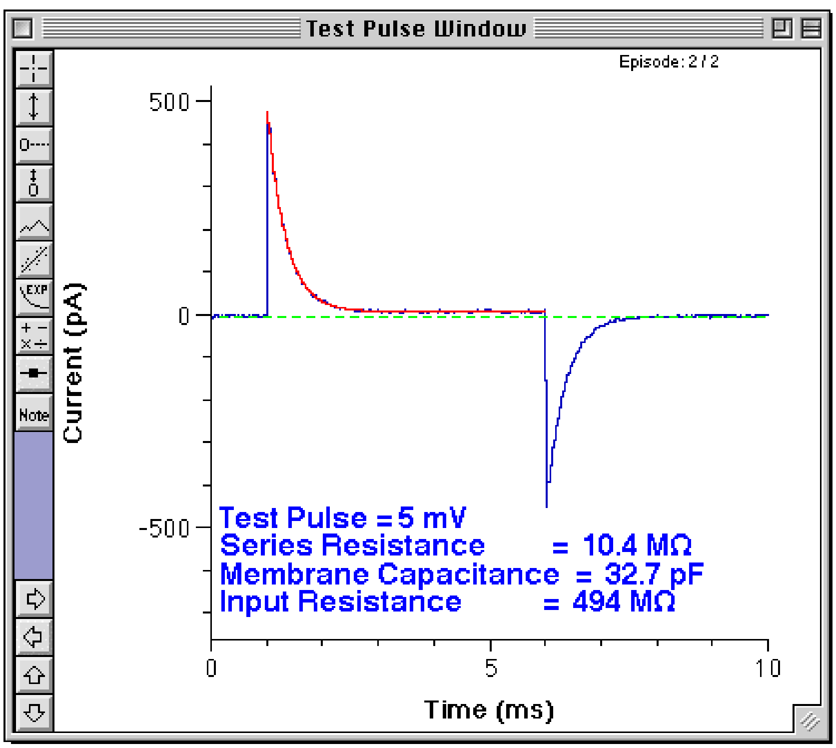
5.4 Test Seal



The Test Seal button activates the scope window that monitors the recording electrode input resistance, or seal resistance. A series of voltage-clamp pulses is delivered, and the current response is monitored. The amplitude of the voltage-clamp pulse, and the seal resistance are displayed at the bottom of the scope window. Hitting the space-bar on the keyboard halts the scope.

The electrode input resistance is calculated from the amplitude of the response to each voltage-clamp pulse. The amplitude is measured by averaging the current over the last half of the pulse response, as indicated by the horizontal red line superimposed on the current response. The amplitude is measured relative to the baseline current before the start of the response, as indicated by the horizontal dashed green line.

5.5 Test Cell



The Test Cell button activates the scope window that monitors the electrode series resistance and the whole-cell membrane capacitance and input resistance. A series of voltage-clamp pulses is delivered, and the current response is monitored. The amplitude of the pulse, and the passive electrical parameters are displayed at the bottom of the scope window. Hitting the space-bar on the keyboard halts the scope.

The passive electrical parameters are calculated by measuring the steady-state current at the end of each pulse response, then fitting an exponential to the first few milliseconds of the response. The fitted exponential and steady-state amplitude are shown as a red line superimposed on the current response. The amplitude is measured relative to the baseline current before the start of the response, as indicated by the horizontal dashed green line.

Several factors will influence the reliability of the Test Cell parameter estimates. It is important that the sampling rate and low-pass filter frequency permit the current decay to be captured without distortion. Also, the test pulse should be long enough for the current response to decay to a steady-state level.

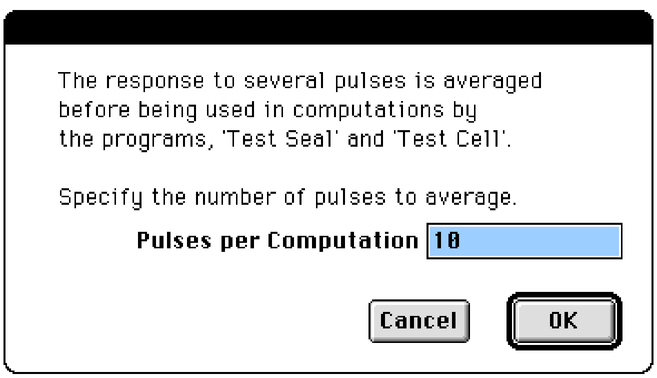
Test Cell will provide a reliable estimate of series resistance and input resistance under most circumstances. Test Cell only provides an accurate estimate of the total membrane capacitance when a cell is ‘isopotential’. In practice this is only true when a cell has no processes (dendrites, axon, etc.), or when the processes are very short or fine. When a cell is isopotential, the current response decays as a single exponential, permitting and accurate estimate of membrane capacitance. If large dendrites or other processes are present, the current decay will follow a multi-exponential time course, because the membrane capacitance is ‘distributed’ along the dendrites. This makes it very difficult to calculate whole-cell capacitance. If the exponential fit is restricted to the first millisecond or so of the response, then the reported capacitance value may represent a useful approximation of the membrane capacitance of the cell body minus the processes. The reported capacitance value will depend on the time range over which the exponential or double-exponential function is fit. This can be adjusted via the Test Setup button, as described in Section 5.7, below.

5.6 Test To Log

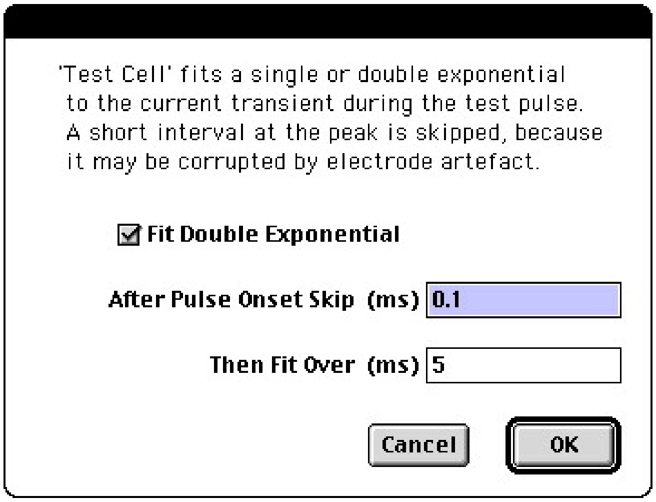
The Test To Log button activates either the Test Seal or the Test Cell scope window, whichever was most recently active. When the scope is halted by hitting the space-bar, the parameter values that are displayed in the scope window will be appended to the log window. The time that the measurement was made will also be noted in the log.

5.7 Test Setup

The Test Setup button brings up two dialogs that adjust the behavior of Test Seal and the Test Cell programs.



Increasing the Pulses per Computation will improve the reliability of the parameter estimates, but will cause the scope window to update more slowly.



A single exponential fit is only suitable for a spherical cell, or a cell with very short, fine process (dendrites, axon, etc.). For most neurons, the current response will decay with a multi-exponential time course, and a double-exponential fit will provide a more accurate estimate of membrane capacitance. Even if the Fit Double Exponential option is on, AxoGraph will automatically revert to a single-exponential fit when appropriate.

5.8 Test Pulse

The Test Pulse button brings up a series of three dialogs requesting the amplitude, onset time and duration of the test pulse. These dialogs are described in detail in Section 3.10, 'Test Pulse'. The pulse amplitude can also be adjusted using 'Hot Keys' (see Section 3.3).

The first dialog requests the pulse amplitude. The second dialog asks whether the onset and duration are to be specified in fixed time units (milliseconds), or as a fraction of the sweep width. If fixed time units are specified, then the third dialog requests the pulse onset time and pulse width in milliseconds. The test pulse duration will remain constant even if the scope sampling rate and sweep width settings are changed. If relative timing is selected, the test pulse will change in duration when the sweep width is changed. The pulse will always occupy the same fraction of the sweep, and the third dialog asks which fraction that will be.

5.9 Test Link

The Test Link feature provides a mechanism for noting the test pulse results to the log window then immediately running a data acquisition protocol. The Test Link button brings up a dialog that requests the file name of an acquisition protocol (see Chapter 6 for an explanation of protocol files). When the Test To Log program is halted by hitting the space-bar, the test results will be directed to the log window, the selected protocol will be activated and data acquisition will commence. See Section 5.6 (above) for a description of the Test To Log program.

6 Protocol Driven Acquisition

6.1 Introduction

The Scope and Chart programs described in Chapters 3 and 4 were designed to emulate familiar laboratory instruments, and so have limited waveform output capabilities. Both programs can only send a simple periodic test pulse to a single output channel. In contrast, the acquisition program described in this chapter takes full advantage of the digitizer's capabilities. The protocol-driven acquisition program can monitor and record signals from **Analog Input** and **Digital Input** channels while sending a complex series of waveforms to multiple **Analog Output** and **Digital Output** channels. Data can also be analysed in real-time as it is acquired.

Data acquisition is controlled by a set of instructions contained in a ‘protocol’. A protocol is a graph file that contains one or more **Analog Output** and **Digital Output** waveforms. It also contains instructions about which **Analog Input** and **Digital Input** channels to record signals from, how to trigger acquisition of each episode, how many episodes to acquire, etc. A protocol window must be open before running a data acquisition program. Detailed information about creating and editing protocols is provided in Chapter 7. Example protocols are supplied in the Acquisition Protocols folder that can be found in the Data Acquisition Package folder inside the AxoGraph 4.9 folder. The function of each of the example protocols is described in Chapter 10.

Data is acquired in a series of separate epochs or ‘episodes’. An episode can be of any length. When the episode is longer than 0.5 seconds, the input signal will be displayed as the data is acquired. For shorter episodes, the data is only displayed when acquisition of that episode is complete. If acquisition is interrupted part way through an episode, the incomplete episode will be discarded.

A protocol may contain one or more waveforms per output channel. As each waveform is output, an episode is acquired. When all waveforms in the protocol have been output, the acquisition program loops back to the first waveform. The protocol specifies how many times the series of output waveforms will be repeated.

The two programs that initiate data acquisition are Preview and Record. Selecting Preview runs a protocol, delivering output waveforms and monitoring the input signals, but does not save the acquired signals. Record runs a protocol, and saves the acquired signals to a new data file. A protocol window must be open before running either of these data acquisition programs.

AxoGraph presents a pop-up menu in the toolbar at the bottom-left of the screen. When the Acquisition item is selected in this pop-up menu, the data acquisition programs are loaded into the toolbar. AxoGraph assumes that data acquisition is about to commence and checks whether a protocol window is open. If no protocols are open, a dialog will appear asking whether to open an existing protocol, or create a new protocol (see Section 6.2). The most common response is to select Open Protocol, then find and open a protocol file (for example, any file in the Acquisition Protocols folder). Next, the Acquisition Monitor window will appear. This window monitors the input signals when a protocol is running.

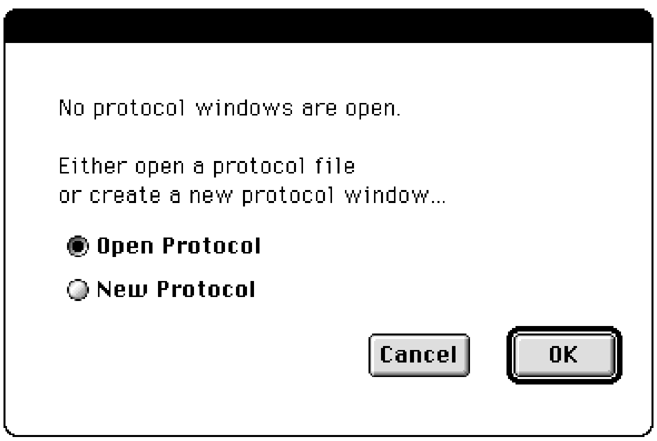
The following sections describe the function of each of the buttons in the Acquisition toolbar.

6.2 Preview

The Preview button initiates data acquisition under the control of a protocol. The waveforms in the protocol window are delivered to the **Analog Output** and **Digital Output** channels, and signals are acquired from the **Analog Input** and **Digital Input** channels specified in the protocol. The input signals are displayed in the Acquisition Monitor window. Acquisition will terminate after the number of repetitions specified in the protocol. Alternatively, hitting the space-bar on the keyboard halts acquisition at any time.

The Preview program is intended for monitoring the response to a protocol without saving the data. It tests whether a protocol is working correctly before committing data to disk.

The name of the protocol that is being run is shown at the top-left of the Acquisition Monitor window, and the episode number is shown at the top-right of the window. The display gain and time range of the monitor window can be changed using the keyboard while the protocol is running (see Section 6.5, ‘Hot Keys’).



A protocol window should already be open before pressing the Preview button. If no protocols are open, then a dialog will appear asking whether to open an existing protocol, or create a new protocol.

If Open Protocol is selected, then a standard open-file dialog appears. Use it to open a protocol file (for example, any file in the Acquisition Protocols folder).

If New Protocol is selected, a series of dialogs appears requesting the information needed to build a basic protocol. These dialogs are described in detail in the next chapter. In particular, see section 7.2, ‘Create a Protocol’.

If several protocols windows are open, the Preview program must decide which one to run. If the front window is a protocol when the Preview button is pressed, then that protocol will be selected. Otherwise, the protocol that was run most recently will be selected. Any of the open protocol windows can be selected by pressing the Protocol button (see Section 6.6, ‘Protocol’).

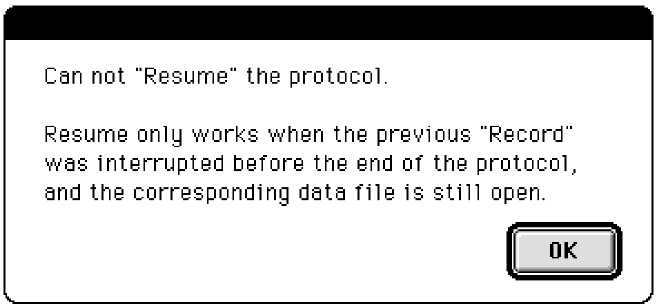
6.3 Record

The Record button runs a protocol and saves the acquired data to disk. Hitting the space-bar halts the protocol. Record behaves exactly like Preview, except that a new graph window is created to receive the acquired data. The name of the new data file will be generated automatically from a root name and an incrementing sequence number. At the completion of the protocol, the new data file is saved to disk and locked to prevent accidental modification at a later date. If the Record button is pressed again, the data window will be closed before a new data file is created.

If the root data file name has not been specified, a series of three dialogs will appear requesting the root name, the sequence number and the location of the folder in which data files should be stored. The root name and destination folder can be changed via the File Name button which brings up the same three dialogs. These dialogs are described in section 6.7, ‘File Name’.

6.4 Resume

If a protocol is interrupted by hitting the space-bar, then the Resume button continues running the protocol from the point at which it was interrupted. The episodes acquired by the Resume program are appended to the data file window.



The Resume button will only work if a previous Record was interrupted, and if the corresponding data file is still open. If these conditions are not met, the following dialog appears.

6.5 Hot Keys

The Hot Keys button opens a documentation window with information about keyboard shortcuts for controlling the display format of the Acquisition Monitor window. Here is a list of the monitor hot keys, and their actions.

space-bar : terminates acquisition

a : Auto-adjust Y axis range to the size of the signal
f : Adjust Y axis to display the full **Analog Input** range
t : Add an event marker (tag) to the log window and data file notes
c : Capture a trace for comparison with subsequent traces
e : Erase monitor
(useful when ‘Erase between sweeps’ is turned off)

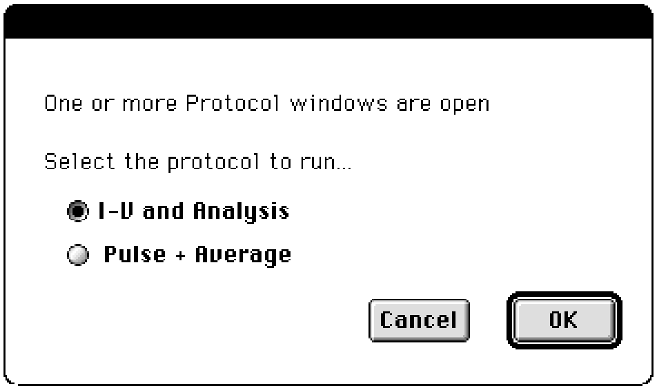
up-arrow : increase Y-axis range (zoom out)
down-arrow : decrease Y-axis range (zoom in)
right-arrow : increase time range (zoom in)
left-arrow : decrease time range (zoom out)

< : Scroll time range to the left
> : Scroll time range to the right

The following keys are active when using an internal trigger.

+ : increase trigger level
- : decrease trigger level

6.6 Protocol

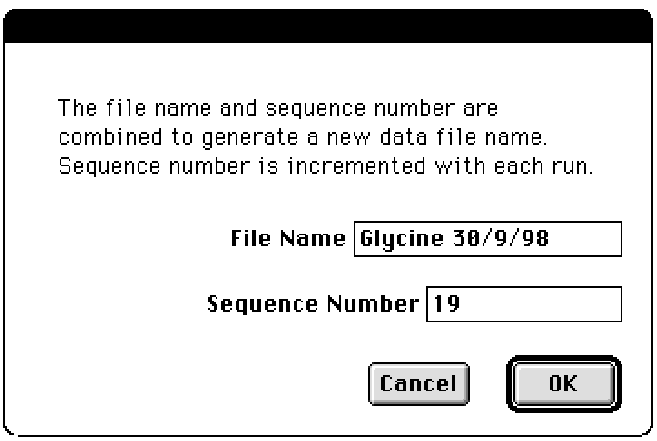


The Protocol button brings up a dialog asking which protocol should be used next time the Preview or Record programs are run.

If no protocols are open, then a dialog will appear asking whether to open an existing protocol, or create a new protocol (see Section 6.2, 'Preview').

6.7 File Name

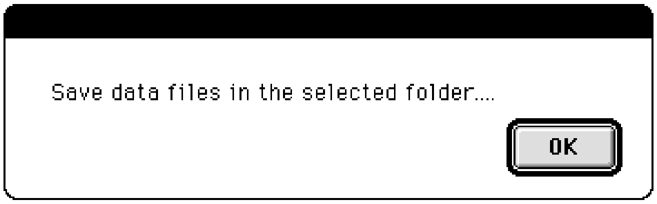
When the Record program is run, a new data file is created to receive the acquired data. The name of the new data file will be generated automatically from a root name and an incrementing sequence number.

