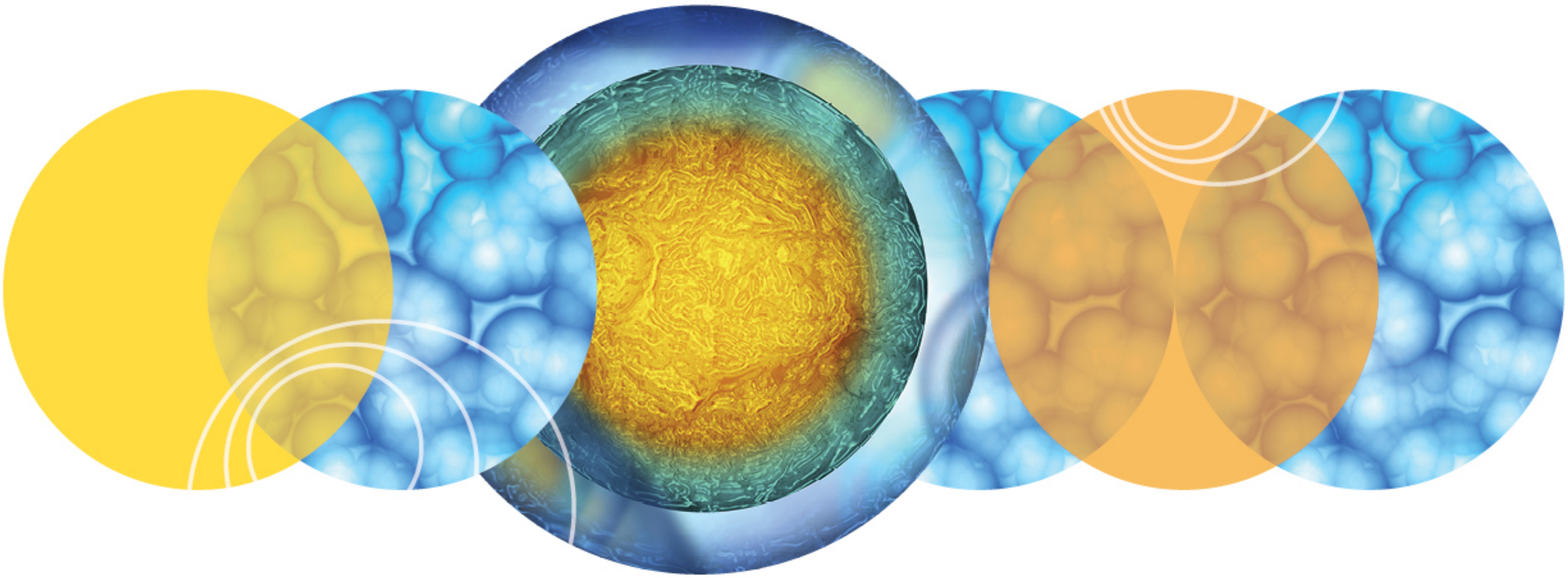

Setting Up Clampex for Data Acquisition

Rev. H

November 2017

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Welcome to the Guide

Setting Up Clampex for Data Acquisition is a step-by-step guide that explains how to integrate Clampex software with your amplifier and digitizer for data acquisition. It describes typical setup configurations for three representative amplifiers—the Axopatch 200B, MultiClamp 700B, and Axoclamp 900A. At the end of the guide, not only will you be ready to acquire data using the explained configurations, you should understand how to set up new configurations tailored to your needs.

We suggest you open Clampex software—and MultiClamp or Axoclamp Commander software if you are following the MultiClamp or Axoclamp software sequence—and toggle between the guide and programs as you move through the guide, using the Alt + Tab key combination.

This guide assumes you are using a Digidata 1550 series (including the 1550, 1550A, and 1550B) digitizer and that you have connected it to your computer. Similarly, if you have amplifiers other than those featured, you should be able to extrapolate the provided instructions to your other amplifiers.

Contents

Configure Digitizer

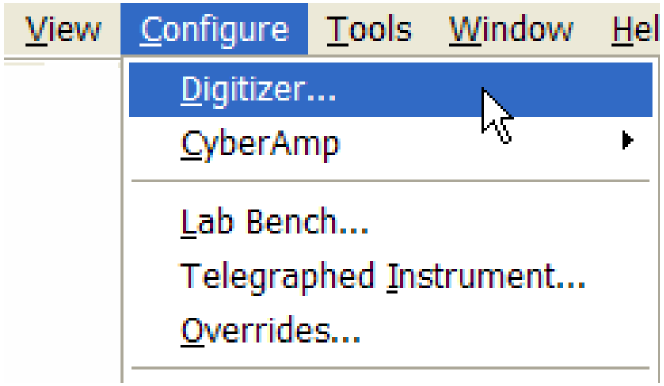
The first step in the setup is to configure Clampex software with a digitizer.

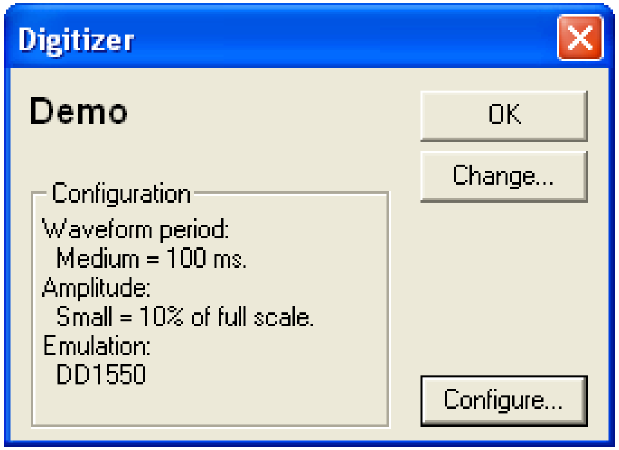
When Clampex software is first installed it is in “Demo” mode. This mode uses simulated data, and is excellent for exploring the application. Now, however, we want to connect to a digitizer for real data acquisition.

You need to have your digitizer connected to the computer and have the digitizer drivers installed. Verify that your software security key (“dongle”) is plugged into a USB port on the computer. Make sure the digitizer is turned on, and then start Clampex software.

Configure Digitizer 1

Select Digitizer from the Configure menu.





The Digitizer dialog opens, showing Clampex software in Demo mode.

Click **Change**.

To continue the configuration, select your model of Digidata digitizer.

Digidata 1550 (including 1550A and 1550B)

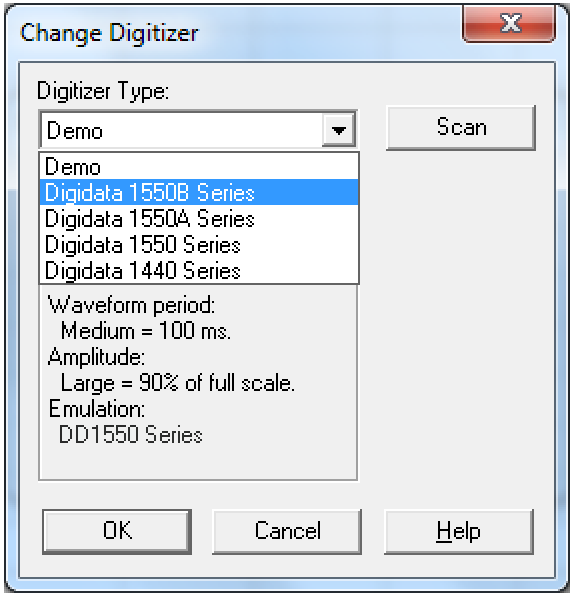
Digidata 1440A

Configure Digitizer 2

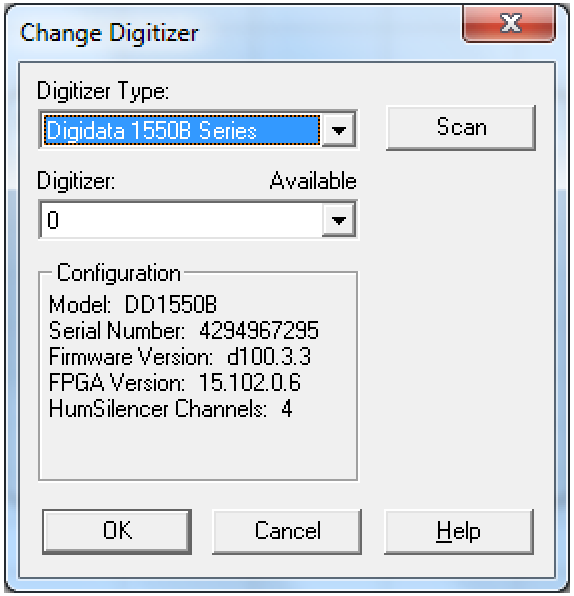
Digidata 1550, 1550A, and 1550B Digitizers

Select **Digidata 1550 Series**, **Digidata 1550B Series**, or **Digidata 1550B Series** from the list.

If Clampex software reports **Not present**, click **Scan**.



Clampex software should then report your Digidata digitizer properties.



There is no calibration necessary with the Digidata 1550, 1550A, or 1550B digitizers.

This completes the configuration of the Digidata 1550, 1550A, or 1550B you have purchased, and it is ready to be used with Clampex software.

If you experience problems, please check the suggestions on the next slide.

Configure Digitizer 3

Digidata 1550, 1550A, and 1550B Digitizers

If clicking **Scan** does not detect the Digidata 1550, 1550A or 1550B digitizer, check the power and the USB 2.0 connections to the digitizer.

The green POWER LED on the front of the digitizer should be illuminated when the power is on and the digitizer is connected to the computer using a USB 2.0 cable. When the digitizer is recognized by Clampex software, the yellow READY LED will turn on as well.