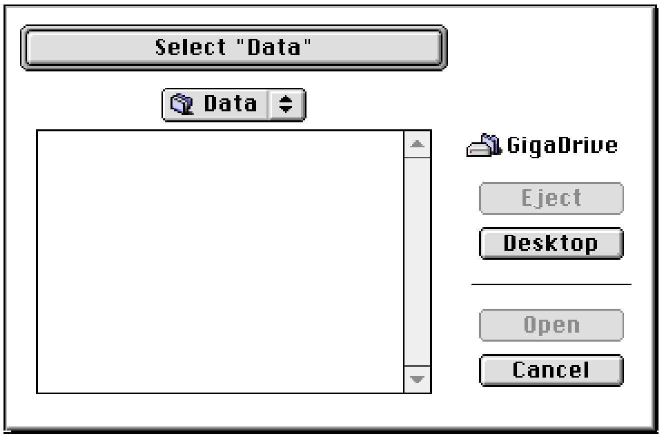


The File Name button initiates a series of three dialogs requesting the root file name, the sequence number and the folder in which data files should be stored.
In this example, the next three data files will be given the names:

Glycine 30/9/98 019
Glycine 30/9/98 020
Glycine 30/9/98 021

After specifying the root data file name and sequence number, two more dialogs appear requesting the destination folder to receive the data files.



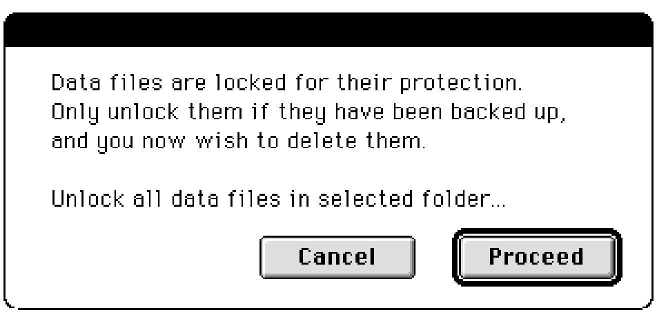


6.8 Deleting Data Files

Data files are locked so that they can not be accidentally modified or deleted. Two mechanisms are available for deleting data files that have been backed up, or are no longer required.

The first mechanism takes advantage of an hidden feature of the Mac OS. The data files are dragged to Trash as usual, but then the "option" key is held down while selecting the menu item, Special ➔ Empty Trash. The option key overrides the error message that normally appears when trying to delete locked files.

The second mechanism uses an AxoGraph program to unlock the data files before dragging them to Trash. The files to be deleted should be gathered in a single folder.



Selecting the AxoGraph menu item, Program ➔ Acquisition ➔ Unlock Data Files brings up the dialog shown at left.

A subsequent dialog is used to identify the folder containing the data files. Every file in that folder will be unlocked, and can then be modified or deleted.

7 Create or Edit a Protocol

7.1 Introduction

Data acquisition is controlled by a set of instructions contained in a ‘protocol’. A protocol is a graph file that contains one or more **Analog Output** and **Digital Output** waveforms. It also contains instructions about which **Analog Input** and **Digital Input** channels to record signals from, how to trigger acquisition of each episode, how many episodes to acquire, etc. A single protocol can output a complex series of waveforms. When necessary, more complex output sequences can be generated by linking several protocols together in a chain, so that they run one after another. This chapter contains detailed information about how to create and edit a protocol, and how to link two or more protocols. Example protocols are supplied in the Acquisition Protocols folder which demonstrate most of the available features (see Chapter 10). The example protocols can by customized or extended using the commands described in this chapter.

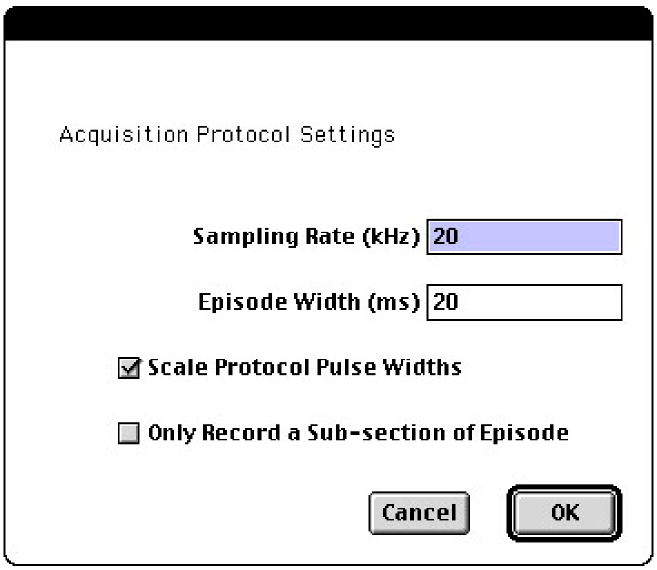
AxoGraph presents a pop-up menu in the toolbar at the bottom-left of the screen. When the Protocol item is selected in this pop-up menu, the protocol editing commands are loaded into the toolbar, and the most recently used protocol window is brought to the front. If no protocols are open, a dialog will appear asking whether to open an existing protocol (for example, one of the protocols supplied in the Acquisition Protocols folder), or create a new protocol (see Section 7.2, ‘Create’). Commands for adding more advanced features to a protocol are loaded into the toolbar by selecting the More Protocol item in the toolbar popup menu. The commands in both these tool bars are always applied to the front protocol window.

The following sections describe the function of each of the buttons in the Protocol and the More Protocol toolbars.

7.2 Create

The Create button creates a new protocol window and initiates a series of up to five dialogs which request the basic information needed to build the protocol. They request the acquisition sample rate and episode width, the input and output channels, the number of waveforms in the output series, and how many times to repeat the output series. These dialogs are described in sections 7.3, 7.4 and 7.5. The information provided via these dialogs is used to build a preliminary protocol in which the output waveforms are set to a flat line at the holding level (typically zero) on each channel (see Section 2.5, 'Holding Levels'). Additional information about the shape of the output waveforms, and the method used to trigger each episode should be added to the preliminary protocol using buttons in the Protocol toolbar. These commands are described in sections 7.6 through to 7.12.

7.3 Timebase



The Timebase button brings up a dialog requesting the data acquisition sample rate and episode width for the front protocol.

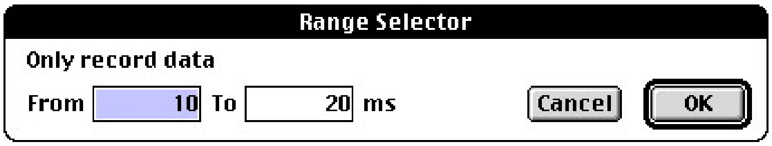
This dialog controls the sample rate used by the data acquisition programs to acquire signals. When several input channels are active, the signal on each channel will be sampled at the requested rate. This dialog also controls the width of each episode in milliseconds.

The sample rate should be at least twice the highest frequency of interest in the signal. The best results are obtained when the signal is low-pass filtered at a frequency equal to half the sample rate.

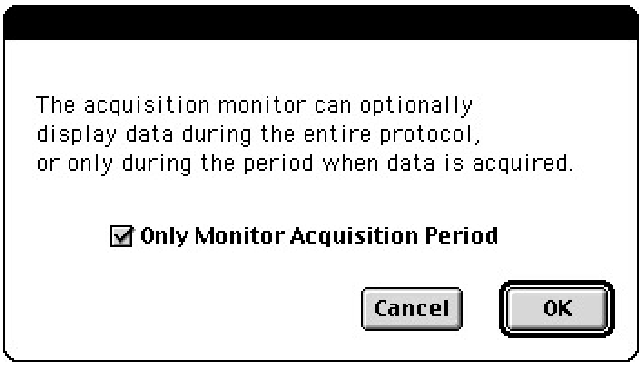
Because of limitations in the digitizer hardware, it is not always possible to sample data points exactly at the requested sampling rate. See Section 11.2, 'Hardware Limitations on the Sampling Rate' for a discussion of these limitations.

When the episode width is changed, the widths of the stimulus pulses (if any) can be handled in one of two ways. Pulse widths can be left unchanged, or they can be scaled to match the change in the episode width. Turing on the Scale Protocol Pulse Widths check box will select the second option.

Some stimulus paradigms require a conditioning stimulus to be delivered well before the test stimulus. Often the response to the conditioning stimulus and the intervening period are of little interest, so only the response to the test stimulus needs to be recorded. Turning on the Only Record a Sub-section of Episode check box in the Timebase dialog, then clicking OK, will present a standard range-selector dialog that asks which sub-section of the protocol to record.

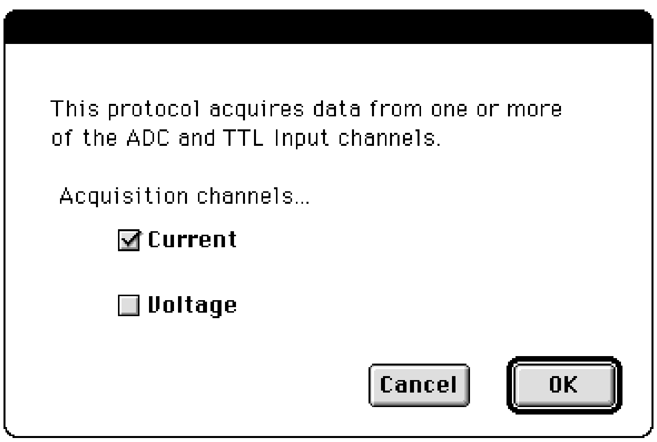


A second dialog asks whether the acquisition monitor window should display the entire width of the protocol, or only the sub-section that is being acquired.



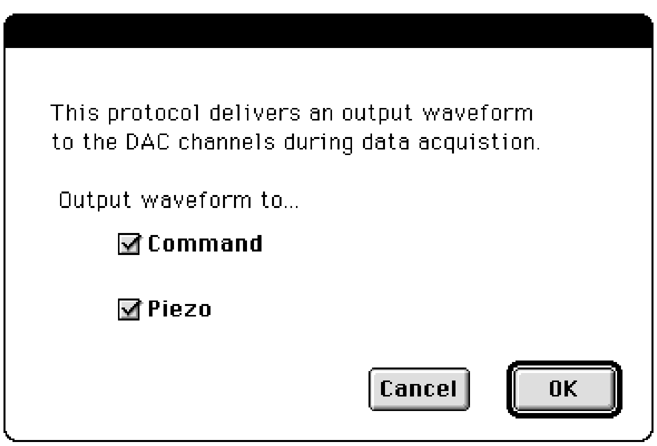
7.4 Channels

The Channels button initiates a series of two dialogs that request the input and output channels to be used by the front protocol. Only channels that have a cable connected are listed in the dialogs (see Section 2.2, ‘Connections’). Channels are listed by name, not by number (see Section 2.3, ‘Channel Names’).



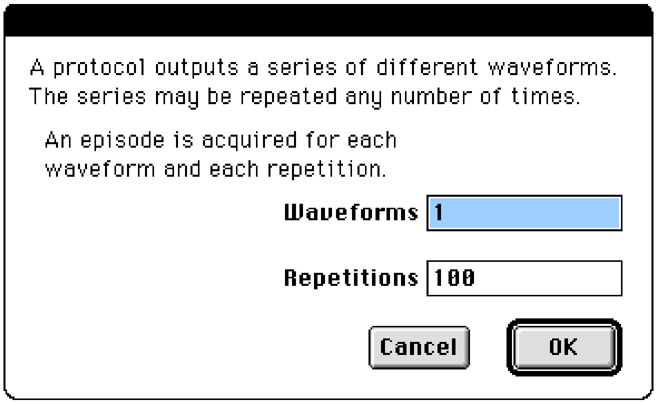
When the protocol is initiated via the Preview or Record programs (see Section 6.2 and 6.3), data will be acquired from the specified **Analog Input** and **Digital Input** channels.

The active **Analog Output** and **Digital Output** channels are specified in the second dialog. This determines which channels will be included in the protocol window.



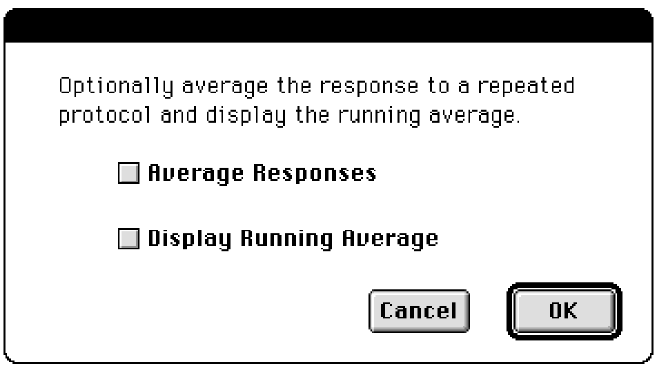
Waveforms can be constructed in the active output channels by adding pulses and ramps (see Section 7.6, ‘Add Pulse’), or by adding custom waveforms that can be any function of time (see Section 7.13, ‘Custom Waveform’).

7.5 Repetitions



If a protocol has active output channels, then it may contain a single output waveform, or a series of different waveforms on each channel. When the protocol is run, the series of waveforms may be repeated any number of times. The Repetitions button brings up a dialog that requests the number of different waveforms in the series, and the number of times to repeat the series.

If a protocol has no active output channels, then the Waveforms field will not appear in this dialog.



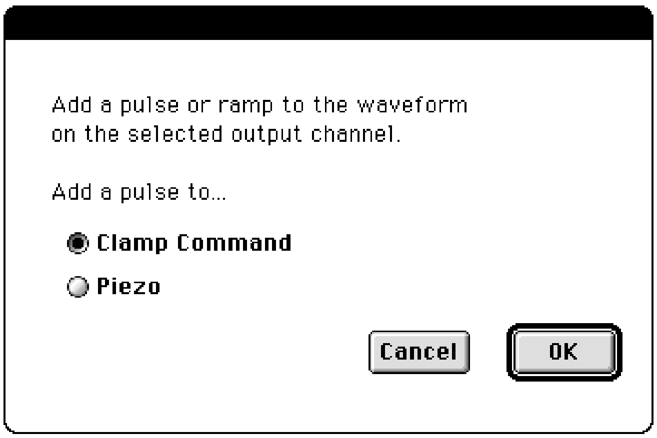
If the specified number of Repetitions is greater than one, then a second dialog will appear asking whether the acquired episodes should be averaged, and whether to display the running average in the Acquisition Monitor window.

If Average Responses is selected, then the ensemble average of the response to each waveform will be calculated when the protocol terminates. For example, if the protocol contains a series of 5 output waveforms, then there will be 5 ensemble average traces per **Analog Input** channel. A new window is created to receive the average traces. This is separate from the data window which receives the raw traces.

If Display Running Average is selected, then the ensemble average is calculated after every repetition of the waveform series. The running average trace is superimposed on the raw trace in the Acquisition Monitor window. If more than one output waveform was specified, then the Display Running Average option will not be available.

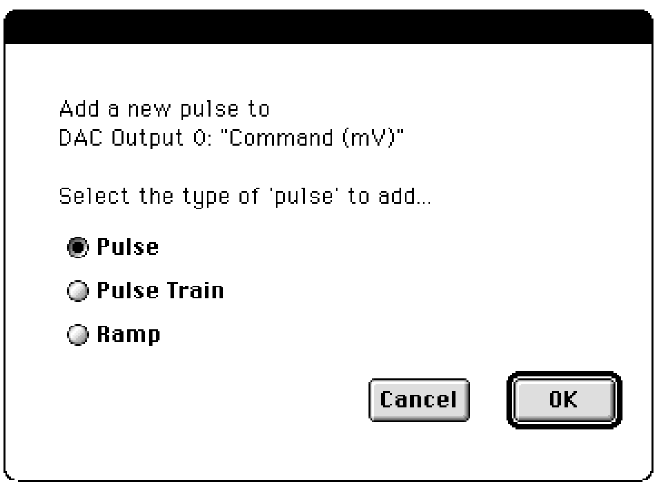
7.6 Add Pulse

When a new protocol is created, all output waveforms are set initially to a flat line at the holding level (usually zero) on each active output channel. The output waveform can be edited in two different ways. The first is by adding square pulses, pulse trains or ramps, as described in this section. The second is by adding a custom waveform which is an arbitrary function of time, as described in Section 7.13, ‘Custom Waveform’.



The Add Pulse button initiates a series of dialogs that request the output channel that the pulse should be added to, the shape and size of the pulse, and the onset time and width of the pulse.

The first dialog only appears if two or more output channels are active. It asks which output channel the new pulse should be added to. Only channels that have a cable connected are listed (see Section 2.2, ‘Connections’). Channels are listed by name, not by number (see Section 2.3, ‘Channel Names’).



The next dialog asks what type of pulse to add.

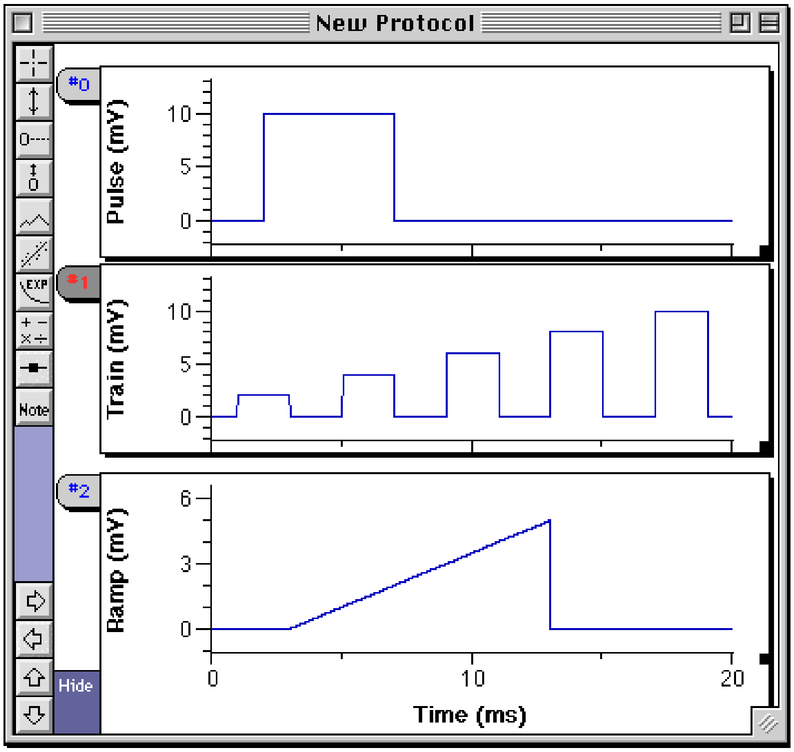
The options are:

Pulse............. a single rectangular pulse

Pulse Train.... several rectangular pulses at regular intervals

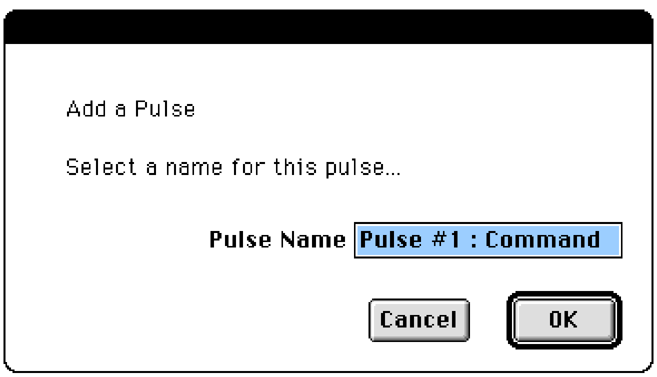
Ramp............ a continuous change in the output signal

Each type of waveform (Pulse, Pulse Train and Ramp) is illustrated in the following protocol window...



The parameters needed to define the shape of a Pulse or a Ramp are identical (amplitude, onset time and width). These parameters are entered via a series of dialogs which is the same for both waveform types. These dialogs are described in the next section.

A different set of parameters is needed to define the shape of a Pulse Train. These parameters are entered via a series of dialogs which is described in a following section (see Section 7.8 'Define Pulse Train Shape').

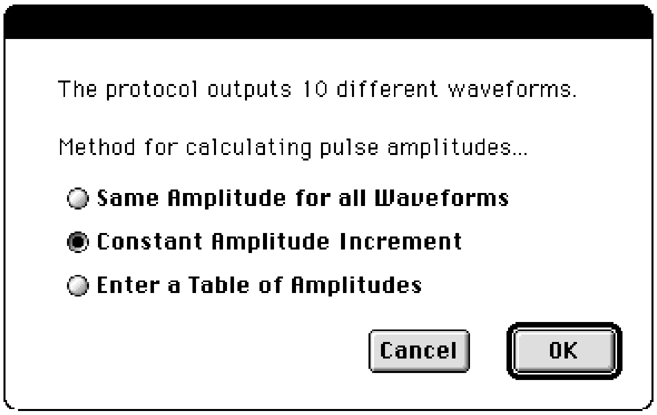


The next dialog is common for all three waveform types. It asks for a name to describe the pulse. A default name is suggested.

Pulse names are useful when a protocol contains a number of pulses on several different channels, and one particular pulse or ramp needs to be edited. The easiest way to identify which pulse to edit in a complex protocol is to give each pulse a distinct name.

7.7 Define Pulse or Ramp Shape

The following series of dialogs will appear if a Pulse or a Ramp has been specified. If the protocol contains only one output waveform, then the first dialog ask for the pulse amplitude and a subsequent dialog asks for the pulse onset time and width. These dialogs are self-explanatory and are not shown.



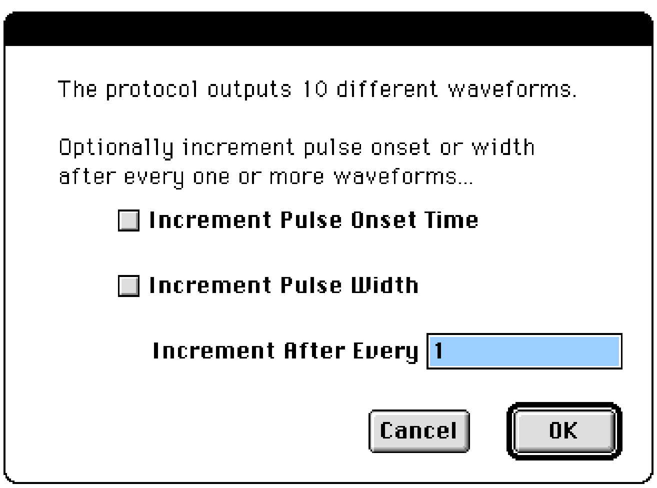
If more than one output waveform has been specified, then a series of three dialogs appears. They request the amplitudes and the timing of the pulse on each waveform. The first dialog asks about the pulse amplitude.

The options are:

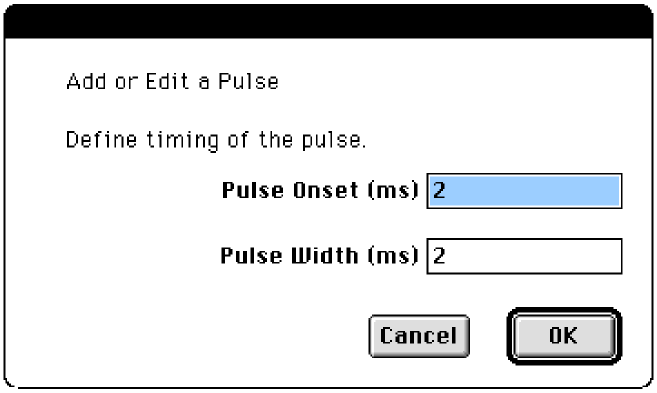
Same Amplitude .... the pulse has the same amplitude on all waveforms
Constant Increment .... the pulse amplitude is incremented after each waveform
Table of Amplitudes ..... the pulse amplitude is different on each waveform, and the individual amplitudes are entered into a table

The next dialog requests the amplitudes of the pulses. The format of the dialog depends on which option is selected in the previous dialog. The pulse amplitude dialog is self explanatory, and is not shown.

The next two dialogs request the pulse onset and width for each waveform.



The first dialog asks whether the pulse onset time or width are to be incremented. These parameters can be incremented after every waveform, or after every few waveforms.



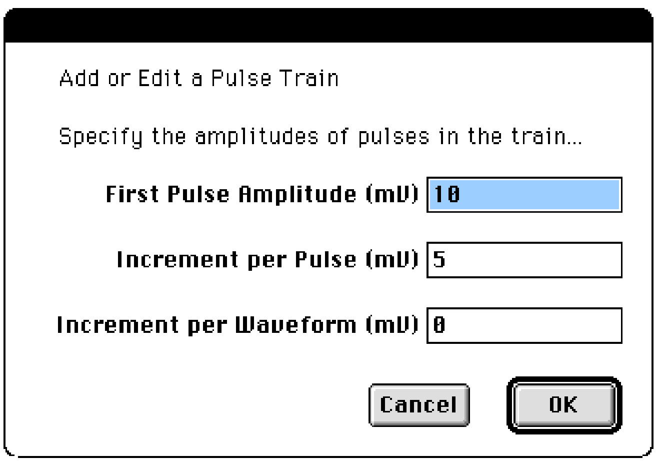
The next dialog requests the pulse onset and width.

If the onset and width increment options were activated in the previous dialog, then this dialog also asks how much to increment the pulse onset and width (increment options are not shown at left).

The shape of the Pulse or Ramp is now completely specified for every output waveform. The waveforms in the protocol window are updated.

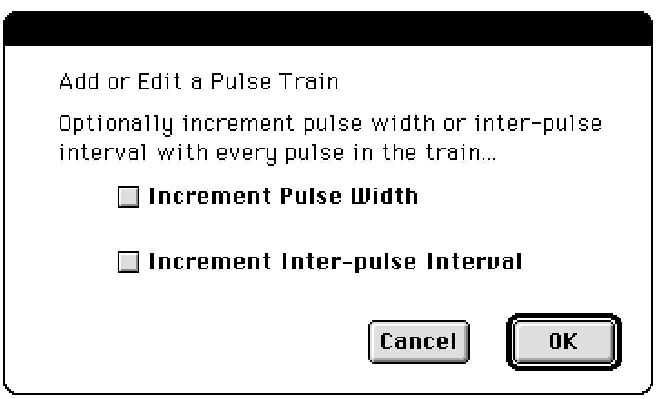
7.8 Define Pulse Train Shape

The following series of dialogs will appear if a Pulse Train has been specified.

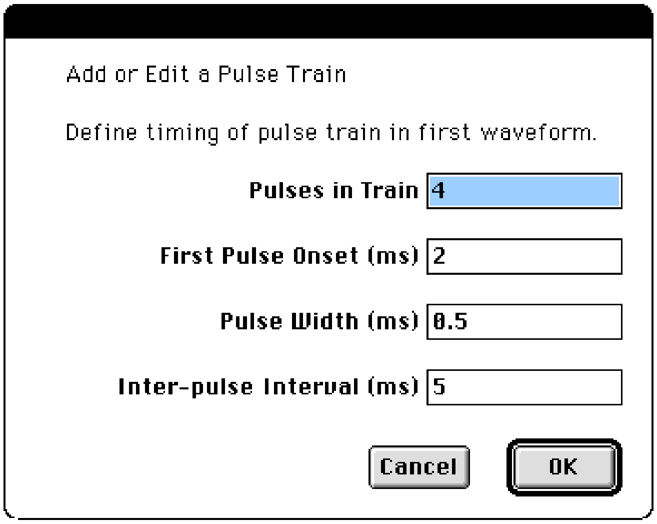


The first dialog asks for the amplitude of the first pulse in the train, and how much to increment the amplitude for each subsequent pulse in the train.

If more than one output waveform has been specified, a third item also appears in this dialog. It asks how much to increment the amplitude of every pulse in the train after each waveform.



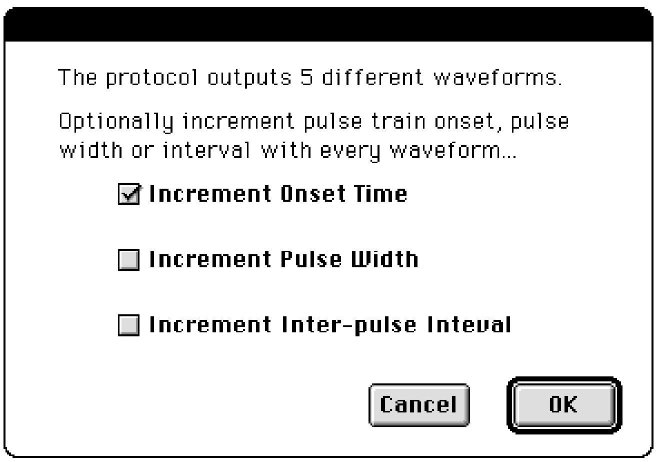
The following two dialogs define the pulse width and inter-pulse interval. The first dialog asks whether the width or the interval are to be incremented for each pulse in the train.



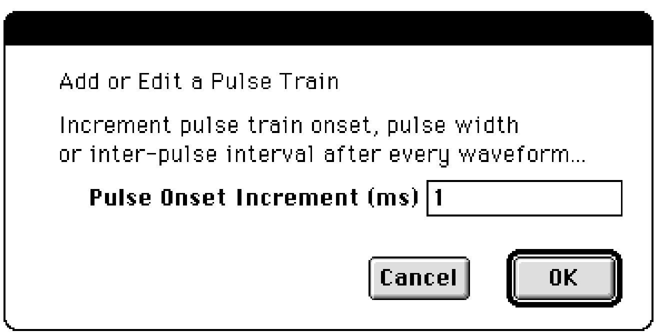
The next dialog requests the number of pulses in the train, and the onset time of the first pulse. It also requests the pulse width and inter-pulse interval.

If the options for incrementing width and interval were activated in the previous dialog, then this dialog will also ask how much to increment the pulse width and inter-pulse interval (increment options are not shown at left).

The next two dialogs only appear if the protocol contains more than one waveform.



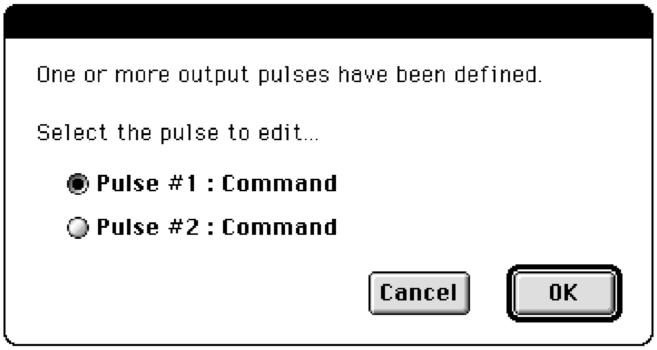
The first dialog asks whether the first pulse onset time, the pulse width or the inter-pulse interval are to be incremented following each waveform.



The next dialog asks how much to increment the first pulse onset time, the pulse width and the inter-pulse interval, if these options were activated in the previous dialog. If no increment options were activated, this dialog does not appear.

The shape of the Pulse Train is now completely specified for every output waveform. The waveforms in the protocol window are updated to display the new pulse trains.

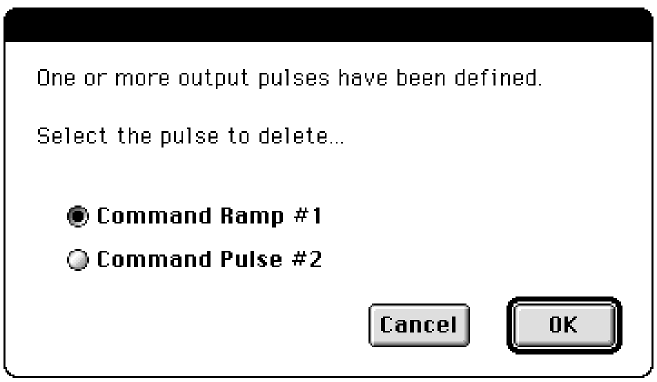
7.9 Edit Pulse



The Edit Pulse button brings up a dialog asking which pulse pulse-train or ramp to modify.

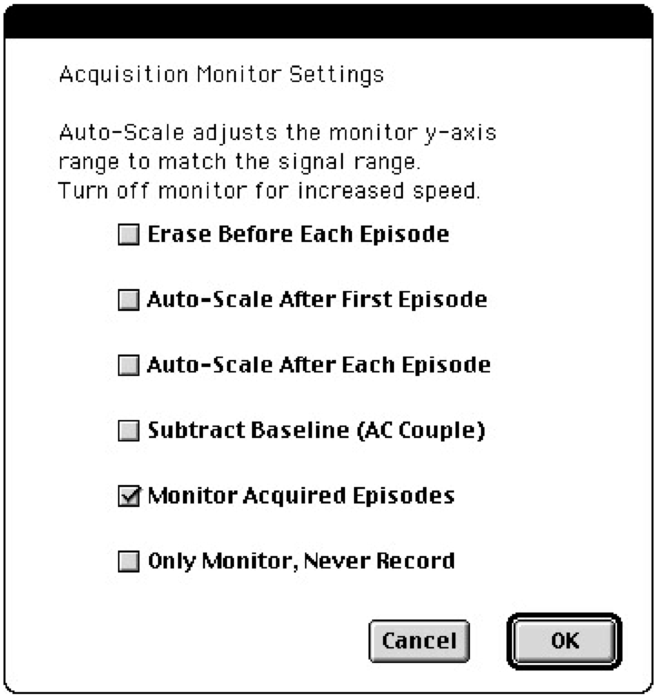
The pulses are listed by name. It is not possible to alter the waveform type (pulse, pulse-train or ramp), but the amplitude and timing parameters can all be changed. These parameters are edited via a series of dialogs that are described in the previous two section (see Sections 7.7 and 7.8).

7.10 Delete Pulse



The Delete Pulse button brings up a dialog that asks which pulse, pulse-train or ramp to delete. The pulses are listed by name. The output waveforms are updated to reflect the change.

7.11 Display



The Display button brings up a series of two dialogs that controls the behaviour of the Acquisition Monitor window.

Erase Before Each Episode If this option is turned on, the monitor will be erased immediately before each episode is displayed. If it is turned off, episodes will be overlaid. The monitor can be erased at any time by hitting the "e" hot-key (see Section 6.5).

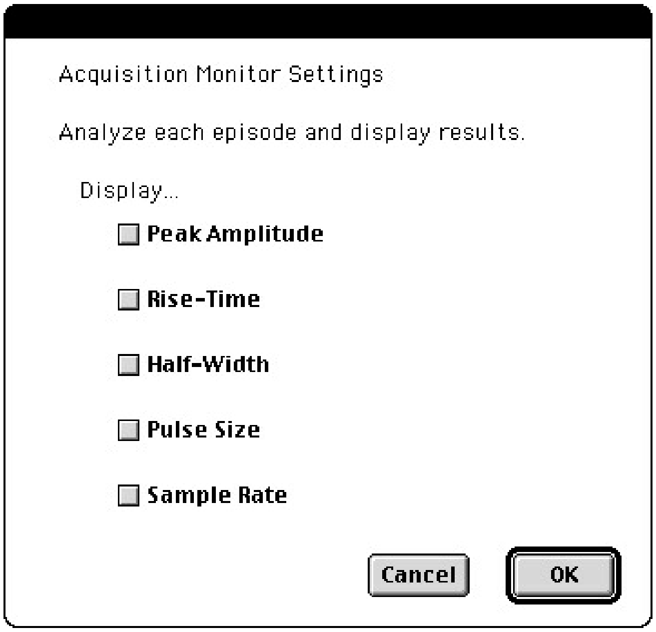
Auto-Scale After First Episode If this option is turned on, the Y axis range of each monitor channel will be adjusted to display the full amplitude of the signal (auto-scale) after the first episode has been acquired. When this option is turned off, the auto-scale function can be performed at any time by hitting "a" hot-key (see Section 6.5).

Auto-Scale After Each Episode If this option is turned on, the monitor will be auto-scaled after every episode. This option will slow the maximum episode repetition rate, and at high repetition rates it will cause the axes to flash. It is only recommended for slow episode repetition rates.

Subtract Baseline (AC Couple) When this option is turned on, the amplitude of the signal on each monitor channel is calculated over a short interval at the start of each episode. This ‘baseline’ amplitude is subtracted from the signal before it is displayed. This procedure emulates the ‘AC couple’ option available on oscilloscopes. It is useful in situations where the signal of interest is superimposed on a larger drifting signal. It stabilizes the signal at the center of the display range. This option only affects the signal displayed in the monitor. It does not affect the signal recorded in the data file.

Monitor Acquired Episodes If this option is turned off, no feedback will be provided during data acquisition. The only reason for turning it off would be to increase performance (see Section 12.4, ‘Disable Acquisition Monitor’).

Only Monitor, Never Record If this option is turned on, the acquisition monitor will display the acquired data, but will not record it to a data file even if the Record button is pressed. A non-recording protocol could be used to deliver a conditioning stimulus as part of a series of linked protocols, or it could be used to create a custom monitoring feature similar to Test Cell.



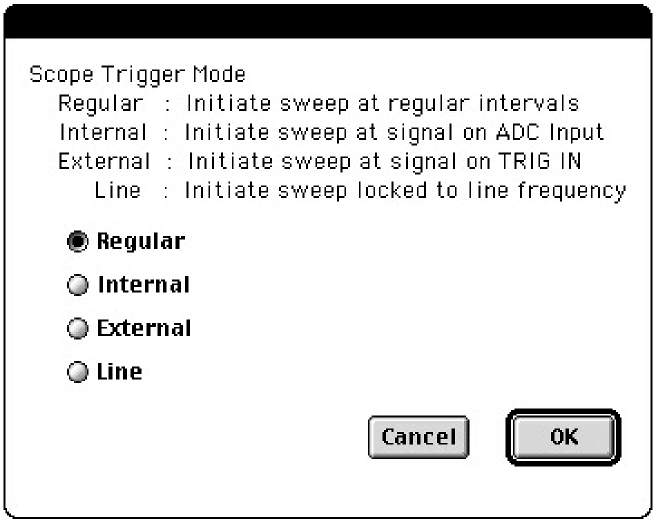
The second Display dialog asks what online analysis feedback to display in the Acquisition Monitor window.

The first three options activate standard programs that analyses each episode immediately after it is acquired. The programs identify the largest event in each episode, and measure three parameters describing that event.

The last two options simply report parameters describing important features of the acquisition protocol.