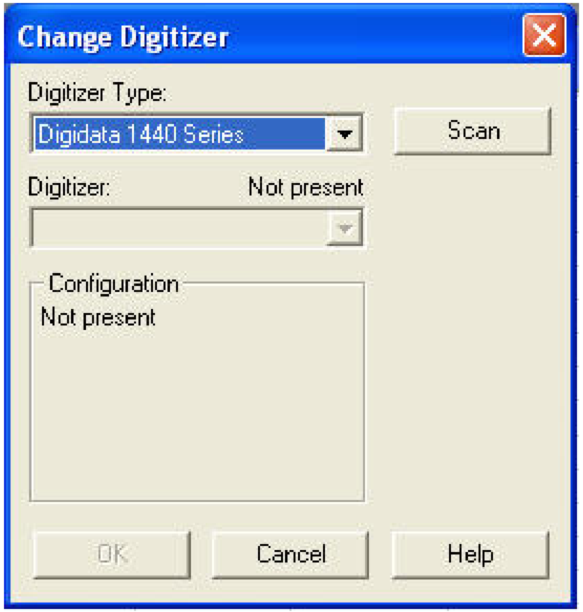
If problems persist, contact Molecular Devices.

Configure Digitizer 4

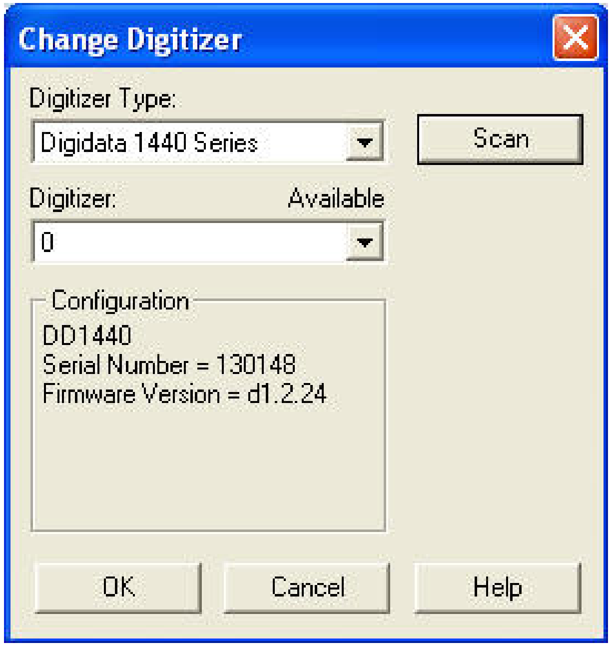
Digidata 1440A

Select **Digidata 1440A Series** from the list.

If Clampex software reports **Not present**, click **Scan**.



Clampex software should then report your Digidata digitizer properties.



There is no calibration necessary with the Digidata 1440A digitizer.

This completes the configuration of the Digidata 1440A digitizer, and it is ready to be used with Clampex software.

If you experience problems, please check the suggestions on the next slide.

Configure Digitizer 5

Digidata 1440A

If clicking **Scan** does not appear to detect the Digidata 1440A digitizer, check the power and the USB 2.0 connections to the digitizer.

The green POWER LED on the front of the digitizer should be illuminated when the power is on and the digitizer is connected to the computer through a USB cable. When the Digidata 1440A digtizer is recognized by Clampex software, the yellow READY LED will turn on as well.

If problems persist, contact Molecular Devices.

Select a Sequence

With the digitizer configured, the next step is to connect the amplifier. Choose the sequence you want to follow according to your amplifier type:

Axopatch Sequence

Follow this sequence if your amplifier telegraphs via cable connections. This includes Axon Instruments' Axopatch series amplifiers, and most non-Axon Instruments amplifiers. Follow this sequence also if your amplifier does not support telegraphs.

MultiClamp Sequence

Follow this sequence if your amplifier is a MultiClamp 700B. Parts of the sequence also apply to MultiClamp 700A and GeneClamp 500B.

Axoclamp Sequence

Follow this sequence if your amplifier is an Axoclamp 900A.

Axopatch Sequence

This sequence describes how to set up two distinct data-acquisition “protocols”, that might be used in whole-cell recording, for an Axopatch 200B amplifier.

With these protocols you will be able to switch between current and voltage clamp and, without any changes to your physical setup, have only to load the appropriate protocol to be sure you are receiving the right signals, with the right units and scaling.

Move through the sequence page by page, or skip sections with the links below—but note that the discussion assumes the setup from earlier sections:

- Digitizer–Amplifier Connections
- Create Signals
- Configure Protocols
- Configure Telegraphs
- Set Scale Factors

Axopatch

Digitizer–Amplifier Connections

In this section we put in place the cabling between the digitizer and Axopatch 200B.

Axopatch Connections 1

We want the following signals:

Voltage Clamp

Digitizer Inputs

- Membrane current—scaled
- Membrane potential—set gain

Digitizer Output

- Command potential

Current Clamp

Digitizer Inputs

- Membrane potential—scaled
- Membrane current—unscaled

Digitizer Output

- Command current

as well as:

Telegraphs

- Gain telegraph for current clamp
- Gain telegraph for voltage clamp

Axopatch Connections 2

Clampex allows for more than one signal to be sent, at different times, on each channel (the relationship between signals and channels is more fully explained in the Create Signals section).

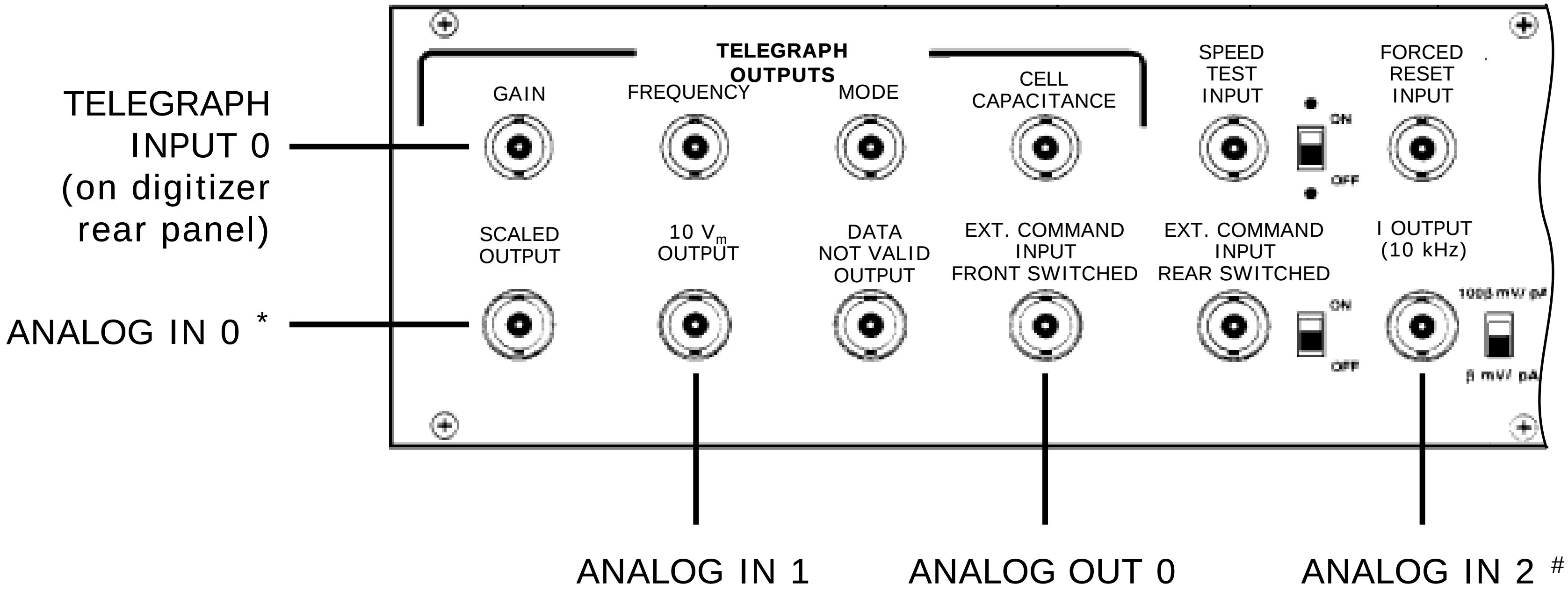
Because we are never in current clamp and voltage clamp at the same time, signals associated with these modes can share channels.

Specifically, the following signals can share channels:

- the scaled input signals for current and voltage clamp
- the command signals for current and voltage clamp
- the telegraphs for current and voltage clamp

The eight signals and telegraphs from the previous slide, then, require only five digitizer-to-amplifier connections. These are described on the next slide.

Axopatch Connections 3



Connect the rear panel BNCs on the Axopatch 200B to the digitizer ports as indicated.

* The SCALED OUTPUT BNCs on the front and rear of the amplifier are equivalent—you may prefer to use the front panel port.

Make sure the switch to the right of the 1 OUTPUT (10 kHz) BNC is in the down position: β mV/pA

Axopatch Connections 4

In this setup, the following connections will carry different signals or telegraphs for voltage- and current-clamp modes:

- SCALED OUTPUT—ANALOG INPUT 0: reads membrane potential in current clamp, and membrane current in voltage clamp
- EXTERNAL COMMAND INPUT—ANALOG OUT 0: for command current in current clamp and command potential in voltage clamp
- GAIN—TELEGRAPH INPUT 0: telegraphs gain irrespective of clamp mode

This leaves:

- 10 V_m OUTPUT—ANALOG IN 1: reads membrane potential in voltage clamp, and
- I OUTPUT—ANALOG IN 2: reads membrane current in current clamp

Finish

With physical connections set up, we now need to configure telegraphs.

Axopatch

Configure Telegraphs

Telegraphs are analog signals sent from the amplifier to Clampex software registering key amplifier settings.

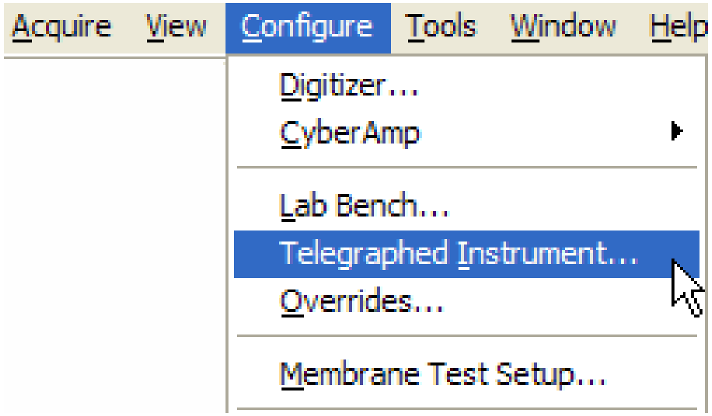
Clampex software recognizes Axopatch 200B telegraphs for gain, lowpass filter frequency and whole cell capacitance neutralization. These are reported in the Real Time Controls in Clampex software, and are written into the header information for data files recorded under those settings.

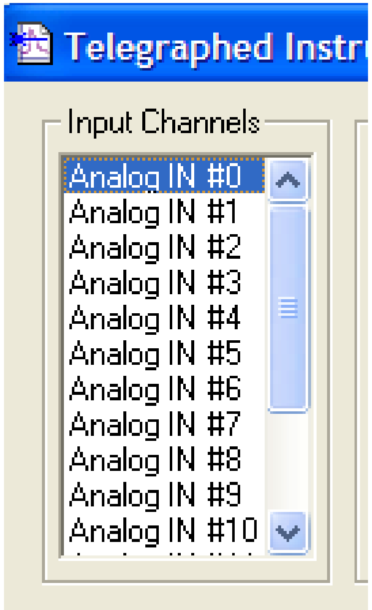
Most importantly, gain telegraphs enable automatic data scaling in Clampex software. When you change the Axopatch 200B amplifier gain settings, Clampex software automatically rescales the Y axis in the Scope window, and similarly sets the Y axis scaling for any data files recorded under the new settings.

We will enable just the gain telegraph in this demonstration.

Axopatch Telegraphs 1

Open Telegraphed Instrument from the Configure menu.



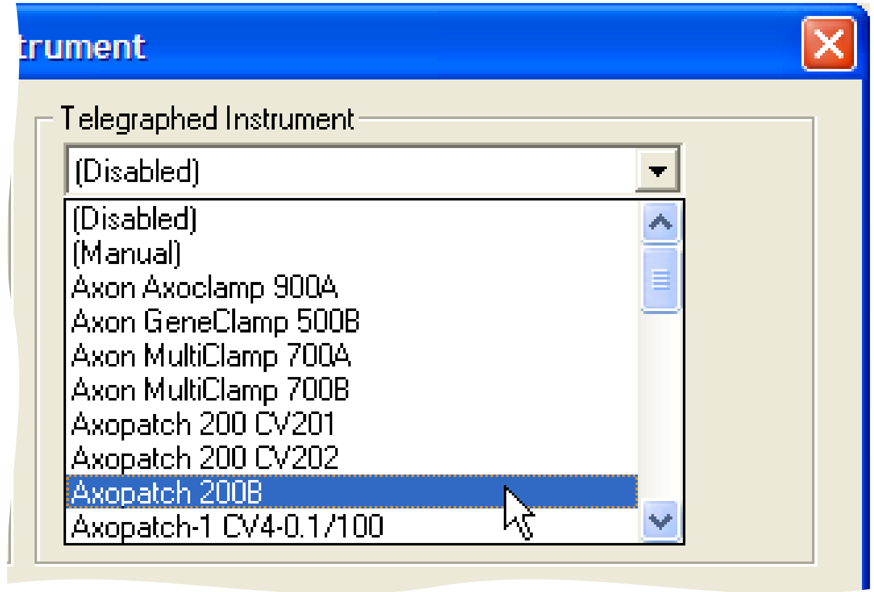


Select the digitizer input channel that you have the Axopatch scaled output connected to. Signals on this channel are the ones that are affected by changes in gain settings, so we must associate our telegraphs with this channel.

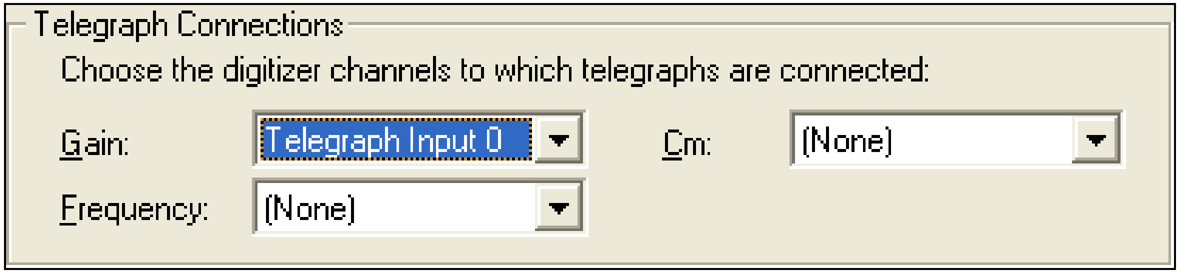
In our case, we have connected the scaled output to Analog IN #0 (Connections), so select this channel.

Axopatch Telegraphs 2

Select Axopatch 200B from the Telegraphed Instrument list box.



The Telegraph Connections section of the dialog is enabled. Here, in the Gain field, select the digitizer telegraph input channel receiving the gain telegraph: Telegraph IN #0.

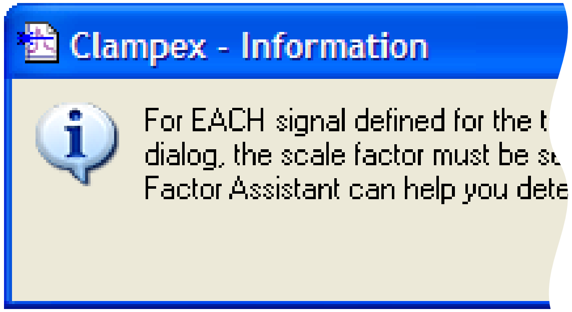


Axopatch Telegraphs 3

This completes the dialog for our purposes. Press OK to exit.

Clampex displays the warning shown at right, alerting us of the need to set scale factors for all signals using the input channel we have set telegraphs for—in our case, Analog IN #0.

We proceed to this in the next two sections.



Finish

We have configured Clampex so that for any data signal received via digitizer channel Analog IN #0, telegraph information about the amplification of that signal is also received. We now have to create signals for this channel so that the telegraphed information is put to use.

Users with different cable-telegraphing amplifiers should follow the procedure outlined here, but select their own machine in the Telegraphed Instrument list box. In the Telegraph Connections section, they will be offered options for the telegraphs supported by their amplifier.

Axopatch

Create Signals

In this section we create the signals required for the two protocols we are going to define, and assign these to input and output channels. We also note some additional telegraph options.

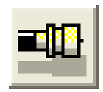
Before starting it is important to be clear on what signals and channels are:

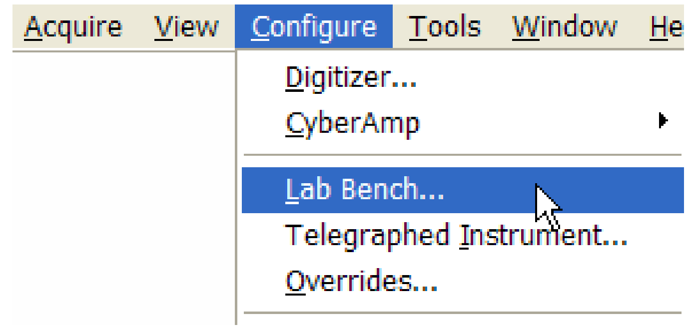
- **Signal:** a set of name, unit, scale factor and offset, by means of which the voltage inputs and outputs at the digitizer are represented in Clampex as the parameter being read at, or delivered to, the preparation.
- **Channel:** a cable connection to the digitizer, identified by the name of the BNC port where connection is made, e.g. Analog IN #0, Digital OUT #2.

As already noted, analog channels can be configured for different signals at different times. In this section we name the signals we will need and give them appropriate units. We set scale factors for these signals in the next section.

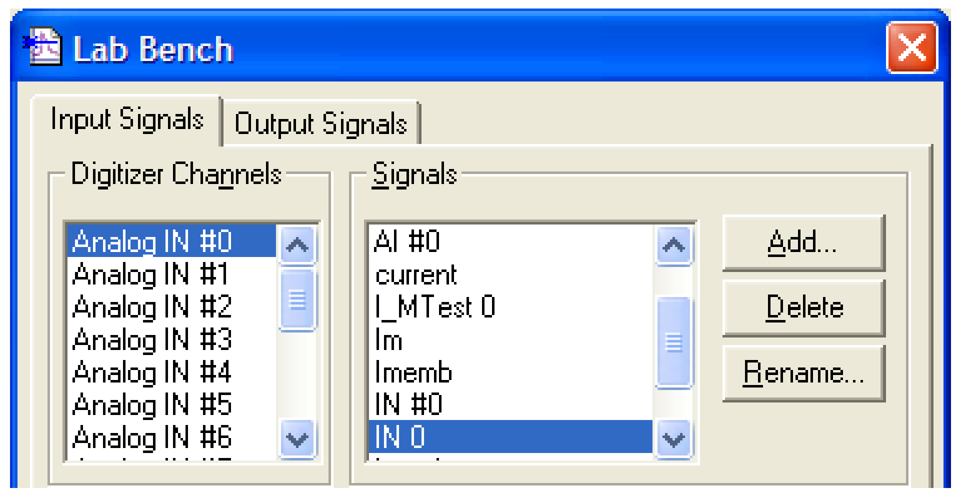
Axopatch Signals 1

Open the Lab Bench from the Configure menu—or use the toolbutton:

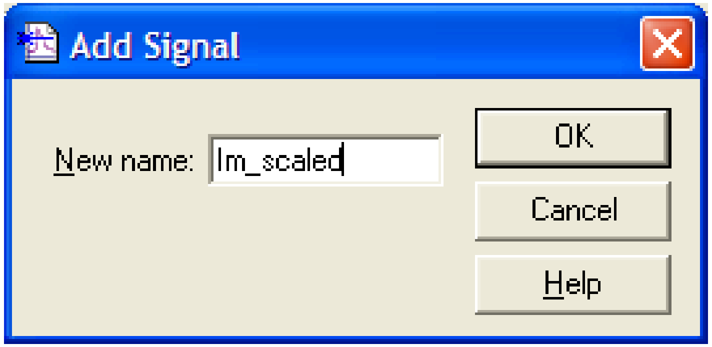




The Lab Bench opens with the Input Signals tab on top, and digitizer channel Analog IN #0 selected. We have the amplifier's scaled output connected to this channel (Connections), so need to create two signals—one each for current and voltage clamp—in association with it.