Peak Amplitude .... The peak amplitude of the event is displayed
Rise-Time .... The 20-80% rise-time of the event is displayed
Half-Width .... The width of the event at 50% of peak amplitude is displayed
Pulse Size .... The amplitude of the first pulse in the protocol is displayed
Sample Rate .... The data acquisition sample rate is displayed in kHz

7.12 Trigger



The Trigger button brings up the Acquisition Trigger Mode dialog. This dialog controls how to initiate (trigger) the acquisition of every episode. There are four options....

Regular
When this mode is selected, episodes are initiated at a regular interval that is specified in a subsequent dialog (not shown). If the episode start-to-start time is set to zero, episodes will be initiated as rapidly as possible. If the Acquisition program can not keep up with the requested episode start-to-start time, a warning dialog will appear when the protocol is halted.

Internal
When this mode is selected, episodes are initiated whenever a signal on one of the **Analog Input** channels crosses a specified threshold. When an Internal trigger is selected, a series of three dialogs appears that request additional information about the trigger threshold, and pretrigger interval. These dialogs were described in a previous chapter (see Section 3.7, ‘Trigger’).

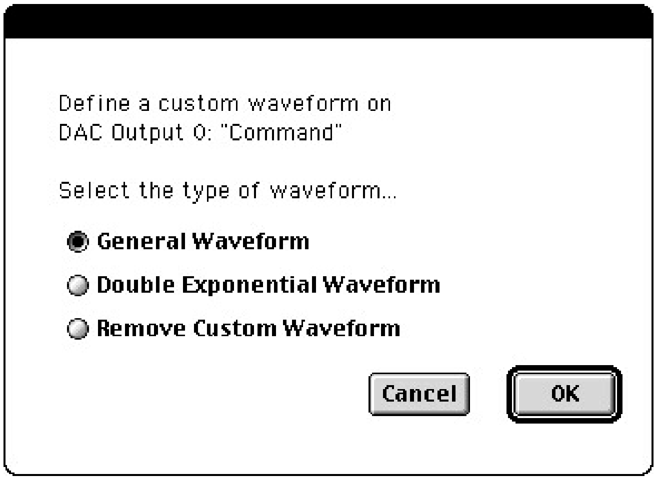
External
When this mode is selected, episodes are initiated whenever a 5 Volt signal appears on the digitizer connector labeled **Trigger Input**. This permits the start of each episode to be synchronized with an external event.

Keyboard
When this mode is selected, an episode is initiated each time the "tab" key is pressed. This permits the start of each episode to be determined manually.

Regardless of which trigger mode is selected, a following dialog will provide the option to Save Trigger Times to Notes. If this option is turned on, the time at which each episode is triggered will be added to the **Notes** field of the data file, when the protocol terminates.

7.13 Custom Waveform

Waveforms on the **Analog Output** channels are not limited to pulses and ramps. They can be set to arbitrary functions of time via the Custom Waveform button. This button initiates a series of two or three dialogs. If the front protocol has two or more active **Analog Output** channels, then the first dialog asks which channel is to receive the custom waveform (dialog not shown).

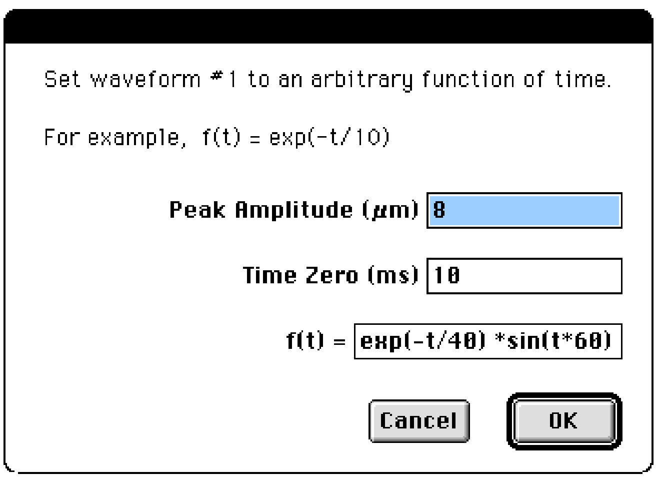


The next dialog asks what kind of custom waveform to add to the **Analog Output** channel. The options are:

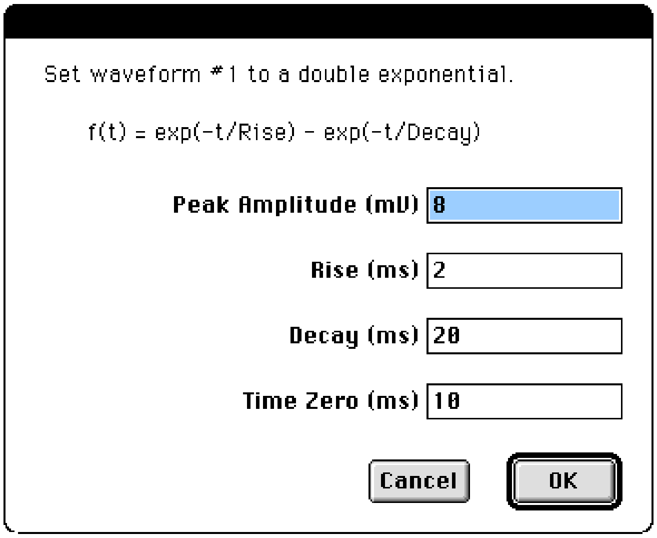
General Waveform
Any mathematical function of time

Double Exponential Waveform
Waveform rises and decays exponentially

If the protocol already contains a custom waveform, a third option appears which permits the removal of all custom waveforms.



The format of the next dialog depends on the response to the previous dialog. If the General Waveform option was selected, the dialog shown at left appears. This dialog requests the peak amplitude of the custom waveform, when to initiate the waveform (time zero), and what function of time the waveform should follow. In this example, the output waveform is defined as a ‘chirp’ function (an exponentially modulated sinusoid). The chirp function starts at 10 ms (time zero), and the preceding 10 ms of the output waveform is zeroed. If the protocol contains a series of waveforms, this dialog will appear once for each waveform.



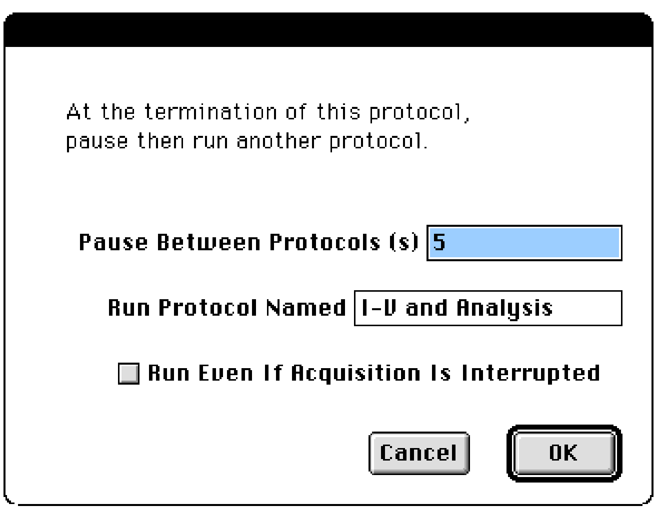
If the Double Exponential Waveform option was selected, the dialog shown at left appears. This dialog requests the peak amplitude, the rise and decay time constants of the double exponential waveform, and when to initiate the waveform (time zero). In this example, the waveform starts at 10 ms (time zero), and the preceding 10 ms is zeroed. If the protocol contains a series of waveforms, this dialog will appear once for each waveform.

When a protocol contains a custom waveform, several limitations are imposed for technical reasons. It is not possible to edit the sample rate, the active **Analog Output** channels, or the pulse parameters, and pulses can not be added or deleted.

The Custom Waveform feature can be used to generate any standard mathematical function. However, some output waveforms may require the summation of several different functions. To achieve more complex output waveform, two or more waveforms can be added together using the Trace Math feature (select the menu item Program ➔ Trace Manipulation ➔ Trace Math). The resulting graph window can then be converted to a protocol by selecting Program ➔ Protocol ➔ Convert to Protocol (see Section 7.17). This feature can take any waveform in any graph window and convert it into a protocol.

7.14 Add a Link

When a protocol terminates, it can automatically initiate another protocol. Several protocols can be linked together to form a chain. The last protocol in the chain can link back to the first protocol, forming a loop. Using this mechanism it is possible to generate output waveform sequences of arbitrary complexity. It also permits the acquisition of a series of episodes with different sample rates and lengths.



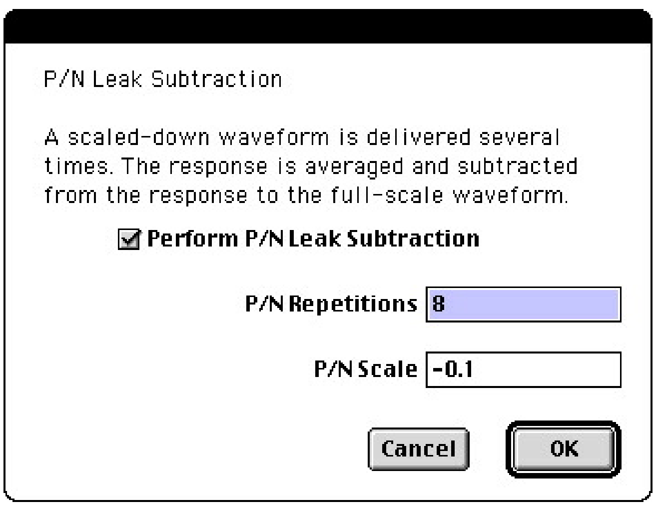
The connection from one protocol to the next is established by adding a ‘link’ to the end of the first protocol. The Add a Link button brings up a dialog that ask for the name of the next protocol to initiate following the termination of the current protocol. It also asks how long to pause before initiating the next protocol, and whether to proceed if the current protocol is interrupted by hitting the space-bar on the keyboard.

7.15 P/N Leak Subtraction

The purpose of P/N leak subtraction is to remove any linear component of the response to a stimulus, and thereby to isolate the non-linear response. It is often used by electrophysiologists to study voltage-activated currents. It is termed 'leak subtraction' because in a voltage-clamp recording, there is always a leak current present (predominantly through voltage-independent potassium channels). This leak current has a linear current-voltage relationship. P/N leak subtraction will remove the leak current from the response to a voltage step, leaving only the voltage-activated current.

The built-in P/N leak subtraction algorithm is relatively simple, and does not permit the raw data to be recorded. Only the leak-subtracted data is displayed in the monitor window and recorded to disk. A more sophisticated form of P/N leak subtraction can be performed using online analysis programs. It permits conditioning voltage steps to be delivered, and raw data to be recorded. An example protocol is supplied with AxoGraph that demonstrates this feature. The protocol is named 'I-V with P/N Leak Subtraction' and is described briefly in a later section (see Section 10.1). The example protocol does not use the built-in P/N feature described here.

The P over N button brings up a dialog that asks whether P/N leak subtraction should be performed on the acquired data. When this feature is activated, each output waveform in the protocol is automatically delivered several times at a reduced amplitude, before being delivered at full amplitude.



In this example, each output waveform is scaled to -1/10th of its original amplitude. It is output 8 times at reduced amplitude, before being delivered at full amplitude. The responses to the reduced amplitude waveforms are ensemble averaged, scaled up 10 times then subtracted from the response to the full amplitude waveform. The resulting signal is displayed in the monitor window, and saved to the data file. Any component of the input signal that scales linearly with the output waveform amplitude will be subtracted away by the P/N procedure. This eliminates any 'passive' or 'leak' component of the response. Only the 'active' voltage-dependent component of the response will remain.

7.16 Extract Protocol

When a data file is saved the parameters defining the protocol that created it are saved in its **Notes** field. The Extract Protocol button reads these parameters and uses them to rebuild the original protocol. It will only work if the front window is a data file. This program may be useful for reconstructing a lost protocol, or for confirming the stimulus conditions that were used to acquire a given data file. For technical reasons, it will not work correctly if the original protocol contained custom waveforms (see Section 7.13).

7.17 Convert To Protocol

Almost any graph file can be converted to a protocol. This permits completely arbitrary output waveforms. For example, a recorded signal could be used as an output waveform. For a graph file to be converted to a protocol, it must meet two requirements. Firstly, the X-axis should have units of time (‘ms’ or ‘s’), and the data points should be regularly spaced. The interval between data points determines the acquisition sample rate. Secondly, the traces should be organized into groups, with the group numbers specifying which **Analog Output** channel the waveforms in that group will be directed to. All groups should contain the same number of traces (episodes). Information on how to group traces is available in the AxoGraph Online Manual, Section 7.4, ‘Combine Traces into Groups’. If a Digidata 1320 series digitizer is in use, then the group numbers should be either 0 or 1, corresponding to the two **Analog Output** channels. For an Instrutech ITC series digitizer, the group numbers should be between 0 and 3.

Selecting the item, Program → Protocol → Convert To Protocol from the AxoGraph menu brings up a dialog that asks whether the front window contains suitable data for conversion. If Proceed is selected, the program performs the conversion by adding protocol information to the **Notes** field of the window, and by changing the names of the groups if necessary, to conform with the **Analog Output** channel names. All protocol parameters will be set to default values. It will be necessary to adjust some of these parameters using the buttons in the Protocol toolbar. For example, the Trigger, Repetition and Channel parameters may need to be changed. For technical reasons, it is not possible to edit the sample rate or the active **Analog Output** channels of the converted protocol, and pulses can not be added.

8 Online Analysis

8.1 Introduction

An acquisition protocol can periodically launch a data analysis program while the protocol is running (online analysis). Analysis programs can operate on the last acquired episode, or on the entire data file. Online analysis can provide immediate graphical feedback about any feature of the acquired data, and can automate routine data analysis tasks.

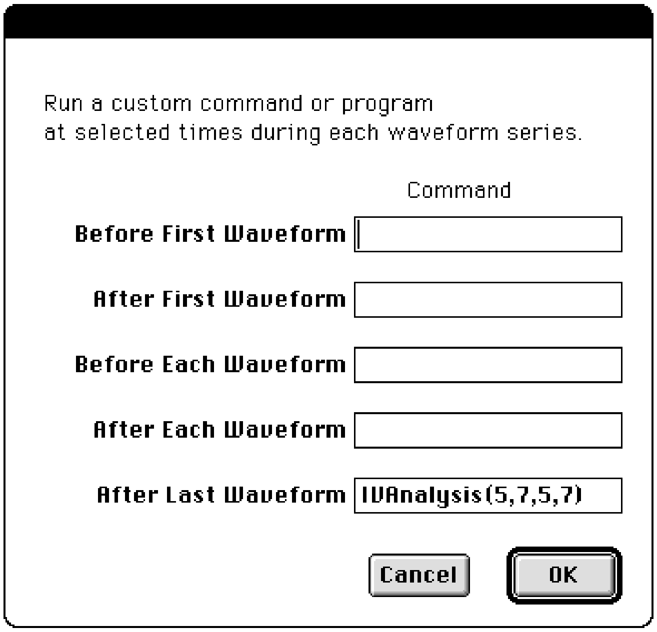
Several different online analysis programs can be run during a protocol, each at a specified time point. A program can only be run before or after an episode, not while an episode is being acquired. A program that is run before the first episode can set up global parameters that control subsequent analysis (e.g. restrict the analysis time range). A program that is launched after each episode can provide graphical feedback about some feature of the acquired data (e.g. the response amplitude). A program that is run after the last episode can analyse the entire data file that has just been acquired and generate a summary graph (e.g. create an amplitude histogram for all the responses).

Several standard online analysis programs are provided as part of the data acquisition package. Some of the example protocols found in the Acquisition Protocols folder incorporate these standard programs (see Chapter 10).

The following sections show how to add online data analysis programs to a protocol, describe the standard online analysis programs, and provide some guidelines and tools for writing new online analysis programs. For general information about writing analysis programs, see the chapter on programming in the AxoGraph Online Manual.

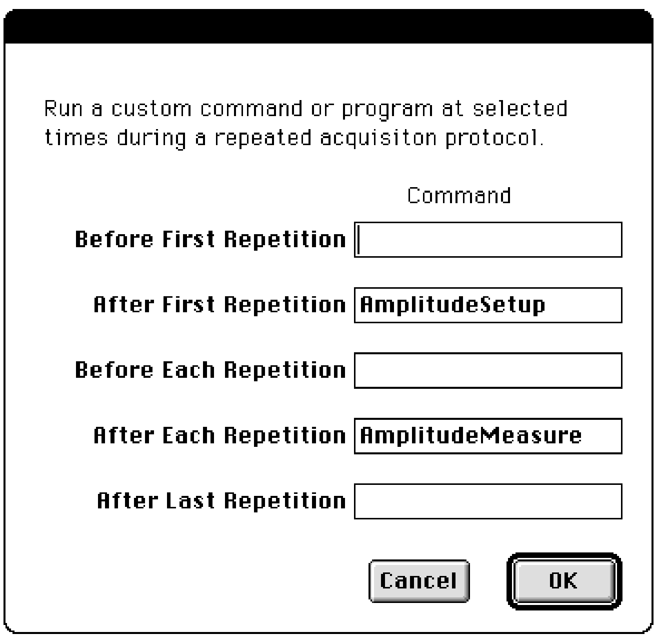
8.2 Add Analysis Programs to a Protocol

Online analysis programs are added to a protocol via the Commands button in the More Protocol toolbar. If several protocol windows are open, the analysis programs are added to the front protocol window.



The Commands button brings up one or two dialogs depending on the number of waveforms, and repetitions specified in the protocol. If a protocol contains more than one output waveform, then the dialog at left will appear requesting the names of programs or procedures to execute at various points during the series of waveforms.

Program names are not case sensitive, and any field can be left blank. This protocol will now run the current-voltage analysis program IVAnalysis after the last waveform in the series has been output and the last episode has been acquired. The program is passed 4 parameters that define the time intervals in milliseconds over which to measure the current and voltage. The program will analyse the entire data file that has just been acquired.



If a protocol repeats a waveform series, then the dialog at left appears requesting the names of programs or functions to execute at various points during the protocol.

Program names are not case sensitive, and any field can be left blank. The protocol will now run the program AmplitudeSetup immediately after the first repetition of the output waveform (or waveform series). This program restricts the time region over which to perform subsequent amplitude measurements. The program AmplitudeMeasure will run after each repetition, and performs the actual amplitude measurement. This program only operates on the most recently acquired episode. It adds each amplitude measurement to a second monitor window that plots the response amplitude versus time.

8.3 Standard Analysis Programs

Several online analysis programs are provided as part of the data acquisition package. These programs are used by some of the example protocols described in Chapter 10. They can be added to any suitable protocol. These programs can also be extended or modified to provide new online analysis functions. Here is a brief descriptions of each of the standard analysis programs.