Axopatch Signals 2



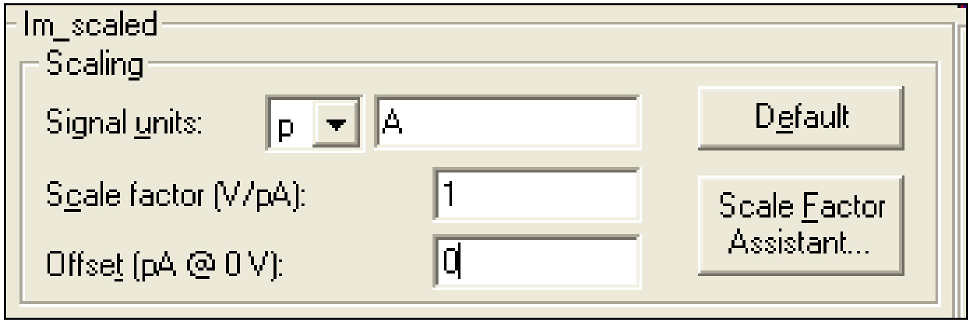
Click the Add button in the Signals section, opening the Add Signal dialog.

Type in “Im_scaled”—the name we will give the scaled membrane current signal for voltage clamp.

Press OK.

With the new signal selected in the Signals list, the rest of the Lab Bench shows options and settings for that signal. First is scaling.

We want to read “Im_scaled” in picoamps— i.e. have picoamps as the Y axis units for the Scope window and subsequently recorded data files.



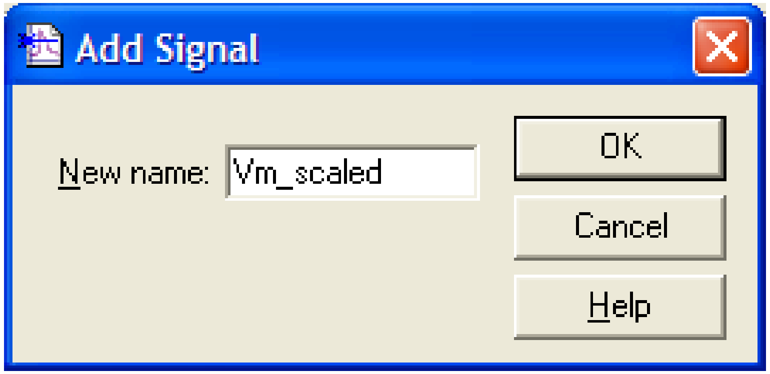
Select “p” from the Signal units list box, and type “A” in the adjoining field.

Axopatch Signals 3

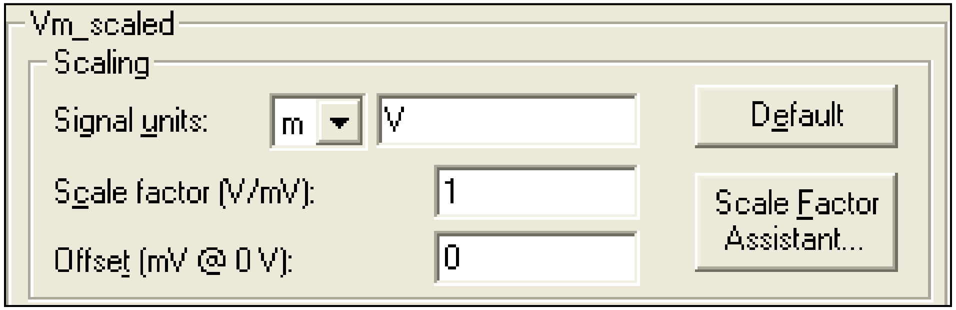
Now we add a signal—still for channel Analog IN #0—for reading membrane potential in current clamp. Press the Add button again.

This time call the signal “Vm_scaled”.

Press OK.



Vm_scaled is to be read in millivolts. Set the signal units appropriately, as we did for “Im_scaled”.

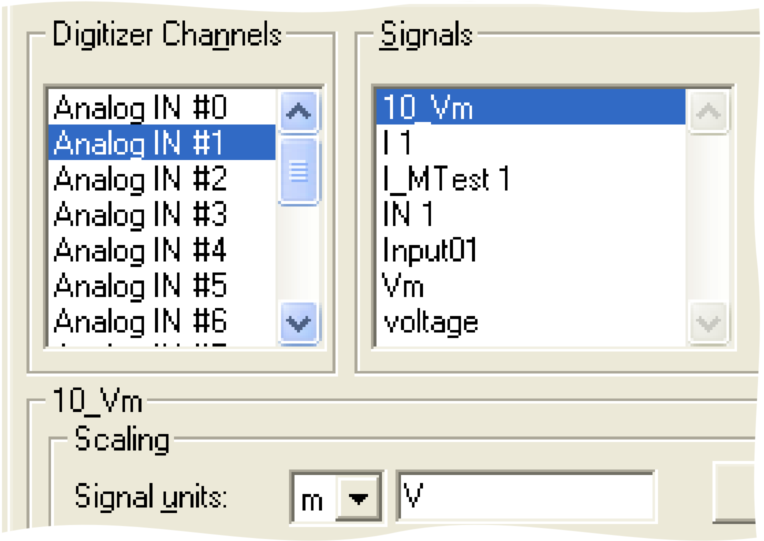


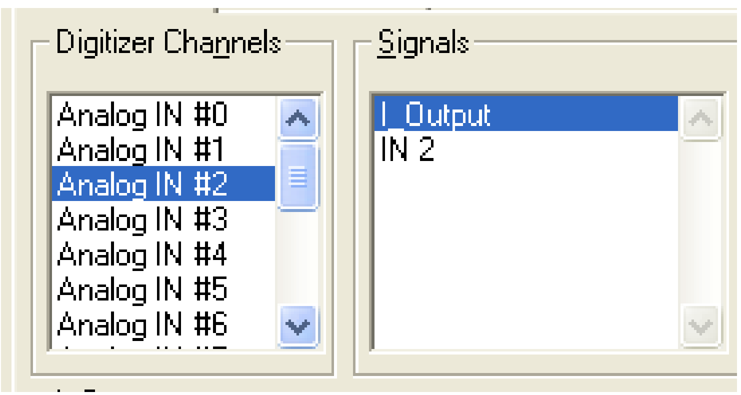
Axopatch Signals 4

There are 2 more input signals to create—one each for the 10 V_m OUTPUT and I OUTPUT ports.

We connected the 10 V_m port to Analog IN #1 (Connections). Select this channel and add a new signal, "10_Vm", as we did for previous signals.

Configure the signal for millivolts.





We connected the I OUTPUT port to Analog IN #2. Select this channel and add new signal "I_Output".

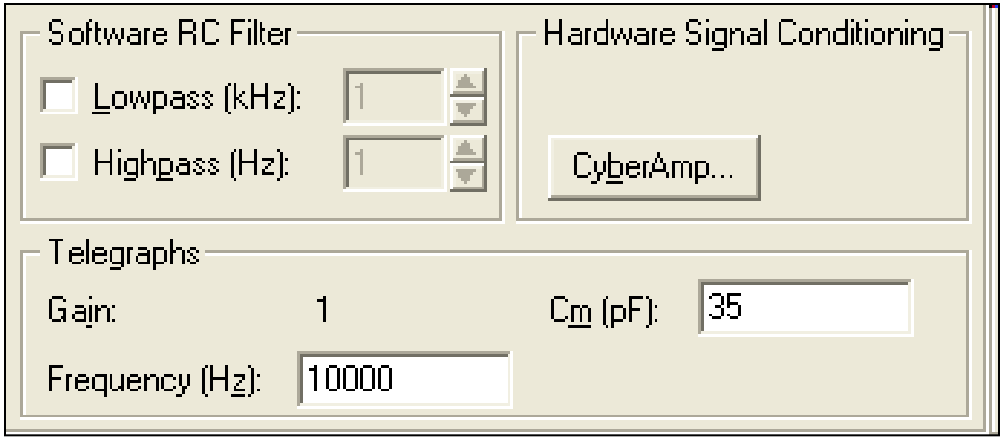
Configure this signal for picoamps.

Axopatch Signals 5

This completes the input signals. Before creating output signals, however, reselect Analog IN #0 as the digitizer channel, and “Im_scaled” as the signal.

Note the options for additional filtering in the lower half of the tab.

In the Telegraphs section, because we have set up a gain telegraph for this channel, the amplifier gain is reported. This combines headstage and output gain, so, for example, a headstage gain β = 0.1 and output gain α = 10 gives a reported gain of one.



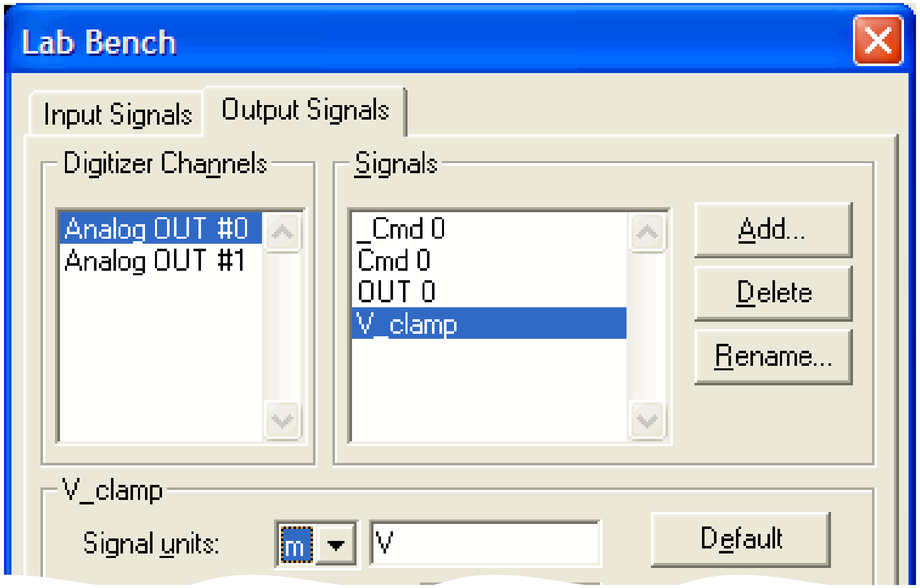
Because we have not set up lowpass filter frequency or whole cell capacitance neutralization telegraphs, we are given the option of typing in values for these.

Axopatch Signals 6

Now we create output signals for the command waveform for each of current and voltage clamp.

Still in the Lab Bench, go to the Output Signals tab. Ensure Analog OUT #0 is selected as the digitizer channel. This is the channel we have connected to the amplifier EXTERNAL COMMAND INPUT FRONT SWITCHED port (Connections).

Just as for the input signals, add a new signal, "V_clamp", and set the units to millivolts. This will carry the command signal for voltage clamp.



Next, still for Analog OUT #0, add "I_clamp", and configure for nanoamps. This is the signal we will use to deliver the command in current clamp.

Axopatch Signals 7

Finish

We have created four input and two output signals, giving them units and associating them with particular digitizer channels:

Voltage Clamp

- Im_scaled
- 10_Vm
- V_clamp

Current Clamp

- Vm_scaled
- I_Output
- I_clamp

Next, and still in the Lab Bench, we must set scale factors for each of these signals.

Axopatch

Set Scale Factors

Clampex must be configured so that voltage differences received and produced by the digitizer represent the actual currents and voltages produced and received by the cell. We have gone some way towards this by defining appropriate units for our signals, but it remains to set scale factors for these.

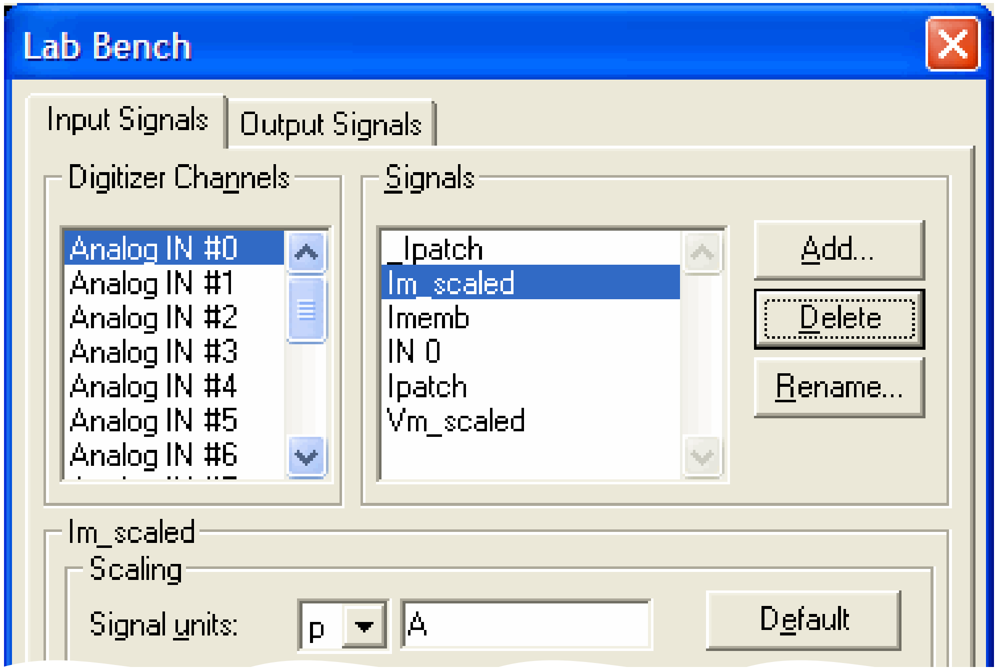
Setting scale factors is greatly simplified with the Scale Factor Assistant. Note however, that although this can be used for all output signals, for input signals it is intended for use with scaled signals only, i.e. signals on channels connected to the amplifier SCALED OUTPUT port. In this section then, we set some scale factors using the Assistant, and some manually.

Axopatch Scale Factors 1

We will set the scale factor for "Im_scaled" first—the signal for reading membrane current in voltage clamp.

We will use the Scale Factor Assistant for this signal.

On the Lab Bench Input Signals tab, with "Im_scaled" (on digitizer channel Analog IN #0) selected, open the Assistant.



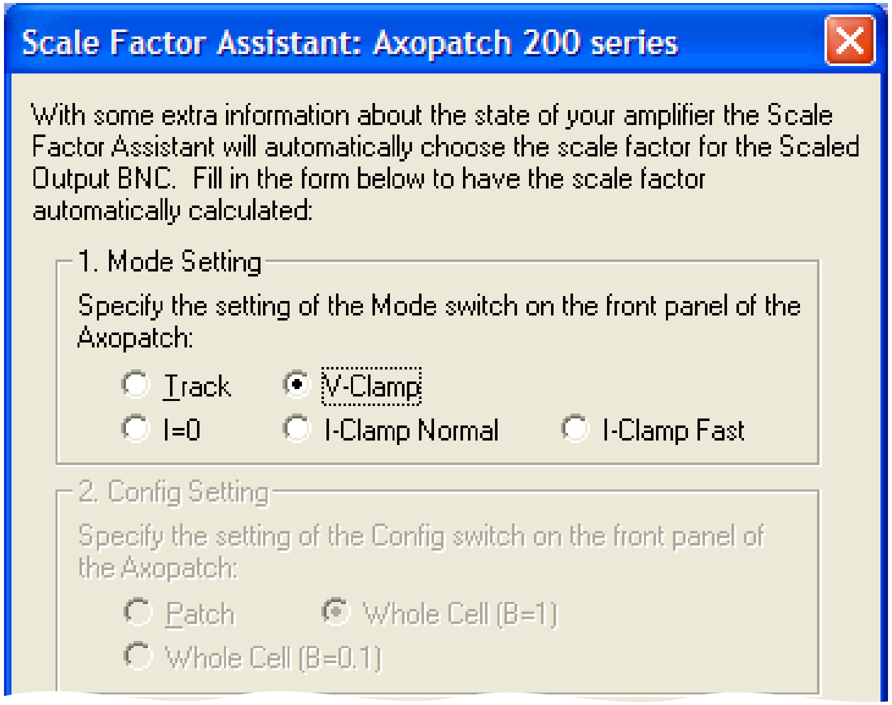
Axopatch Scale Factors 2

Because we indicated the amplifier type when we configured the gain telegraph for this channel, the Assistant automatically opens with the correct dialog for the Axopatch 200.

Most of the work in the Assistant consists of simply copying amplifier settings into the dialog.

Mode Setting

"Im_scaled" is for use in voltage clamp, so select "V-Clamp" for the amplifier mode option.



Config Setting

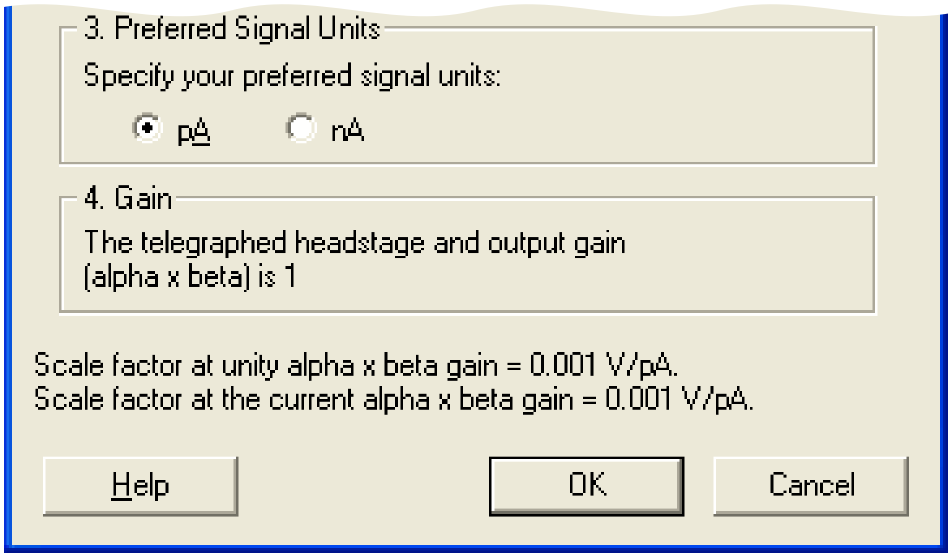
This section, where you would otherwise indicate the headstage gain that you are using is disabled, as the combined headstage and amplifier gain is being telegraphed.

Axopatch Scale Factors 3

Signal Units

In section 3 choose whether to read cell current in picoamps or nanoamps. In fact we have already set this value in the previous section, so keep the "pA" setting we entered there.

Entering "nA" here does not affect the scale factor, but simply causes it to be expressed in terms of this unit.



Gain

The amount of gain applied to a signal is important for calculating the scale factor. In our case the total amplifier gain—i.e. the combined headstage (b) gain and amplifier output (a) gain—is telegraphed to Clampex, and this value is reported, just as in the Telegraphs section of the Lab Bench Inputs tab.