IVAnalysis (CurrentMin,CurrentMax,VoltageMin,VoltageMax)
This program produces a current-voltage (I-V) plot from a data file consisting of a series of current transient recorded on one **Analog Input** channel, in responses to a series of voltage pulses recorded on a second **Analog Input** channel. The names of the two input channels should contain the words 'Current' and 'Voltage' so that the program can determine which is which. The 'IVAnalysis' program name should be added to the Command dialog field After Last Waveform if the protocol is not repeated, or to the dialog field After Last Repetition if the protocol is repeated (see previous section). This will guarantee that a complete data file is available for analysis. The program name is followed by four numerical parameters (in brackets separated by commas). These specify two time ranges over which to measure the current and voltage amplitudes. The time range parameters are given in milliseconds. The average amplitude of each episode is calculated over the specified time range, and the current amplitudes are plotted against the voltage amplitudes in a new graph window.

IVAnalysisRequestRange
This is very similar to the previous program, but does not require the four time parameters that define the current and voltage measurement regions. Instead, when the protocol is run, the program poses a dialog requesting the measurement regions. The example protocol titled I-V with Analysis uses this program.

IVAnalysisNoVoltageRecord
This is similar to the previous program, but is designed for a protocol which does not record the voltage pulses on an **Analog Input** channel. Instead, the amplitudes of the voltage pulses are obtained from the acquisition protocol. The voltage pulses are delivered to an **Analog Output** channel by the protocol. When the protocol is run, a dialog requests the time range for measuring the current amplitude.

AmplitudeSetup
This program, in cooperation with the following program, monitors the amplitude of a response that occurs within a specified time range during each episode. The protocol should specify multiple repetition of a single output waveform (a stimulus). The 'AmplitudeSetup' program name should be added to the Command dialog field After First Repetition. It creates a new graph window titled Amplitudes that will monitor the response amplitude while the protocol is running. It resizes the standard Acquisition Monitor Window, so that both monitor windows are visible on the screen. It then poses two dialogs that requests the time range over which to calculate the baseline signal, and the time range within which the peak of the response will occur. Peak amplitude is measured relative to the baseline signal.

If an Amplitudes monitor window already exists, the amplitude measurements will be appended to this window. The range dialog will not appear, and the amplitude measurement range that was specified previously will be used. To change the measurement range, the monitor window must be closed before the protocol is run.

AmplitudeMeasure
This program works in conjunction with the previous program. It measures the response amplitude after each episode is acquired, and adds it to the Amplitudes monitor window. The 'AmplitudeMeasure' program name should be added to the Command dialog field After Each Repetition. The example protocol titled Pulse with Amplitudes demonstrates the use of these two programs.

RsAndAmplitudeSetup
This program is very similar to ‘Amplitude Setup’. In cooperation with the following program, it monitors two parameters of each episode: the series resistance of the preparation (Rs) and the amplitude of a response that occurs within a specified time range during each episode. The protocol should specify multiple repetition of a single output waveform on each of two channels (a test pulse and a stimulus). One **Analog Output** channel should deliver a voltage pulse that will be used to monitor the series resistance. This should be the first pulse added to the protocol (see Section 7.6, ‘Add a Pulse’). The second pulse should be used to trigger a stimulus. The ‘RsAndAmplitudeSetup’ program name should be added to the Command dialog field After First Repetition. It creates a new graph window titled Amplitudes and Rs that will monitor the response amplitude and series resistance while the protocol is running. It then poses two dialogs that requests the time ranges for the baseline and the peak amplitude measurements.

RsAndAmplitudeMeasure
This program works in conjunction with the previous program. It measures the response amplitude and series resistance after each episode is acquired, and adds these to the Amplitudes and Rs monitor window. The ‘RsAndAmplitudeMeasure’ program name should be added to the Command dialog field After Each Repetition. The example protocol titled Pulse + Stim and Rs demonstrates the use of these two programs.

EventRateSetup
This program, in cooperation with the following program, monitors the average rate of occurrence of spontaneous events with amplitudes larger than a specified threshold during each episode. The protocol should specify multiple repetitions and have no output waveform. The ‘EventRateSetup’ program name should be added to the Command dialog field After First Repetition. It creates a new graph window titled Event Rate that will monitor this parameter while the protocol is running. It then poses a dialogs that requests the amplitude threshold for the events. It also requests the minimum time between events so that an events with noise on its peak is not counted as two events, and the maximum expected number of events per episode which is used to adjust the y-axis range of the Event Rate monitor window.

EventRateMeasure
This program works in conjunction with the previous program. It measures the rate of occurrence of spontaneous events after each episode is acquired, and adds the result to the Event Rate monitor window. The ‘EventRateMeasure’ program name should be added to the Command dialog field After Each Repetition. The example protocol titled Spontaneous Events with Rate demonstrates the use of these two programs.

PonNSetup
This program, in cooperation with the following program, performs P/N leak subtraction. The purpose of P/N leak subtraction is to remove any linear component of the response to a stimulus, and thereby to isolate the non-linear response. It is often used by electrophysiologists to study voltage-activated currents. It is termed ‘leak subtraction’ because in a voltage-clamp recording, there is always a leak current present (predominantly through voltage-independent potassium channels). This leak current has a linear current-voltage relationship. P/N leak subtraction will remove the leak current from the response to a voltage step, leaving only the voltage-activated current.

P/N leak subtraction works as follows. Each output waveform in the protocol is delivered several times at a reduced amplitude, before being delivered at full amplitude. The responses to the reduced amplitude waveforms are ensemble averaged, scaled up then subtracted from the response to the full amplitude waveform. Any component of the input signal that scales linearly with the output waveform amplitude will be subtracted away by the P/N procedure. This eliminates any ‘passive’ or ‘leak’ component of the response. Only the ‘active’ voltage-dependent component of the response will remain. The resulting leak-subtracted signal is displayed in a separate P/N analysis window.

The reduced amplitude waveforms may be superimposed on a long step to a different holding level. A hyperpolarized holding level is typically used to inactivate any voltage-dependent channels that may be open at the resting level.

The P/N protocol must be set up to contain 4 pulses in the following order...

1) Pre-pulse Holding Level
2) Pre-pulse Train
3) Test Holding Level
4) Test Pulse

The analysis region is determined from the pre-pulse train. Its length is equal to the pulse width plus the interpulse interval. The pulse is at the center of this region. The 'EventRateSetup' program name should be added to the Command dialog field Before First Repetition.

PonNMeasure

This program works in conjunction with the previous program. It performs P/N leak subtraction after each episode is acquired, and adds the resulting waveform to the P/N results window. The 'PonNMeasure' program name should be added to the Command dialog field After Each Repetition. The example protocol titled I-V with P/N Leak Subtraction demonstrates the use of these two programs.

8.4 Writing New Analysis Programs

For general information about writing an online analysis program, see the chapter on programming in the AxoGraph Online Manual. Online programming documentation that can be accessed by selecting Program → Programming Help... Three online analysis programs are presented below. They illustrate techniques for accessing data from the most recent episode, analysing it and reporting the result.

The first program displays the baseline noise standard deviation in the Acquisition Monitor window. To activate this program, save it as a text file in the Plug-In Programs folder. Next, select Program → Reload Plug-Ins. Finally, enter the program name 'OnlineNoiseSD' in the After Every Repetition field of the online analysis dialog.

```
LocalLanguage C

/* Custom analysis program calculates and displays
   the standard deviation (SD) of the signal measured
   over the first 10% of each acquired episode */

void OnlineNoiseSD
{
/* Declare local variables */
   short window, trace;
   float yArray[0];
   float xMin, xMax;
   float noiseSD, theScale;
   string yUnits;

/* Get the first 10% of the front trace */
   GetFront (window, trace);
   GetXRange (window, xMin, xMax);
   xMax *= 0.1;
   yArray = yRange(window, trace, xMin, xMax);

/* Calculate the SD */
   noiseSD = SD(yArray);

/* Get the displayed Y-axis units and scale factor */
   DisplayedYUnits(window, trace, yUnits);
   DisplayedYScale(window, trace, theScale);

/* Display the noise SD using the Y-axis units */
   DrawMove (xMin, Mean(yArray)+2*noiseSD);
   DrawSetSize (12);
   DrawString (concat(noiseSD*theScale:3,yUnits));
}
```

When the protocol is run, the Acquisition Monitor window will display the baseline noise SD superimposed on each episode.

Note that the above program is exactly the same as the Scope custom analysis program presented at the end of Chapter 3. The program works in both contexts because it applies the analysis and directs the feedback to the front graph window. When the Scope program is running, this will be the Scope Window. When a protocol is running this will be the Acquisition Monitor window.

The standard analysis programs AmplitudeSetup and AmplitudeMeasure will be presented below. But first, several global variables and a utility procedure are introduced.

The standard online analysis programs take advantage of several global parameters defined in a file named Custom Analysis Globals. Here is a brief description of each of these global variables ...

acqRefMin, acqRefMax .... baseline time range
acqAnalysisMin, acqAnalysisMax .... the peak measurement time range
acqRsMin, acqRsMax .... time range for calculating series resistance
acqRsPulseSize .... series resistance test pulse amplitude
acqAnalysisWindow .... destination window for the analysis results
acqAnalysisEpisodes .... a counter for number of episodes analysed
acqAnalysisData .... an array that accumulates the analysis results
acqRsData .... an array that accumulates the Rs results
acqAnalysisAverage .... an array for ensemble average calculation

These variables are defined as global for two reasons: 1) so that they can be accessed by all the online analysis programs, and 2) so that the time range values will default to their most recent settings in the range dialogs.

The standard online analysis programs also use several global variables that are created and maintained by the data acquisition programs...

monitorWindow .... the window number of the acquisition monitor
dataWindow .... the window number of the data file (if running Record)
prRepetitions .... the number of times the active protocol is repeated
prEpisodes .... the number of episodes in the data file
prPulseOnset[0] .... the onset time of the first pulse (in ms)
prPulseWidth[0] .... the width of the first pulse (in ms)
prPulseAmplitude[0] .... the amplitude of the first pulse (in output channel units)

Several of the standard online analysis programs take advantage of a utility procedure NewAnalysisWindow. This procedure is passed a window name, a boolean variable (set to ‘true’ if a new analysis window is created), and the number of traces in the analysis window (= the number of different analysis results to monitor). This procedure creates a new monitor window in which to plot analysis results during data acquisition. It resizes both the new window, and the Acquisition Monitor window so that both are visible on the screen. If the monitor window is already open, it updates its X-axis range so that it can receive new measurements.

Two of the standard online analysis programs described in Section 8.3,
are presented below. The original source code for these programs can be found in the file Measure Amplitude. This file is located in the folder... Plug-In Programs : Acquisition Programs : Protocol.
A description of the function of these programs, and instructions for attaching them to a protocol are given in Section 8.3, above.

```
LocalLanguage C

/*---------------------------------------------------------

   Online analysis program calculates and displays
   the peak amplitude of the signal measured
   over a specified time range of each sweep.

----------------------------------------------------------*/

/* Define the monitor window name as a global variable because it is
   used by both the AmplitudeSetup and AmplitudeMeasure programs */

   string AmplitudeFileName;
   AmplitudeFileName = "Amplitudes";


void AmplitudeSetup
{
   boolean newWindow;

/* Check that this protocol is repeated at least twice. The global
   variable "prRepetitions" defines the number of repetitions */
   if (prRepetitions <= 1) Exit;

/* Create a new monitor window using the NewAnalysisWindow utility routine */
   NewAnalysisWindow (AmplitudeFileName, newWindow, 1);
```

```
    SetFront (acqAnalysisWindow,1);
    SetFront (monitorWindow,1);

    if (newWindow) {
    /* If a new monitor window was created, request new baseline
    and peak amplitude measurement regions.
    The parameters acqRefMin, acqRefMax, acqAnalysisMin, acqAnalysisMax
    are global variables available for use by any acquisition program */
    GetRange (monitorWindow, "Subtract Baseline", acqRefMin, acqRefMax);
    GetRange (monitorWindow, "Measure Peak Amplitude",
    acqAnalysisMin, acqAnalysisMax);
    }
}


void AmplitudeMeasure
{
    float refArray[0];
    float dataArray[0];

/* Check that this protocol is repeated at least twice. */
    if (prRepetitions <= 1) Exit;

/* Check the analysis window number is correct.
    It might change if a protocol is halted then Resumed */
    if (WindowTitle(acqAnalysisWindow) != AmplitudeFileName) {
    NewAnalysisWindow (AmplitudeFileName, newWindow, 1);
    }

/* Get the selected regions of the most recently acquired signal
    (the signal displayed in the Acquisition Monitor window) */
    refArray = YRange (monitorWindow, 1, acqRefMin, acqRefMax);
    dataArray = YRange (monitorWindow, 1, acqAnalysisMin, acqAnalysisMax);

/* Adjust the size of the global array that
    receives the amplitude measurements */
    SetArraySize(acqAnalysisData,acqAnalysisEpisode+1);

/* Perform the amplitude measurement, add it to the measurements array
    and increment the episode counter */
    acqAnalysisData[acqAnalysisEpisode] = Max(Abs(dataArray-Mean(refArray)));
    acqAnalysisEpisode++;

/* Pass the measurements array to the Amplitudes monitor window */
    YData(acqAnalysisWindow,1) = acqAnalysisData;

/* Redraw the Amplitudes monitor window */
    DontRecalculateYAxis (acqAnalysisWindow);
    DrawTrace (acqAnalysisWindow,1,eraseFirst);
}
```

9 The Protocol Launch List

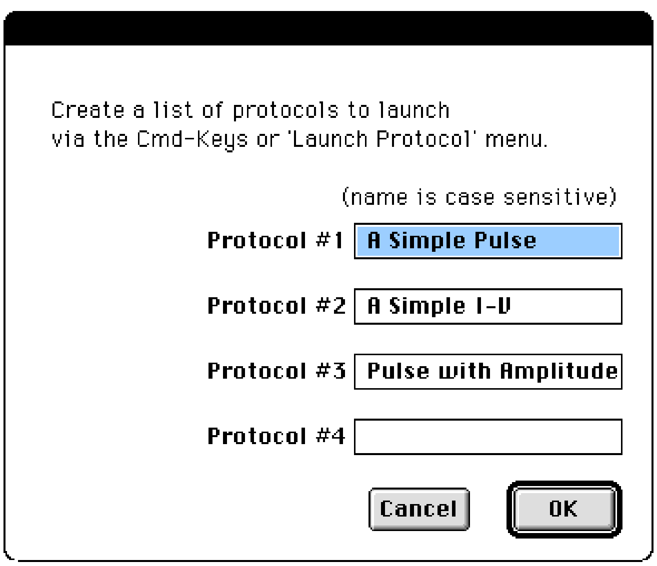
9.1 Introduction

Protocol files can be opened manually via a standard file dialog, then selected and run via buttons in the Acquisition toolbar (see Chapter 6). An alternative mechanism is provided that can open and run a protocol with a single keystroke. This is termed 'launching' the protocol. The launch mechanism requires that the protocol file reside in a specific folder, and the file name must be a member of a predefined 'launch list'. Setting up the launch list takes a little work, but the benefit is faster and more convenient access to protocols that are used most frequently.

The three programs for setting up and editing the launch list are listed under the Launch Protocol submenu of the Program menu. Each of the programs is described in the following sections.

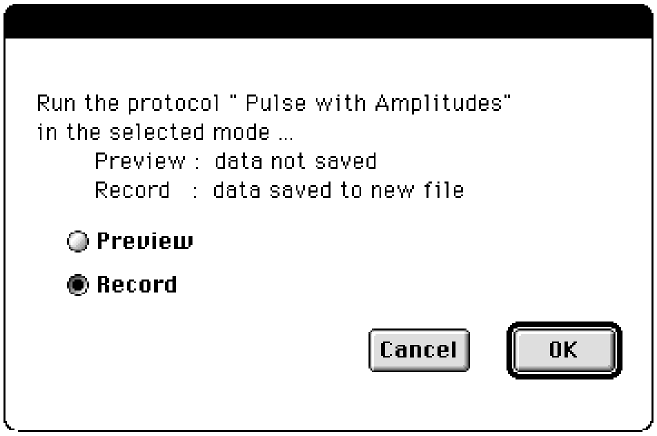
9.2 Edit Launch List

Selecting Program → Launch Protocol → Edit Launch List brings up the launch list dialog containing 8 fields (only 4 are shown here). A protocol file name can be entered in each field. The name is case sensitive and must include any trailing or leading blanks. Each of the protocols on the launch list should be saved in the Acquisition Protocols sub-folder of Acquisition Package.



In this example, the first protocol on the list is named ' A Simple Pulse', and it can be launched by selecting, Program → Launch Protocol → Launch #1 or by holding down the Command key and typing "1" ("Cmd-1").

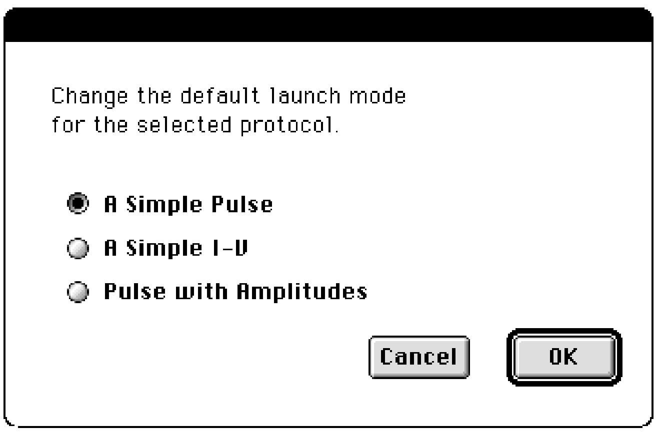
The second protocol named ' A Simple I-V' can be launched by selecting Launch #2 or by typing "Cmd-2".
Similarly for the other protocols on the launch list.



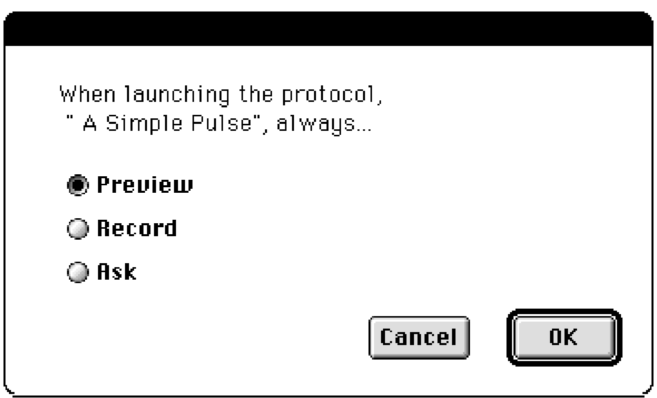
When a protocol is launched via a launch list command or keyboard shortcut, the protocol window will be opened, and data acquisition will be initiated in either Preview or Record mode. A dialog will appear asking which mode to use.

This dialog can be bypassed for faster launching. Bypass requires that the launch mode for a given protocol be predefined, as described in the next section.

9.3 Default Launch Mode



Some protocols should always be launched in either Preview or Record mode. A default launch mode can be predefined for each protocol on the launch list. Selecting Program → Launch Protocol → Default Launch Mode
brings up a dialog which selects one protocol on the launch list.



The subsequent dialog defines the default launch mode for the selected protocol.

9.4 Open Listed Protocols

Selecting Program → Launch Protocol → Open Listed Protocols opens each of the protocols on the launch list. This is a convenient way to open all the protocols required for a given experiment with a single menu selection.

9.5 Remove the Launch List Features

If the launch list programs described above are not required, they can be removed to simplify the Program menu. Open the Acquisition Programs folder (inside the Plug-In Programs folder). Drag the Launch Protocol folder to Trash, or store it in the AxoGraph folder. Quit and re-run AxoGraph.

10 Example Protocols

10.1 Introduction

Twenty-one example protocols can be found in the Acquisition Protocols sub-folder of the Acquisition Package folder. They demonstrate many of the advanced features of the data acquisition package. These protocols can be extended or customized using the protocol editing tools described in Chapters 7 and 8.

The example protocols assume a minimal configuration of connections to the digitizer. They assume that **Analog Outputs** 0 and 1 both have a connection, and that **Analog Input** channels named 'Current' and 'Voltage' are connected. A few of the example protocols also assume that **Digital Output** 0 is connected.

A brief outline of the example protocols is given in the next section. This is followed by a more detailed description of each protocol.

10.2 Brief Overview of Each Protocol

A Simple Pulse Acquires up to 500 episodes from an input channel while sending a square voltage pulse to an output channel.

Pulse + Average Acquires and averages 50 episodes from an input channel while sending a square voltage pulse to an output channel.

Pulse + Amplitudes Acquires up to 500 episodes from an input channel while sending a voltage pulse to an output channel. Analyses each acquired episode and monitors the response amplitude in a separate window.

Pulse + Stimulus and Rs Acquires up to 500 episodes from an input channel while sending voltage pulses to two output channels. Analyses each acquired episode and monitors the response amplitude and the series resistance.

Pulse + Slope Acquires up to 500 episodes from an input channel while sending a voltage pulse to an output channel. Analyses each acquired episode and monitors the slope of the response over a selected time region.

Pulse + Two Amplitudes Acquires up to 500 episodes from an input channel while sending a voltage pulse to an output channel. Analyses each acquired episode and monitors the response amplitude at two selected time points.

A Simple I-V Delivers a series of 20 square voltage pulses covering a range of amplitudes from -40 to +150 mV to an output channel. Records the current and voltage responses on two input channels.

I-V with Analysis Delivers a series of 20 square voltage pulses covering a range of amplitudes to an output channel. Records the current and voltage responses on two input channels, then analyses them to generate an I-V plot.

I-V using Command Table Delivers a series of 10 square voltage pulses to an output channel, and records the current and voltage responses on two input channels. An arbitrary series of pulse amplitudes is specified in a table.

I-V with P/N Leak Subtraction Delivers a series of 10 voltage pulses to an output channel, and records the current responses. The linear or 'passive' component of the current response is automatically subtracted to reveal any non-linear or 'voltage-dependent' component.

Paired-Pulse AP with P/N Delivers a voltage-clamp waveform consisting of two action potentials separated by 10 ms. The paired action potentials are preceded by a series of 8 pairs of inverted scaled-down action potentials. The output waveform is repeated up to 5 times. The recorded response to each waveform is analysed online using P/N leak subtraction. The linear or 'passive' component of the current and voltage responses is automatically subtracted to reveal non-linear or voltage-dependent' components.

Ramp Then Link to I-V Delivers a voltage command waveform that ramps over the range from -100 to +100 mV. Repeats the waveform 10 times, and records the current and voltage responses on two input channels. When complete, this protocol automatically runs another protocol.

Keyboard Trigger Acquires up to 100 episodes from an input channel while sending a square pulse to an output channel. Each episode is triggered via the keyboard (by hitting the "tab" key).

Trigger on Event Acquires up to 500 episodes from an input channel, but does not output any signal. Each episode is triggered when the slope of the signal on the input channel exceeds a threshold.

Chirp Acquires up to 100 episodes from an input channel while sending a 'chirp' waveform to an output channel. This custom waveform consists of a 1 kHz sinusoid with an amplitude that decays exponentially.

LTP Induction (Pairing) Delivers a train of 100 pulses at a rate of 100 Hz to trigger a presynaptic stimulator. Simultaneously, delivers a voltage command pulse of +50 mV to depolarize the postsynaptic neuron. This 'paired' stimulus is delivered every 10 seconds, and is repeated 5 times.

Toggle Digital Output #0 Record a series of up to 500 episodes. Before the start of each episode, toggle the setting of **Digital Output** 0. This is achieved using online analysis commands.

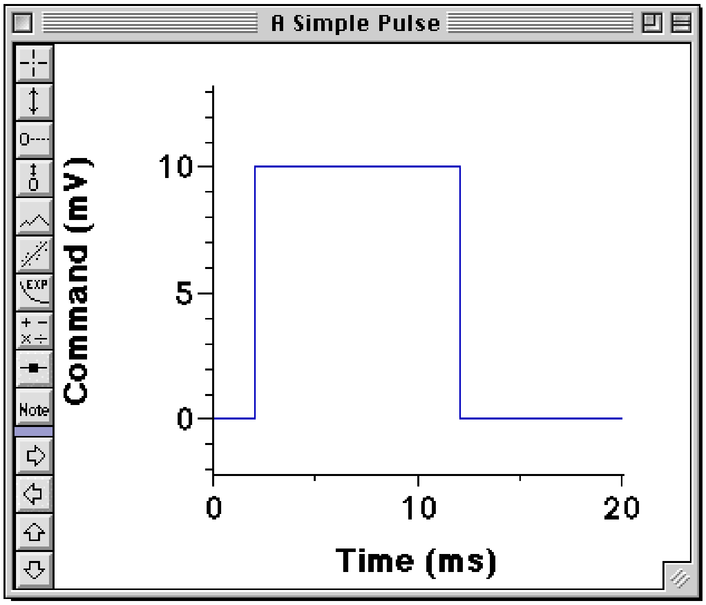
Stimulus Preceding Episode Delivers two rectangular pulses to an output channel. Only the response to the second stimulus is recorded. The first pulse is the 'conditioning' stimulus, and it is delivered 1 sec before the start of the recorded episode. The second pulse is the 'test' stimulus, and the response is recorded over the last 0.5 sec of the protocol.

Multi-Channel Complex Delivers a series of 6 complex waveforms to 3 output channels, and records the current and voltage responses on two input channels. The series is repeated 4 times for a total of 24 episodes.

Spontaneous Events with Rate Acquires up to 100 episodes from an input channel, but does not output any signal. Searches each acquired episode for spontaneous events and monitors the event rate in a separate window.

Monitor Cm Acquires up to 500 episodes from an input channel while sending a square voltage pulse to an output channels. Analyses each acquired episode and calculate the cell's membrane capacitance.

10.3 Detailed Description of Each Protocol



A Simple Pulse

This protocol sends a 10 mV, 10 ms voltage command pulse to **Analog Output** 0. The pulse is repeated up to 500 times, and episodes are initiated as rapidly as possible. The protocol acquires the signal at 20 kHz from the **Analog Input** channel named 'Current', and displays it in the Acquisition Monitor window. See Section 7.4, 'Channels' for a description of how to add or remove channels from a protocol. This protocol can be halted at any time by hitting the space-bar. It will halt automatically when 500 episodes have been acquired.

Pulse + Average

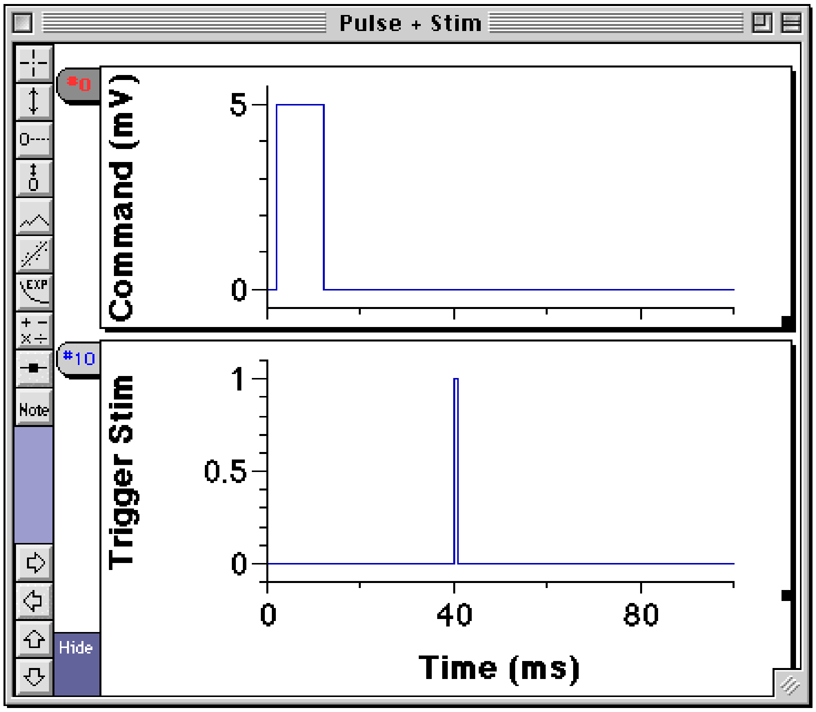
This protocol is identical to the previous protocol, except that the 10 mV pulse is repeated only 50 times, and the ensemble average of the acquired signal is calculated. The running average is superimposed on the individual acquired signals in the Acquisition Monitor window, and the final ensemble average is saved in a separate data file when the acquisition program is halted. See Section 7.5, 'Repetitions' for a description of how to activate online averaging.

Pulse + Amplitudes

This protocol sends a 5 mV, 10 ms voltage command pulse to **Analog Output** 0. This output pulse is repeated up to 500 times, and an episode is initiated every 0.4 seconds (regular trigger mode). The protocol acquires the signal from the **Analog Input** channel named 'Current'. The Pulse + Amplitudes protocol runs an online analysis program after each episode. The program analyses the response recorded on the input channel and measures the amplitude relative to the average current in a 'baseline' period preceding the response. Both the baseline and the amplitude measurement periods are defined by placing cursors in the monitor window after the first episode has been acquired. The measured amplitudes are plotted in a separate monitor window that is created when the protocol is first run. The online amplitude measurement program is a standard part of the data acquisition package, and it can be modified to monitor any parameter of the response (see Chapter 8 'Online Analysis'). To add this online analysis feature to a protocol, click the Commands button then enter 'AmplitudeSetup' in the After First Episode field, and 'AmplitudeMeasure' in the After Each Episode field (see Section 8.3 'Standard Analysis Programs' for additional details).

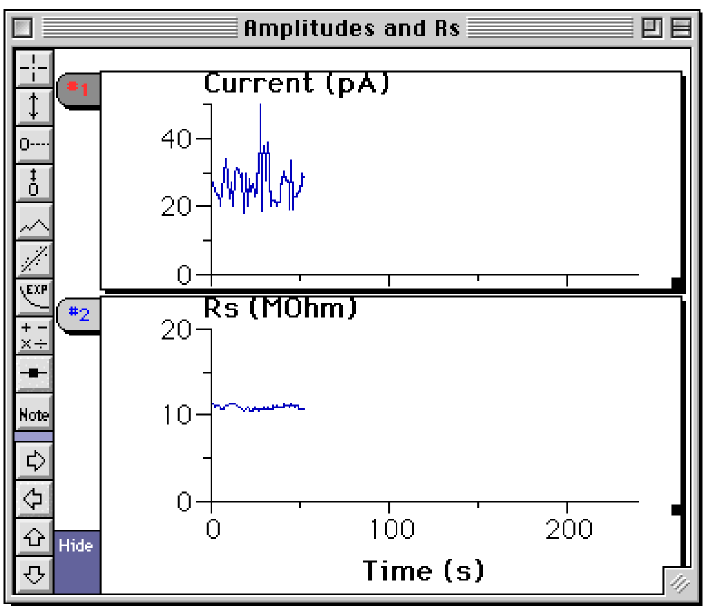
Pulse + Two Amplitudes

This protocol is similar to the previous protocol, but it measures the amplitude at two different points in the response. It sends a 5 mV, 10 ms voltage command pulse to **Analog Output** 0, and analyses the current response recorded on the 'Current' channel. The analysis program measures average amplitude over two separate time periods relative to the average current in a 'baseline' period. These time periods are specified manually. The amplitude at both time points is plotted in a separate monitor window. The analysis program is a standard part of the data acquisition package (see Chapter 8 'Online Analysis').



Pulse + Stimulus and Rs

This protocol sends a 5 mV, 10 ms voltage command pulse to **Analog Output** 0, and after a delay sends a 1 ms **Digital Output** pulse (+5 V) to **Digital Output** 0. This pair of output pulses is repeated up to 500 times, and an episode is initiated every 1 second (regular trigger mode). The protocol acquires the signal from the **Analog Input** channel named 'Current'. See Section 7.4, 'Channels' for a description of how to add or remove channels from a protocol.

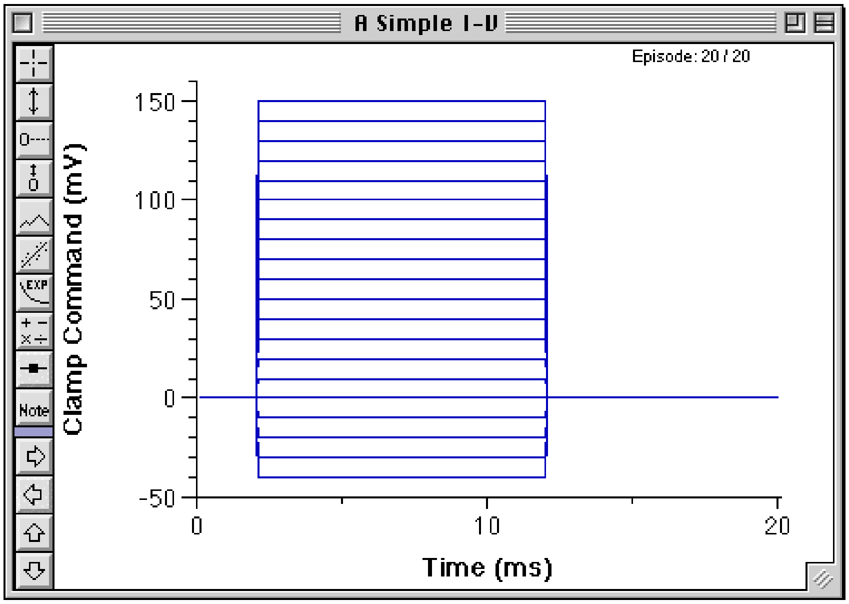


The Pulse + Stimulus and Rs protocol also runs an online analysis program after each episode. The program analyses the response recorded on the 'Current' channel to the 5 mV voltage command pulse. It calculates the 'series resistance' (Rs), from the size of the applied voltage step (Vcmd) and the peak amplitude of the resulting current transient (Iresp), using the formula, Rs = Iresp / Vcmd. The program also measures the peak amplitude of the current (Ipeak) over a selected time period (for example, following the Trigger pulse on **Digital Output** 0). It calculates the amplitude relative to the average current in a 'baseline' period. Both the baseline and the peak time periods are defined by placing cursors in the monitor window after the first episode has been acquired. The value of Rs and Ipeak are plotted in a separate monitor window that is created when the protocol is first run.

The analysis program that measures Rs and Ipeak is a standard part of the data acquisition package, and it can be modified to monitor any parameter of the response. See Chapter 8 'Online Analysis' for information on adding analysis programs to a protocol, and on writing or editing custom analysis programs.

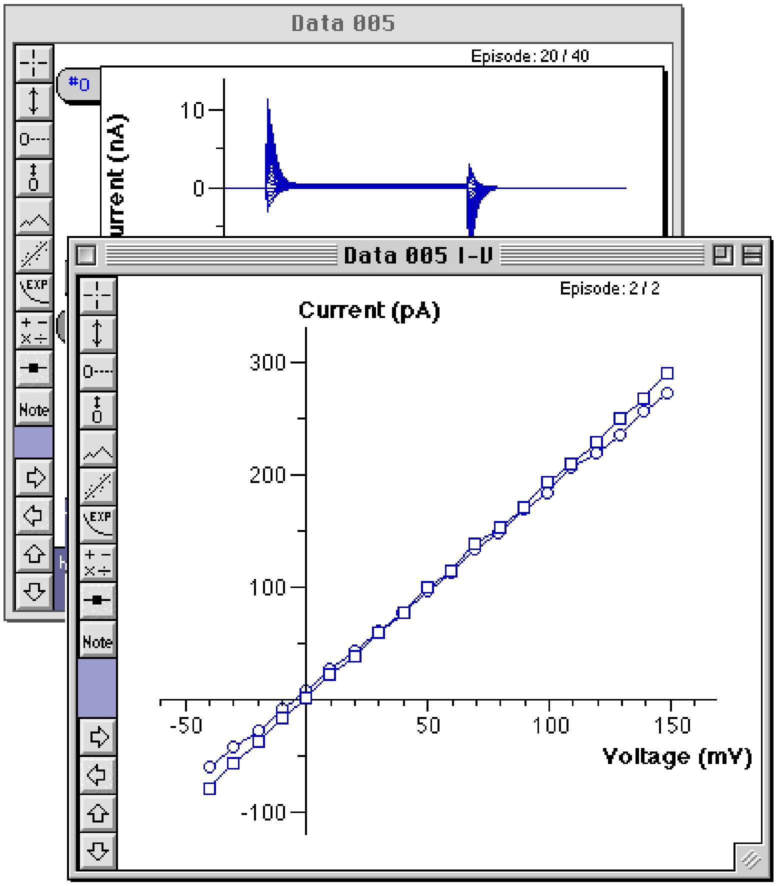
Pulse + Slope

This protocol is similar to the previous protocol, but it measures the slope of the response over a selected time regions. It sends a 5 mV, 10 ms voltage command pulse to **Analog Output** 0, and analyses the current response recorded on the 'Current' channel. The analysis program fits a regression line over a selected time period. The slope of the regression line is plotted in a separate monitor window. The analysis program is a standard part of the data acquisition package (see Chapter 8 'Online Analysis').



A Simple I-V

This protocol sends a series of 20 different voltage pulses to **Analog Output** 0. An episode is initiated every 0.5 seconds (regular trigger mode). The first pulse is -40 mV in amplitude, and subsequent pulses are incremented by +10 mV per episode to a maximum of +150 mV. This series of pulses is delivered twice, for a total of 40 episodes. The protocol acquires signals from the **Analog Input** channels named 'Current' and 'Voltage'. The acquired episodes describes the current-voltage (I-V) relationship of the preparation. See Sections 7.6 and 7.7 for a description of how to add an incrementing series of pulses to a protocol.