Axopatch Scale Factors 4

Two scale factor values are reported at the bottom of the Assistant:

- Scale factor at unity alpha × beta gain = 0.001 V/pA

  This is the scale factor that applies when the combined headstage and output gain is one.

  This value depends on amplifier circuitry and never changes (though it can be expressed in terms of nanoamps if this is selected as your preferred unit).

  Since we have the gain telegraph enabled, this is the value that will be reported in the Lab Bench scale factor field.

- Scale factor at the current alpha × beta gain = [ ]

  This is the scale factor that will be used if data are acquired under the current gain settings on the Axopatch. With the gain telegraph enabled, this value does not appear anywhere else in Clampex.

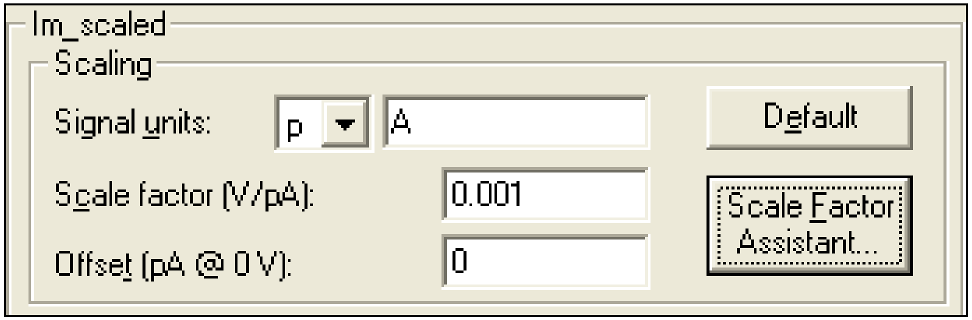
  This scale factor changes if you change the gain setting, but you need to close and reopen the Assistant in order to have this reported in the Assistant.

Press OK to close the Scale Factor Assistant.

Axopatch Scale Factors 5

The scale factor at unity α × β gain—0.001 V/pA—has been written into the scale factor field.

When gains telegraphs have been enabled—as in the current case—this value is displayed no matter what the gain on the amplifier is set to. The scaling applied to incoming data is automatically adjusted for the gain at the time of acquisition, but the value reported in this field does not change.



- There is generally no need to set offsets for the amplifiers used in electrophysiology, so this completes setup for "Im_scaled". We now move on to the second scaled and telegraphed input signal, "Vm_scaled", for which we will again use the Scale Factor Assistant.

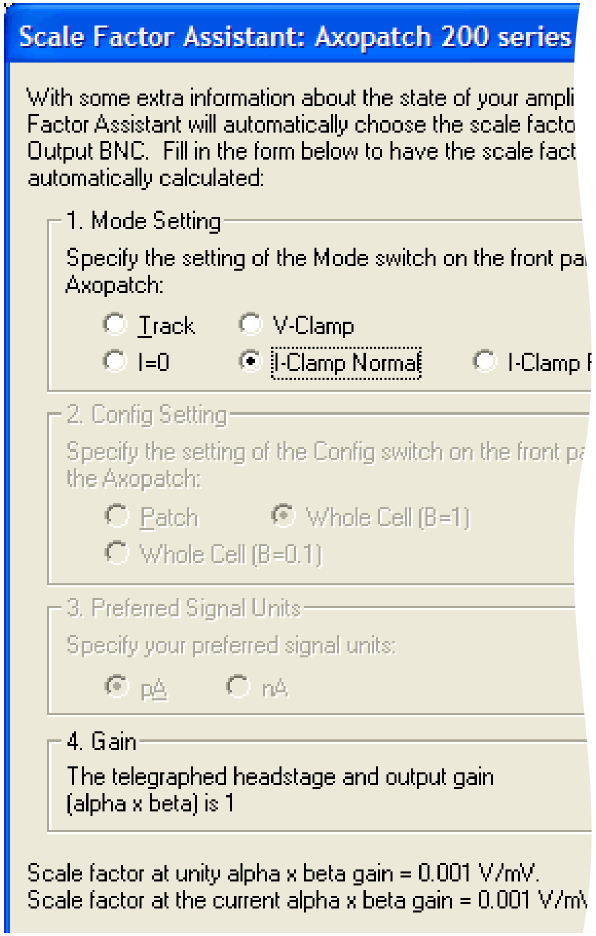
Axopatch Scale Factors 6

Still with Analog IN #0 as the digitizer channel, select “Vm_scaled” and again open the Assistant.

This time, the signal is for use in current-clamp mode “I-Clamp Normal”, so select this in the Mode group.

This is all you need to do. Again, the amplifier’s total gain setting is reported and used to calculate the second of the scale factors at the bottom of the Assistant. And again, only the unity-gain scale factor is reported in the Lab Bench, though incoming data will be scaled for the gain at the time of acquisition.

The scale factor at unity gain is 0.001 V/mV, i.e. 1 mV/mV, which is what we expect when there is no amplification.



Axopatch Scale Factors 7

Setting Scale Factors Manually

The scale factor for a signal is found by taking the unity-gain scale factor for the amplifier port the signal will use, and multiplying by the amount of amplification applied.

For the Axopatch 200B, this procedure is summarized in either the names given to the BNC ports, or in information provided beneath the ports on the amplifier panel.

For example, the SCALED OUTPUT port has information:

- **I: α β mV/pA**
- **V_m: α mV/mV**.

The first of these means that, for current, when combined headstage and output gain is one ($\alpha \times \beta = 1$) the Axopatch outputs one millivolt per picoamp input, or 0.001 V/pA.

The second means that for voltage, with output (α) gain of one (headstage gain is not relevant in this case), the Axopatch outputs one millivolt per millivolt, or 0.001 V/mV.

Note that these are the unity-gain scale factors reported by the Scale Factor Assistant for the previous two signals.

We must now apply this to our two remaining input signals: “10 _Vm” and “I_Output”.

Axopatch Scale Factors 8

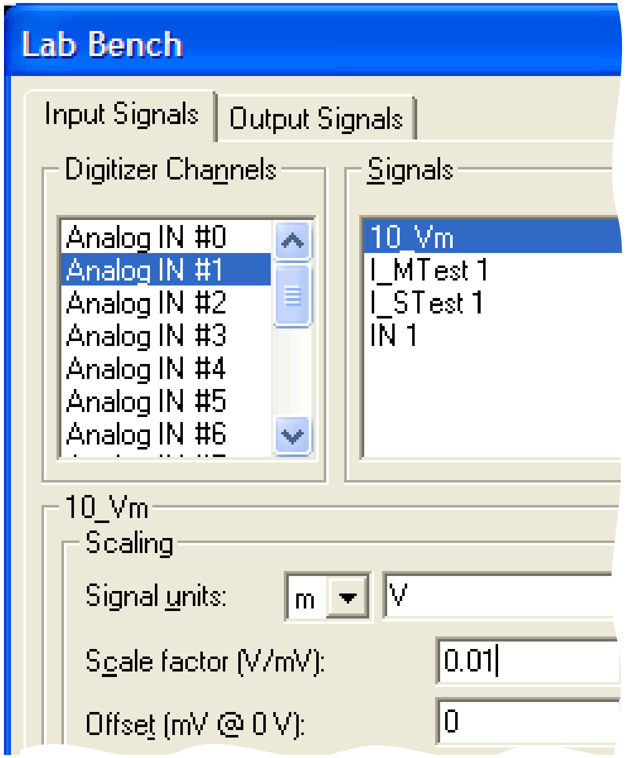
10_Vm

We created "10_Vm" for digitizer channel Analog IN #1. Select this channel and the signal.

Analog IN #1 is connected to the 10 V_m port on the amplifier. This port outputs membrane voltage with a set gain of 10. A unity-gain scale factor of one millivolt per millivolt (0.001 V/mV) multiplied by the set gain value:

$$0.001 \text{ V/mV} \times 10 = 0.01 \text{ V/mV}$$

This is the scale factor for signals read from this port. Enter 0.01 in the scale factor field.



Axopatch Scale Factors 9

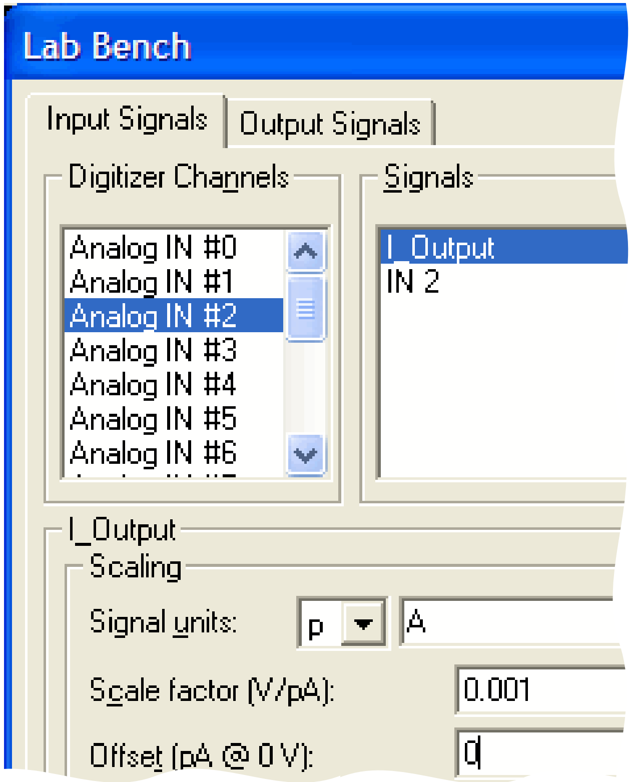
I_Output

Select Analog IN #2 digitizer channel, and "I_Output". The channel is connected to the
I OUTPUT port on the amplifier, which outputs membrane current at one of two scaling options reported on the panel: 100 β mV/pA and β mV/pA.

We have the switch in the down position (β mV/pA), and the Config switch on the front panel should be set at "Whole Cell β = 1". The scaling factor we set, then, is:

1 mV/pA = 0.001 V/pA

Set this value in the scale factor field.