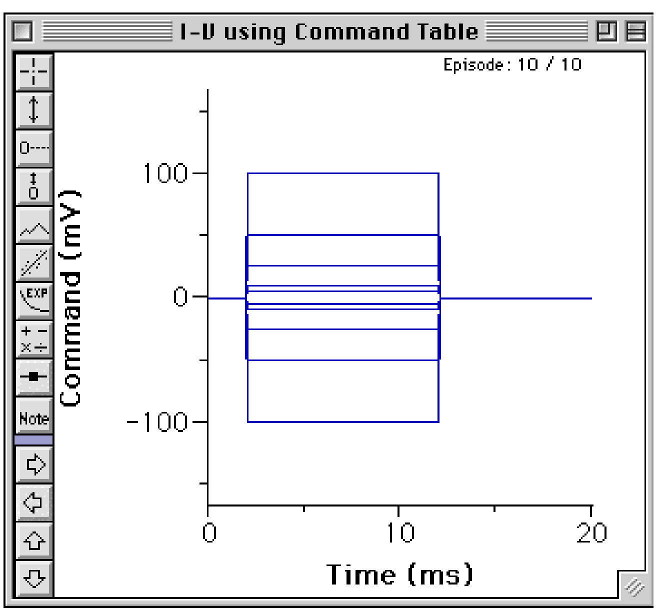
I-V with Analysis

This protocol is identical to the previous protocol, except that an analysis program is run after the last episode is acquired. This analysis program is applied to the recorded data file. It only runs when the Record option is used, not with Preview. The I-V analysis program generates a new graph window containing a plot of the current recorded on one channel versus the voltage recorded on the other (I-V plot). The current and voltage are measured over two different time periods selected by placing cursors in the monitor window.

The slope of the I-V plot is inversely proportional to the input resistance of the preparation. A typical result is shown.

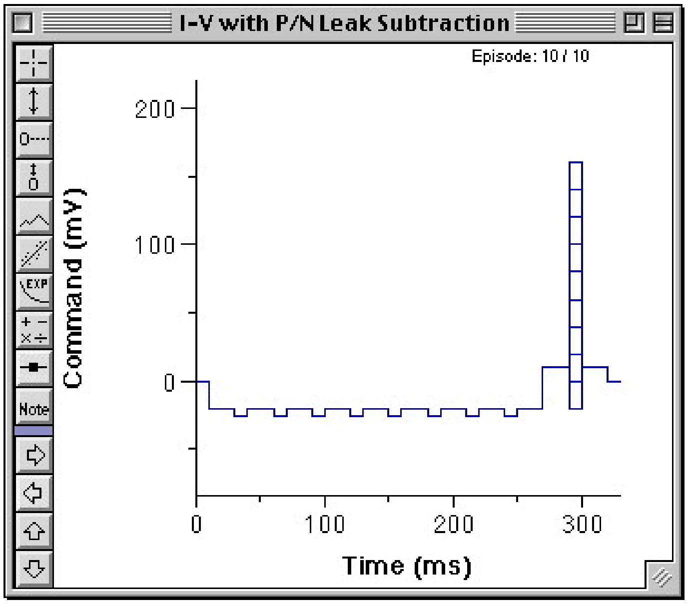
Note that there are two traces in the I-V plot. These correspond to the 1st and 2nd repetitions of the series of voltage pulses.

The I-V analysis program is a standard part of the data acquisition package, and it can be modified to perform any kind of analysis on the acquired data file (see Chapter 8 ‘Online Analysis’).



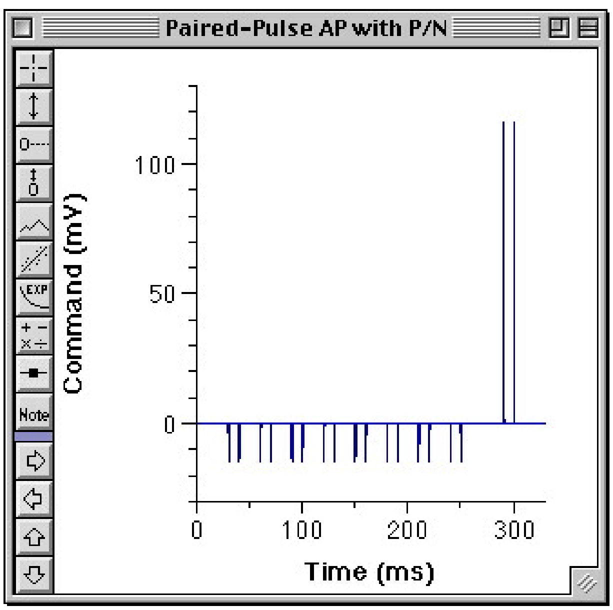
I-V using Command Table

When delivering a series of pulses to an **Analog Output** channel, it is not always desirable to increase the pulse amplitude in constant increments. For example, a logarithmic series, or random series of amplitudes may be required. In this example protocol, each pulse amplitude is specified by a table entry (see Section 7.7). A series of 10 voltage command pulses is directed to **Analog Output** 0, and the pulse amplitudes are (in mV) -100, 100, -50, 50, -25, 25, -10, 10, -5 and 5. Any series of pulse amplitudes can be specified in a table. See Section 7.7 ‘Define Pulse or Ramp Shape’ for a description of how to add a series of pulses to a protocol with amplitudes specified in a table.



I-V with P/N Leak Subtraction

This protocol delivers a series of 10 waveforms to **Analog Output** 0. Each waveform consists of a test pulse preceded by a train of 8 smaller inverted pulses that are superimposed on a long negative step. The response to these 8 pre-pulses is used to perform 'P over N' (or P/N) leak subtraction, which removes the 'passive' or linear component of the response to the test pulse (see Section 7.15). P/N leak subtraction is performed as follows. The 8 responses to the pre-pulses are averaged, scaled then subtracted from the response to the test pulse. The resulting signal is displayed in a separate P/N analysis window. The monitor window and the data file record the raw responses to both pre-pulses and test pulse. The P/N result window contains only the non-linear component of the response to the test pulse. The P/N analysis programs are named 'PonNSetup' (which is set to run before the first waveform) and 'PonNMeasure' (which is set to run after each waveform). See Chapter 8 'Online Analysis', for information on how to add online analysis programs to a protocol. To use these online programs, the P/N protocol must be set up to contain exactly 4 pulses in the following order:
1) Pre-pulse Holding Level, 2) Pre-pulse Train, 3) Test Holding Level, 4) Test Pulse.

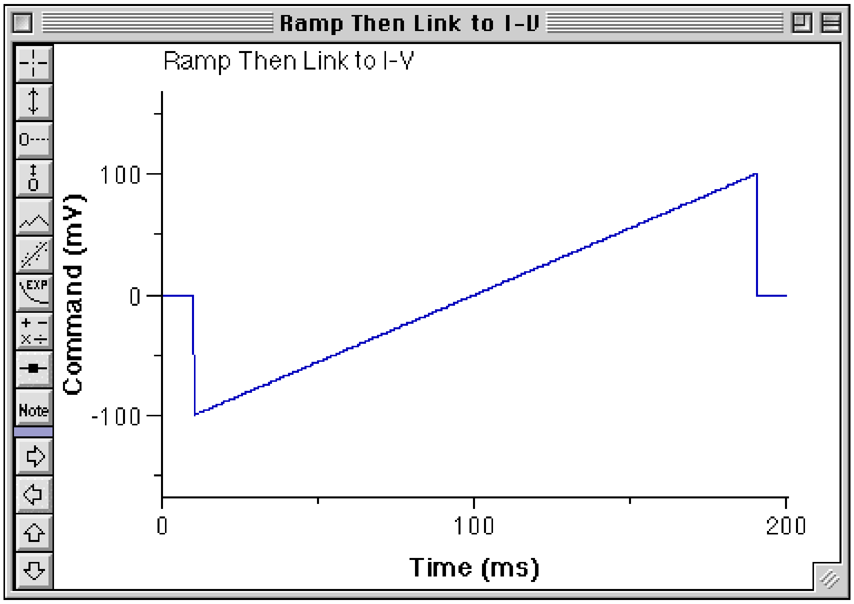


Paired-Pulse AP with P/N

This protocol delivers a voltage-clamp waveform consisting of two action potentials (AP's) separated by 10 ms (test stimulus). The paired action potentials are preceded by a series of 8 pairs of inverted scaled-down action potentials (pre-pulses). The output waveform is repeated 5 times. The recorded response to each waveform is analysed using P/N leak subtraction (see description of previous protocol). The linear or 'passive' component of the current response is automatically subtracted to reveal non-linear or 'voltage-dependent' components.

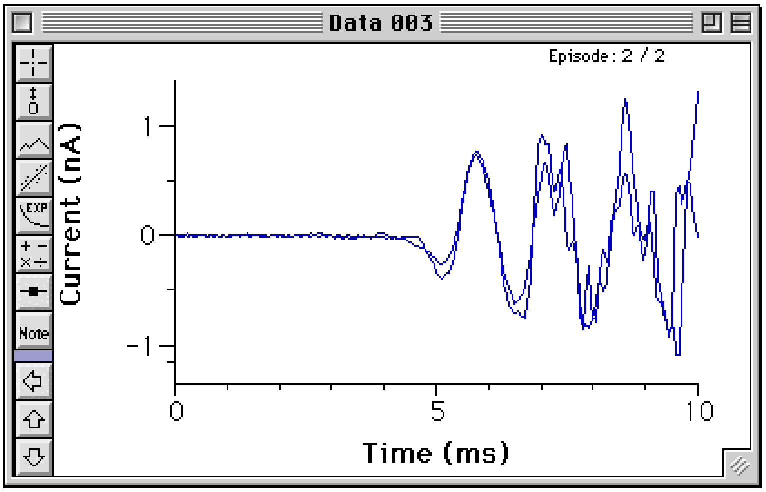
This protocol uses the online P/N analysis programs PonNSetup' and PonNMeasure' (see Chapter 8 'Online Analysis').

This protocol is relatively difficult to set up. The online P/N analysis programs expect the protocol to contain 4 pulses in the following order: 1) Pre-pulse Holding Level, 2) Pre-pulse Train, 3) Test Holding Level, 4) Test Pulse. So the first step is to build a protocol in this format. For example, the protocol named I-V with P/N Leak Subtraction could be copied then edited so that it only contained one waveform, but was repeated 5 times. The second step is to replace the waveform containing rectangular pulses, with a synthetic waveform containing pairs of AP's. This can be done using AxoGraph's built-in programming environment.



Ramp Then Link to I-V
This protocol outputs a voltage command ramp superimposed on a square pulse to **Analog Output** 0. The pulse is -100 mV in amplitude, and the ramp is +200 mV in amplitude. Both the pulse and the ramp start at 10 ms and are 180 ms wide. Their superimposition results in a voltage command waveform that sweeps over the range from -100 to +100 mV. The waveform is repeated 10 times. The protocol acquires data from the **Analog Inputs** named ‘Current’ and ‘Voltage’. The acquired data estimates the current-voltage (I-V) relationship. See Section 7.6 for a description of how to add a ramp to a protocol.

An important feature of this protocol is that it contains a ‘link’ to another protocol. When all 10 ramps have been delivered, and the data saved to a new file, the acquisition program pauses for 5 seconds then runs a second protocol named ‘I-V with Analysis’. Two or more protocols can be linked in this way to form a chain or loop, and thereby generate output sequences of arbitrary complexity. See Section 7.14 ‘Add a Link’ for a description of how to add a link to a protocol.

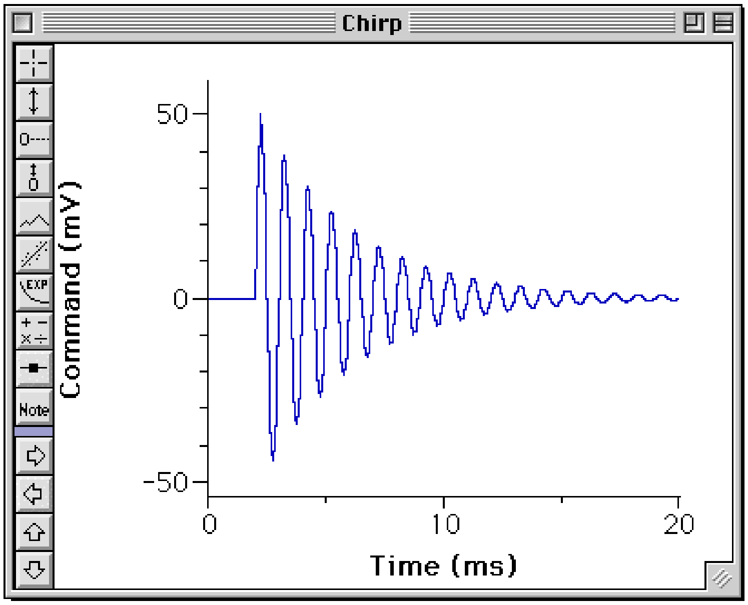


Trigger on Event
This protocol does not output any signal, but it acquires up to 500 episodes from the **Analog Input** named ‘Current’. Each episode is triggered when the slope of the signal on the input channel exceeds a threshold of 1 nA / ms (internal trigger mode). The slope is calculated over a 0.5 ms interval that is slid along the input signal. The 5 ms period preceding the trigger is also acquired. See Section 7.12 ‘Trigger’ for a description of how to specify internal trigger mode for a protocol.

This figure shows the first 10 ms of a data file produced by the ‘Trigger on Event’ protocol. Two episodes were triggered when the signal slope exceeded 1 nA/ms. The protocol specifies a 5 ms pre-trigger interval and a 0.5 ms slope calculation region, so the region that triggered the acquisition is displayed from 5.0 to 5.5 ms.

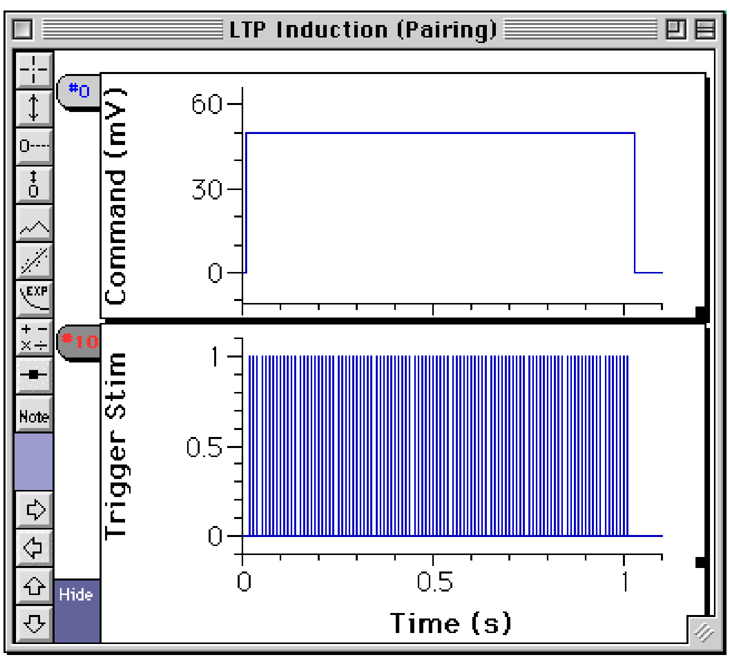
Keyboard Trigger

This protocol sends a 10 mV, 10 ms voltage command pulse to **Analog Output** 0. The pulse is repeated up to 100 times. Each episode is initiated via the keyboard by hitting the "tab" key (keyboard trigger mode). The protocol acquires the signal from **Analog Input** 0. The time at which each episode is triggered is saved in the 'Notes' field of the data file. See Section 7.12 'Trigger' for a description of how to specify the keyboard trigger mode for a protocol.



Chirp

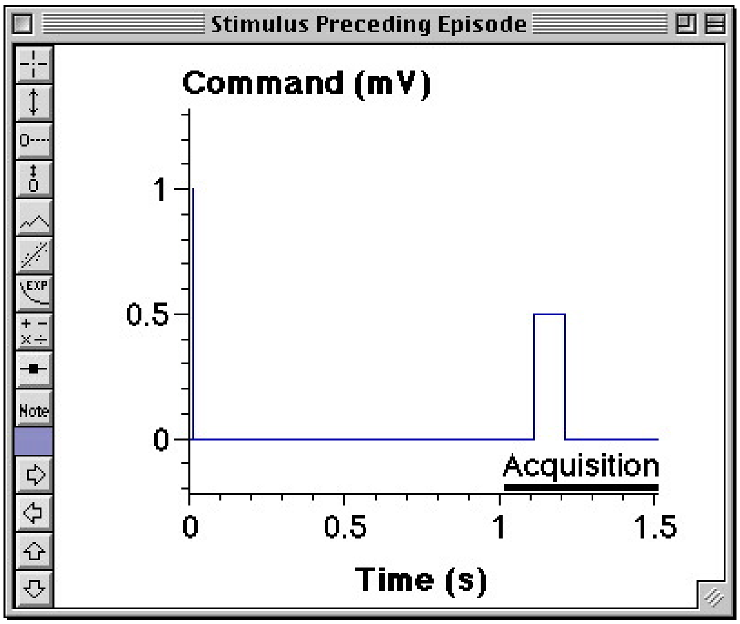
This protocol sends a 'chirp' waveform to **Analog Output** 0. The custom waveform consists of a 1 kHz sinusoid with an amplitude that decays exponentially. The initial amplitude of the sinusoid is 50 mV and it decays with a 4 ms time constant. The waveform is repeated up to 100 times. See Section 7.13, 'Custom Waveform', for information on adding a custom waveform to a protocol.



LTP Induction (Pairing)

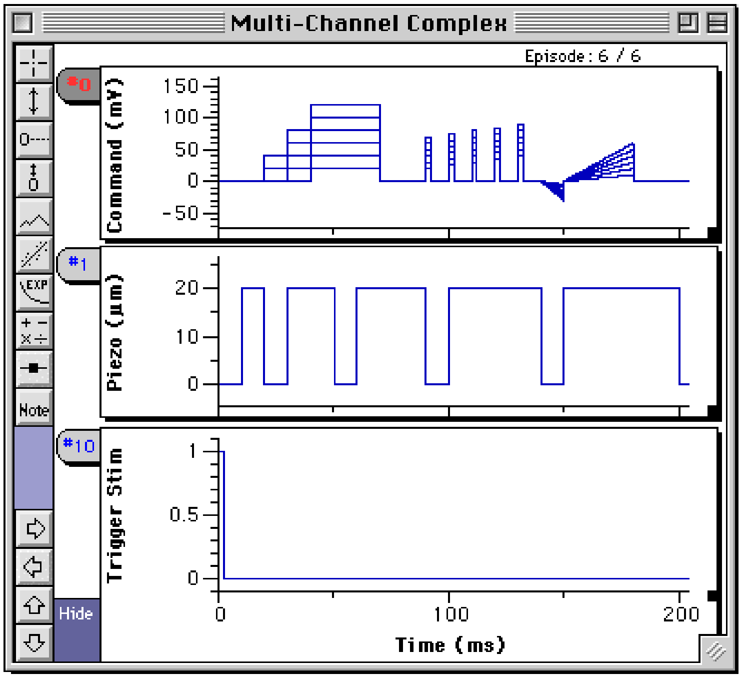
The phenomenon of long-term potentiation (LTP) of a synaptic contact can be induced by 'pairing' presynaptic stimulation with depolarization of the postsynaptic neuron. This protocol delivers a train of 100 pulses at a rate of 100 Hz to **Digital Output** 0 to trigger a presynaptic stimulator. Simultaneously, a voltage command pulse of +50 mV is directed to **Analog Output** 0 to depolarize the postsynaptic neuron. This 'paired' stimulus is delivered every 10 seconds, and is repeated 5 times. The protocol acquires current and voltage data from the **Analog Inputs** named 'Current' and 'Voltage' during the paired stimulus. See Section 7.6 'Add Pulse' for a description of how to add a train of pulses to a protocol.

Toggle Digital Output #0
This protocol does not output any signal, but it acquires up to 500 episodes, each 20 ms second long, from the **Analog Input** named 'Current'. Before the start of each episode the protocol runs an online program that toggles the setting of **Digital Output** 0 between low (0 Volts) and high (5 Volts). The purpose of this protocol is to demonstrate the command for setting the levels of **Digital Output** channels. This command can be used to set the levels of any of these channels, including the channels that can only be accessed at the rear panel of the digitizer. However, it can only be run between episodes. It can not be used to change a **Digital Output** setting while an episode is being acquired.



Stimulus Preceding Episode
This delivers two rectangular pulses to **Analog Output** 0, and records the response on **Analog Input** 0. Only the response to the second stimulus pulse is recorded. The first pulse is the 'conditioning' stimulus, and it is delivered 1 sec before the start of the recorded episode. The second pulse is the 'test' stimulus, and the response is only recorded over the last 0.5 sec of this protocol. Data acquisition can be restricted to a sub-section of a protocol via the Timebase button (see Section 7.3).

In this protocol, the monitor window is set to display the entire 1.5 second time range. The monitor range can be restricted to display only the acquisition sub-section via the Timebase button (see Section 7.3).



Multi-Channel Complex

This protocol demonstrates how pulses, pulse trains and ramps can be concatenated to build complex waveforms (see Sections 7.6 to 7.9). It delivers a series of 6 waveforms to 3 output channels while acquiring 6 episodes from the **Analog Inputs** named 'Current' and 'Voltage'. The first pulse on the 'Command' channel has an amplitude of 20 mV on the first episode. The pulse amplitude increments by 20 mV with each episode. On every second episode, the onset time is incremented by 10 ms, and the pulse width is reduced by 10 ms. This pulse is followed by a train of 5 pulses. The amplitude of the first pulse in the train is 20 mV, and the amplitude is incremented by 5 mV for each pulse in the train. The amplitude of all pulses in the train are incremented by 10 mV with each episode. The pulse train is followed by two successive ramps. The amplitude of both ramps increments with each episode. The 'Piezo' channel delivers a pulse train which consists of 5 pulses, each 20 mV in amplitude. The width of the first pulse is 10 ms, and the width is incremented by 10 ms for each pulse in the train. The interpulse interval is constant at 10 ms. A **Digital Output** pulse is delivered at the start of each episode to the 'Trigger Stim' channel.

Spontaneous Events with Rate

This protocol does not output any signal, but it acquires up to 100 episodes, each 1 second long, from the **Analog Input** named 'Current'. It runs an analysis program after every episode that scans along the acquired signal searching for spontaneous events. The value of spontaneous event rate is plotted in a separate monitor window that is created when the protocol is first run. The analysis program that measures the event rate is a standard part of the data acquisition package, and it can be modified to monitor any parameter of the response (see Chapter 8 'Online Analysis').

Monitor Cm

This protocol sends a 5 mV, 10 ms voltage command pulse to **Analog Output** 0. The output pulse is repeated up to 1000 times, and episodes are initiated as rapidly as possible. The protocol acquires the signal from the **Analog Input** channel named 'Current'. An online analysis program is run after each episode. The program assumes that the recorded current is from a voltage-clamped spherical cell, and that whole-cell capacitance neutralization is not active. The program analyses the amplitude and decay of the current transient recorded and estimates the total membrane capacitance (Cm) of the cell. The measured Cm values are plotted in a separate monitor window that is created when the protocol is first run. The online Cm measurement program is a standard part of the data acquisition package (see Chapter 8 'Online Analysis'). To add this online analysis feature to a protocol, click the Commands button then enter 'MonitorCmSetup' in the After First Episode field, and 'MonitorCm' in the After Each Episode field. These programs assume the first pulse in the protocol is a rectangular test pulse.

11 Precise Acquisition Timing

11.1 Stability and Accuracy of Regular Trigger

The regular trigger mode can be used for both the digital oscilloscope and protocol driven data acquisition (see Sections 3.7 and 7.12). In this mode, acquisition of a sweep or episode is initiated at regular intervals. The line trigger mode also initiates sweeps at a regular interval that is a multiple of the power line cycle time (1/50th or 1/60th second).

The precise timing of the sweep initiation is determined with reference to a microsecond clock on the computer motherboard. The data acquisition software reads this clock at the start of the first sweep, then calculates the time in microseconds at which to start each subsequent sweep. The clock is read inside a tight loop and when it reaches the calculated start time, the sweep is initiated.

The precision of this trigger scheme is limited by the accuracy and stability of the computer's clock, and by the software's ability to read it in a tight loop without interruption. The clock on a Macintosh motherboard was designed primarily for tracking the day and date. It is reasonably accurate, but may drift by a few seconds per day. This corresponds to a few milliseconds per minute, and is enough to prevent tight time lock to the power line cycle in 'line' trigger mode over a minute or more.

The Macintosh operating system can interrupt a running program to perform low-level system tasks, such as monitoring a network connection. If an interrupt occurs close to the sweep initiation time, this could prevent precise triggering. For optimum performance, it is recommended that the network connection is inactivated when acquiring data. This can be done by restarting the computer while holding down the "shift" key.

The acquisition software always notes the time that a sweep is actually triggered, and compares this with the requested trigger time. If regular triggering is not achieved, this will be reported when the acquisition is terminated.

11.2 Hardware Limitations on the Sampling Rate

Most digitizers place limitations on the available sampling rates because they are driven by an internal clock with a fixed tick rate. The clock in the Digidata 1320 and 1321A tics every 0.1 µs, the ITC-16 clock ticks every 1.0 µs, and the ITC-18 every 1.25 µs. The sample interval must be a multiple of the clock interval, and can not be less than 2 µs (= 500 kHz) for the Digidata 1321A, 4 µs (= 250 kHz) for the Digidata 1321A, or 5 µs (= 200 kHz) for the ITC digitizers. A 5 µs interval corresponds to 5 ticks on an ITC-16, or 4 ticks on an ITC-18.

A table of the available sample intervals and sample rates

Digidata 1320

Interval (µs)	Rate (kHz)
4	250
4.1	243.90
4.2	238.10
4.3	232.56
4.4	227.27
4.5	222.22
4.6	217.39
4.7	212.71
...	...
...	...

Digidata 1321A

Interval (µs)	Rate (kHz)
2	500
2.1	476.19
2.2	454.55
2.3	434.78
2.4	416.67
2.5	400
2.6	384.62
2.7	370.37
...	...
...	...

ITC-16

Interval (µs)	Rate (kHz)
5	200
6	166.67
7	142.86
8	125
9	111.11
10	100
11	90.909
12	83.333
...	...
...	...

ITC-18

Interval (µs)	Rate (kHz)
5	200
6.25	160
7.5	133.33
8.75	114.29
10	100
11.25	88.889
12.5	80
13.75	72.727
...	...
...	...

This table assumes that only 1 **Analog Input** channel and 1 **Analog Output** channel are active. The situation is more complicated when there are several active input or output channels. The ITC can only process one input and one output channel every N clock ticks. If there are 2 input channels or 2 output channels, the effective sample interval is 2N ticks. This places additional limits on the available sampling rates, including the maximum sampling rate. In general, the maximum multi-channel sampling rate, is given by...

maximum multi-channel rate = (maximum single-channel rate / maximum channels) kHz

where 'maximum channels' is equal to the number of input channels or the number of output channels, whichever is greater.

All **Digital Output** channels are processed simultaneously, so any number of active **Digital Output** channels are counted as only 1 output channel when calculating 'maximum channels'. A similar argument applies for the **Digital Input** channels.

A case study demonstrating the restrictions on the available sampling rates due to hardware limitations: An acquisition protocol samples data from 1 **Analog Input** channel while sending signals to 2 **Analog Output** channels and 2 **Digital Output** channels. The 2 **Digital Output** channels are counted as a single output channel, so there are 3 effective output channels. There is only 1 input channel, so maximum channels = 3. The protocol requests a sample rate of 20 kHz (=50 µs). The ITC needs to process all three output channels in this interval, or one channel every (50 / 3) = 16.6667 µs. This inter-channel interval is not permitted, because it is not an integer multiple of the clock tick interval.

• For a Digidata 1320 or 1321A, the inter-channel interval corresponds to 166.667 ticks. The Digidata delivers the closest available sampling rate by processing one output channel every 167 ticks. This gives an effective sample interval of 167 x 3 x 0.1 = 50.1 µs, which corresponds to 19.960 kHz.

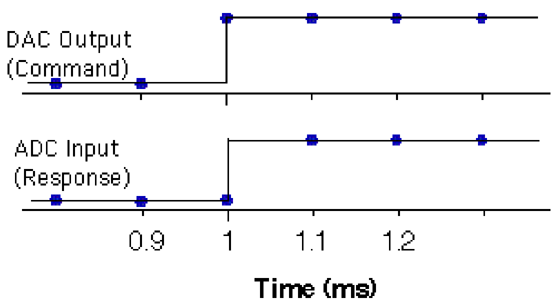
• For an ITC-16, the inter-channel interval corresponds to 16.6667 ticks. The ITC-16 delivers the closest available sampling rate by processing one output channel every 17 ticks. This gives an effective sample interval of 17 x 3 x 1.0 = 51 µs, which corresponds to 19.6078 kHz.

• For an ITC-18, the inter-channel interval corresponds to 13.3333 clock ticks. The ITC-18 delivers the closest available sampling rate by processing one output channel every 13 ticks. This gives an effective sample interval of 13 x 3 x 1.25 = 48.75 µs, which corresponds to 20.5128 kHz.

11.3 Timing of Input and Output Events

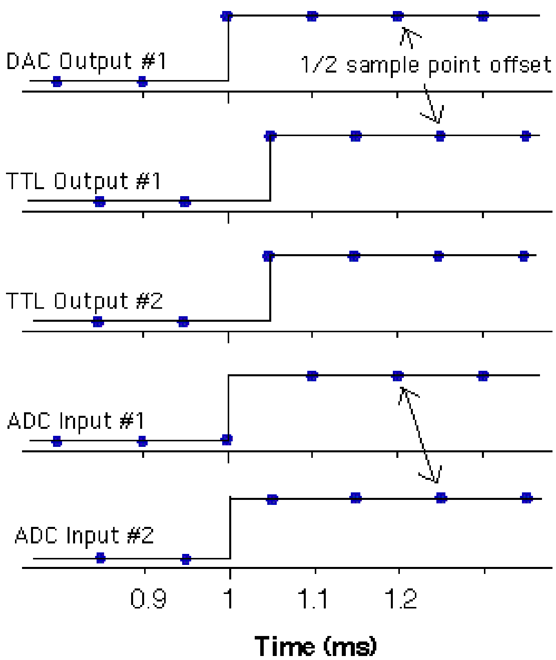
In some situations it is important to know the precise timing of events on the active input and output channels. When several input or output channels are active, the timing of events will be slightly different for an ITC-16 compared with an ITC-18.

The ITC-18 hardware separates the jobs of 'processing' a channel (moving a numerical value in or out), and 'updating' a channel (changing the output voltage or sampling the input voltage). Although only one input and one output channel can be processed at a time, all channels can be updated simultaneously. Consider a protocol with a sample rate of 10 kHz, 2 **Analog Input** channels, 1 **Analog Output** and 2 **Digital Output** channels. The protocol specifies that a step output is applied to the **Analog Output** channel and both **Digital Output** channels at 1 ms after the start of the acquisition sweep. At exactly 1 ms, both input channels are sampled and all 3 output channels are stepped to a new voltage.



Even if a response occurs instantaneously it will not be sampled on the **Analog Input** channels until the next sample point at 1.1 ms. In general, the first point of the response to a step command is not sampled until 1 sample interval after the step is applied. The figure at left shows the sampled **Analog Input** and the **Analog Output** numerical values (blue circles), and the actual voltage signals (black lines). This figure assumes that the response is instantaneous, and that an ITC-18 is used.

Both the ITC-16 and the Digidata 1320 series are less sophisticated than the ITC-18. These digitizers process and update a channel at the same instant. When several channels are active, they will be updated at different times within the sample interval. Consider a protocol with a sample rate of 10 kHz, 2 **Analog Input** channels, 1 **Analog Output** and 2 **Digital Output** channels. The protocol specifies that a step output is applied to the **Analog Output** channel and both **Digital Output** channels at 1 ms after the start of the acquisition sweep. At exactly 1 ms, the first **Analog Input** is sampled and the **Analog Output** is stepped to a new voltage. Then at 1.05 ms, the second **Analog Input** is sampled and the two **Digital Output** channels are stepped to a new voltage. Thus, the second **Analog Input** channel and the **Digital Output** channels are offset in time by 1/2 a sample interval relative to the first **Analog Input** channel and the **Analog Output** channel.



The figure at left shows the **Analog Input**, the **Analog Output** and **Digital Output** values (blue circles), and the actual voltage signals (black lines). The figure assumes that the response to the **Analog Output** is instantaneous, and that an ITC-16 is used. Note that the 1/2 a sample interval offset (arrows in this schematic) is not shown in the protocol graph or in the acquired data graph.

To work out the precise sequence of events when using multiple channels of an ITC-16 or Digidata, the channel processing sequence is required. All active **Analog Input** and **Analog Output** channels are processed and updated in ascending numerical order, followed by the active **Digital Output** channels.

12 Optimizing Performance

12.1 Memory and Network Settings

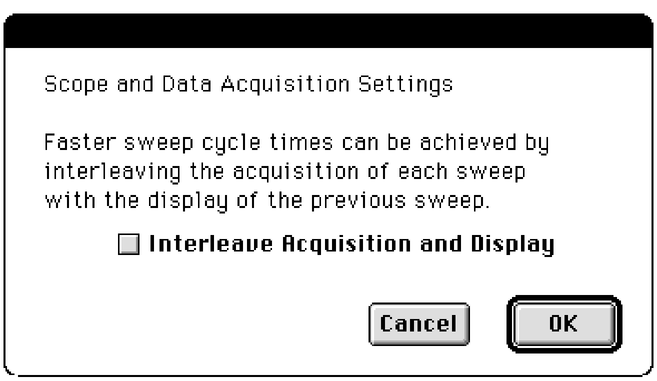
AxoGraph acquires data to memory, so the maximum recording time is limited by the available memory. This can be increased simply by quitting all other programs before launching AxoGraph. It can also be increased by installing additional memory - a minimum system configuration of 32 MByte is recommended.

The amount of available memory can also be increased by activating virtual memory, however this incurs a performance penalty and is not recommended. The maximum sustained sampling rate, and the maximum sweep rate will both be reduced by a few percent initially. However, the performance penalty increases dramatically as the amount of acquired data approaches the limits of physical memory. Once this limit is reached, the acquisition program will come to a grinding halt, and may freeze causing the loss of all the acquired data. This is a flaw of the current implementation of virtual memory. It is therefore recommended that virtual memory is switched off during data acquisition. This can be done via the Memory control panel. It can also be done by restarting the computer while holding down the “shift” key, which has the additional benefit of inactivating all system extensions and freeing up several MBytes of system memory.

When an acquisition program is running, AxoGraph does not share CPU time with any other program running in the background. However, the Mac operating system can interrupt the acquisition program to perform low-level tasks. Network tasks are of particular concern. For optimum performance, it is recommended that the network connection is inactivated. This can be done via network control panels, by physically unplugging the connection, or by restarting the computer while holding down the “shift” key.

12.2 Interleave Acquisition and Display

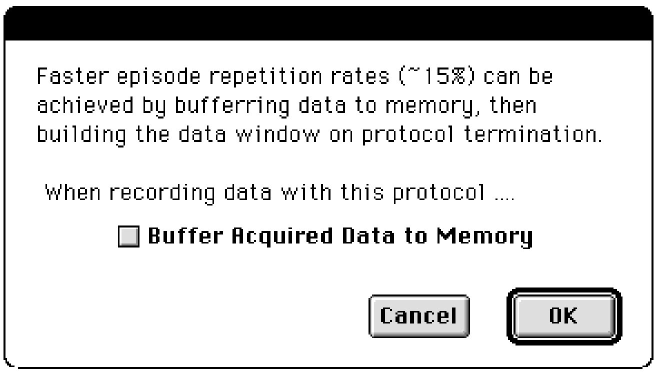
By default, the digital oscilloscope and the protocol-driven data acquisition programs work in a sub-optimal manner. Acquisition of a sweep can not begin until the display of the previous sweep is complete. For Instrutech ITC series digitizers, faster repetition rates can be achieved by initiating the acquisition of a new sweep while the previous sweep is being displayed. However, it is recommended that interleave is turned off unless very fast sweep repetition rates are required (regular trigger interval is set to zero). The performance gain is generally less than 25%, and at lower repetition rates (regular trigger interval > sweep width + 200 ms) interleave introduces a lag between data acquisition and screen display that may be annoying.



To interleave acquisition and screen display, select Program → Acquisition Options → Performance and turn on the Interleave Acquisition and Display check box in the resulting dialog. This option will not appear when a Digidata 1320 series digitizer is connected.

12.3 Buffer Acquired Data to Memory

By default, protocol-driven data acquisition displays each episode in the Acquisition Monitor window, then saves it to a separate data file window as soon as it is acquired. This second step involves the adjustment of the data window's display parameters, and incurs a performance penalty. Faster repetition rates can be achieved by buffering the acquired data to memory, then moving it into the data window when the protocol terminates. However, it is recommended that buffering is turned off unless very fast episode repetition rates are required. The performance gain is generally less than 15%, and buffering can cause data to be lost if memory overflow occurs during acquisition.



To buffer acquired data to memory, select Program → Protocol → Buffer and turn on the Buffer Acquired Data to Memory check box in the resulting dialog.

12.4 Disable Acquisition Monitor

The maximum episode repetition rate is limited by the time it takes to acquire an episode, plus the time it takes to de-multiplex the acquired episode and copy it to the data window, plus the time it takes to display the episode in the monitor window. Under many circumstances, the display step is the slowest. The episodes repetition rate can be increased by disabling the display of each episode during acquisition. Acquired episodes will still be displayed, but only when acquisition terminates.

To disable the acquisition monitor, select the Protocol item in the toolbar pop-up menu. This will bring the current protocol window to the front, or request a protocol filename if no protocol windows are open. Press the Display button in the Protocol toolbar to bring up the Acquisition Monitor Settings dialog (see Section 7.11). Turn off the Monitor Acquired Episodes check box.