This completes the scale factors for all the input signals.

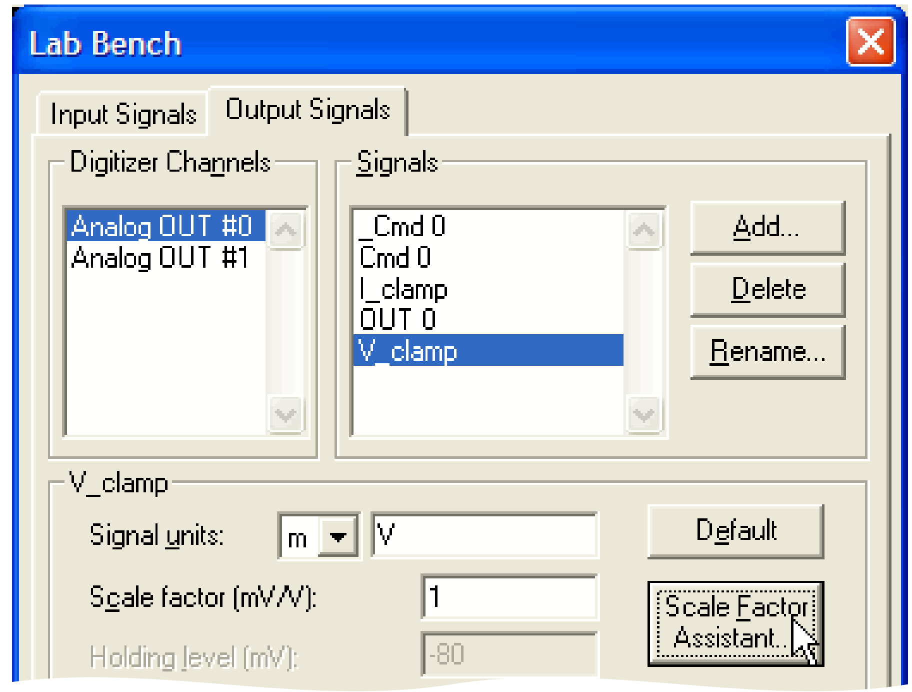
Now for the two output signals.

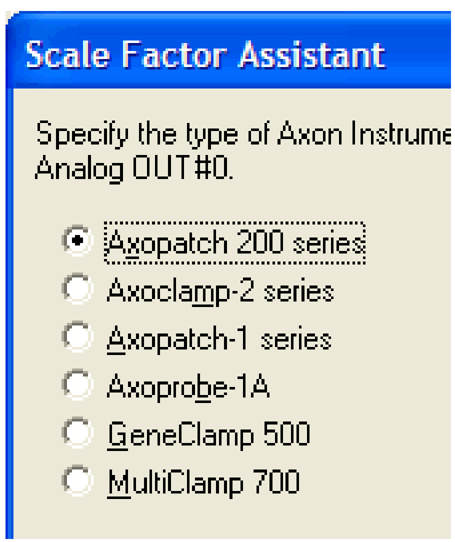
Axopatch Scale Factors 10

Command Signals

We will use the Scale Factor Assistant to set the scale factor for the voltage clamp command signal first.

On the Output Signals tab, select Analog OUT #0 and the signal "V_clamp".





Open the Scale Factor Assistant and select "Axopatch 200 series" from the first dialog box.

Axopatch Scale Factors 11

The signal “V-clamp” is for use in voltage clamp, so select “V-Clamp” in the Mode Setting section.

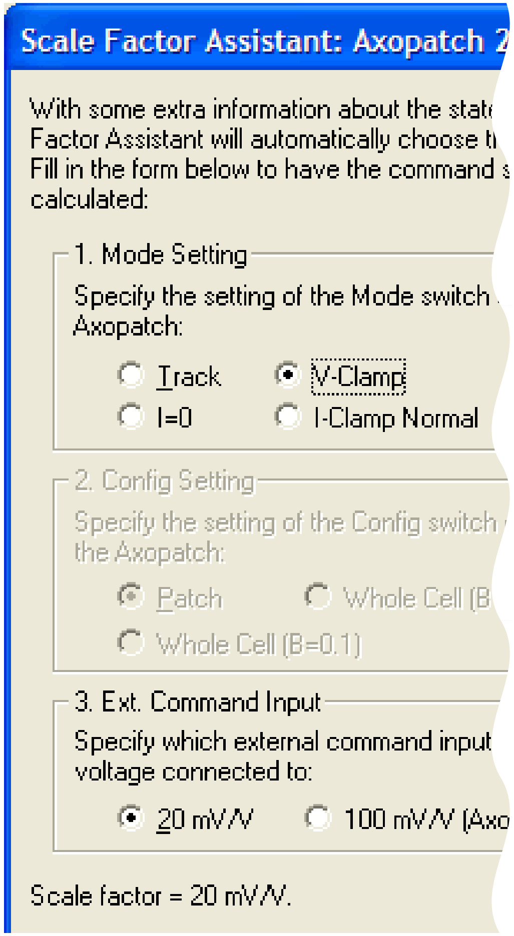
This leaves just the Ext. Command Input section to complete.

The Axopatch 200B has two command input ports—the selection here informs the Assistant of the one we have connected our command cable to. We used the front-switched port.

Reading off the panel we see that in voltage-clamp mode this port has a set scaling of 20 mV/V.

The rear-switched port, in contrast, scales command signals at 100 mV/V.

Select the 20 mV/V option. This is reported at the bottom of the dialog. Press Finish to close the Assistant and transfer the scale factor to the Lab Bench.



Axopatch Scale Factors 12

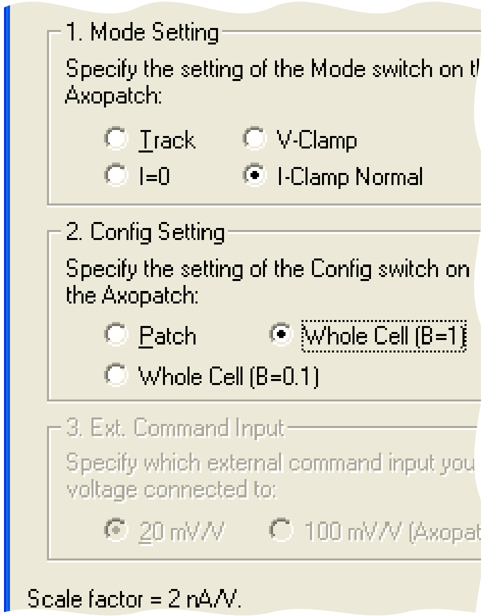
For “V_clamp“, the choice of amplifier input BNC was the sole determinant of the scale factor, and this could have been easily read off the panel and entered manually. For current clamp, headstage β gain becomes relevant.

Select the signal “I_clamp” in the Lab Bench and open the Scale Factor Assistant. Again, choose the Axopatch 200 series and go to the next dialog.

Select “I-Clamp Normal” as the mode, as “I_clamp” is the command signal for current clamp.

As we are passing current with this signal the Config setting, switched on the front panel of the Axopatch, is relevant. It determines the headstage β gain. We are setting up for whole cell recording, with β = 1. Select this option.

The calculated scale factor (2 nA/V) is reported at the bottom of the Assistant, and in the Lab Bench scale factor field when the Assistant is closed (next slide).

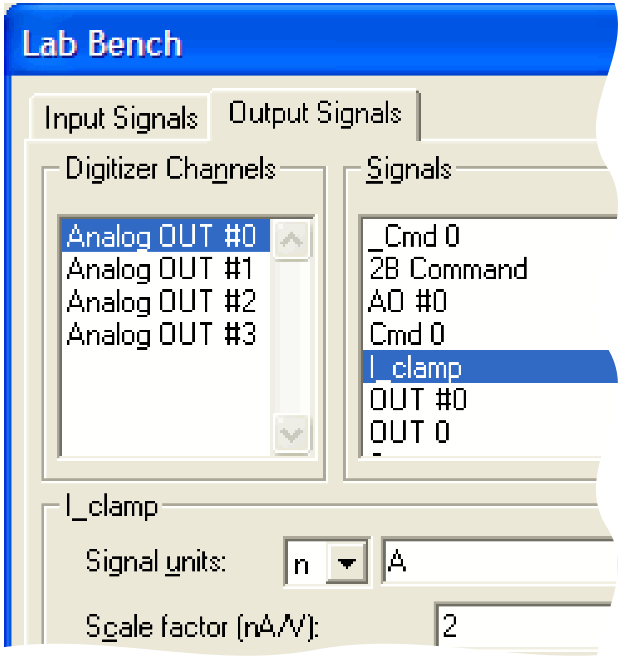


Axopatch Scale Factors 13

Again, we might have set the scale factor manually. Under the front-switched EXT. COMMAND BNC on the amplifier the scaling rate for current clamp is given as:

$$2 \div \beta \text{ nA/V}$$

We have β = 1, hence a scale factor of 2 nA/V.



Finish

We have completed all that we need to do in the Lab Bench. We have created all the signals we wanted, and set scale factors for these. Press the OK button to close the Lab Bench with the new signals intact.

It remains now to assemble the new signals into two groups, according to their use for current or voltage clamp.This is done in the protocol editor.

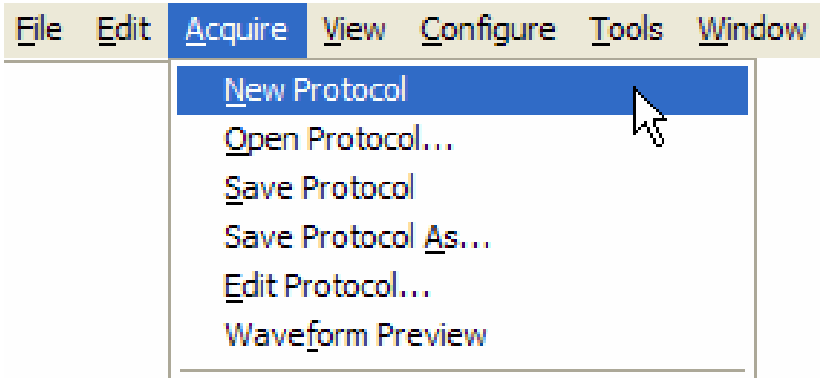
Configure Protocols

Protocols in Clampex are complete sets of acquisition parameters, including options for command waveforms and preliminary data analysis. Particular signals, defined in the Lab Bench, are specified for each protocol.

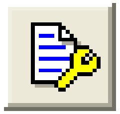
In this section we create two simple protocols, one each for current and voltage clamp, incorporating the signals we have just defined.

Axopatch Protocol 1

Open the protocol editor by selecting New Protocol in the Acquire menu.



Note: If a previously saved protocol is not loaded in Clampex, it uses a place-holder protocol, labeled "(untitled)". If this is currently loaded you can open the editor to create a new protocol by selecting Edit Protocol, or by clicking the toolbutton:



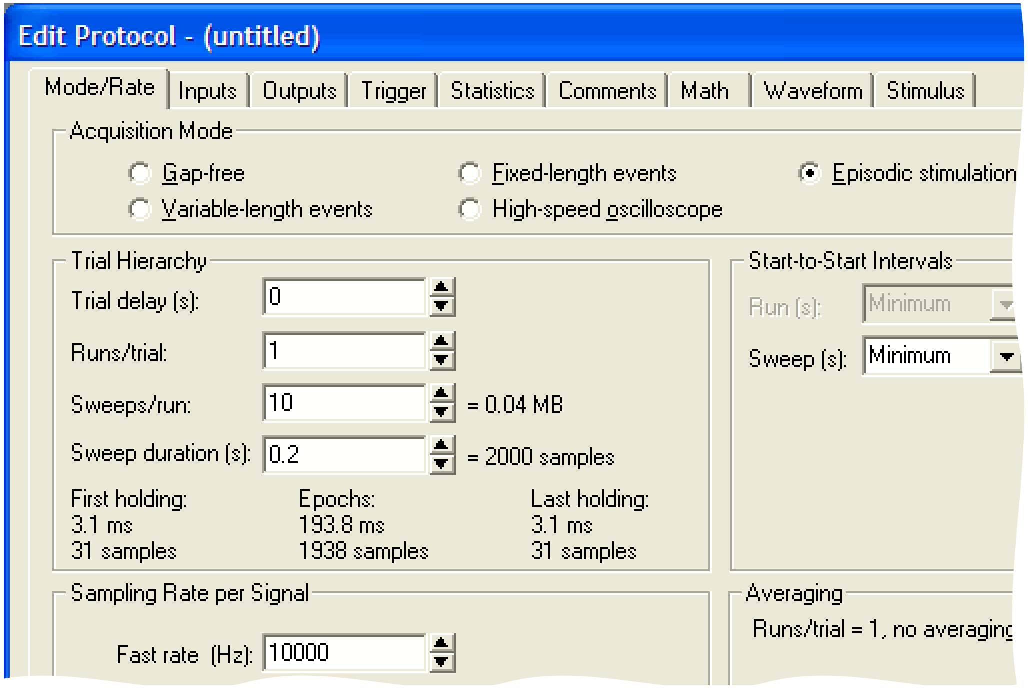
The currently loaded protocol is reported in the status bar at the bottom of the main Clampex window.

We will begin by setting up the protocol for voltage clamp.

Axopatch Protocol 2

The front tab of the protocol editor has controls for, amongst other things, acquisition mode, sampling rate, and trial hierarchy.

The default acquisition mode is episodic stimulation—the only mode that allows a command waveform to be generated. We want to generate a command, so leave this setting. In fact, all the default settings on this tab can be left as they are, but take time to note key parameters such as the Sampling Rate (10 kHz), the number of samples per sweep, and the number of sweeps per run.



The sweep start-to-start interval is set at Minimum, so each new sweep starts as soon as the previous one is finished.

Axopatch Protocol 3

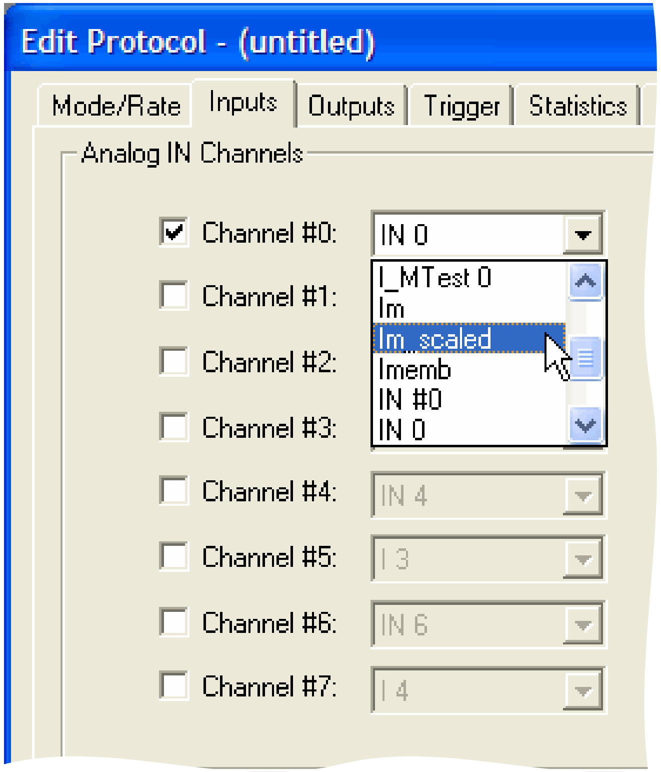
Next go to the Inputs tab.

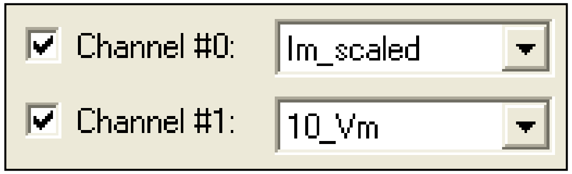
Here you select digitizer input channels for the protocol, and the signals you want to carry on these.

For voltage clamp, we want two input signals—one scaled signal for membrane current, and a second unscaled signal to monitor membrane voltage. We created these in the Lab Bench—"Im_scaled" and "10_Vm"—associating them with channels 0 and 1. Now we incorporate them into the protocol.

Channel #0 should be already checked. Open the list box beside it and select "Im_scaled".

Then check Channel #1 and select "10_Vm".



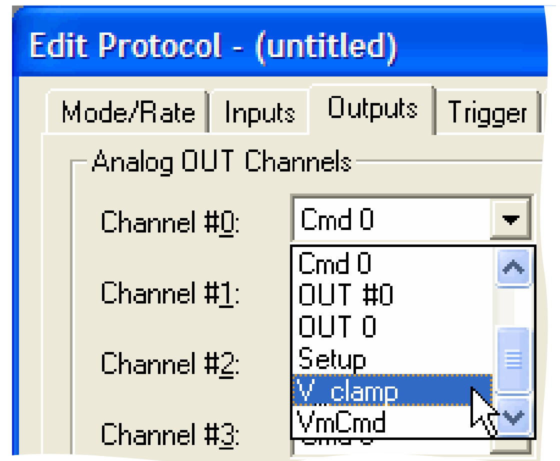


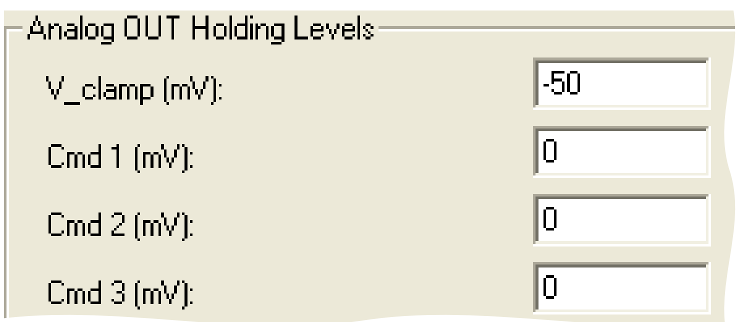
Axopatch Protocol 4

Go to the Outputs tab.

We configured the signal “V_clamp” to deliver the voltage clamp command waveform, on output channel #0.

Select “V_clamp” from the Channel #0 list box.





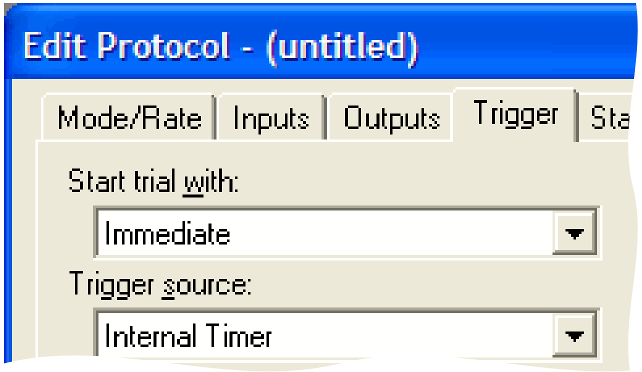
Additionally, we will set a holding level for this output. This is maintained all the time the protocol is loaded except for when specific output commands are generated.

Enter –50 in the V_clamp holding level field. The units are millivolts, from our Lab Bench configuration of this signal.

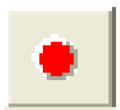
Axopatch Protocol 5

Although we will not make any changes for the purposes of our protocol, it is worth taking a quick look at the trigger settings.

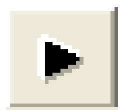
Go to the Trigger tab.



Default settings give "Immediate" trial starts. This means Clampex is armed for data acquisition as soon as you select Record, or View Only, from the Acquire menu—or click the toolbuttons:



Record



View Only

The default trigger source is "Internal Timer".
This triggers the command waveform and data acquisition immediately the trial is started, continuing through to the end of the trial automatically.

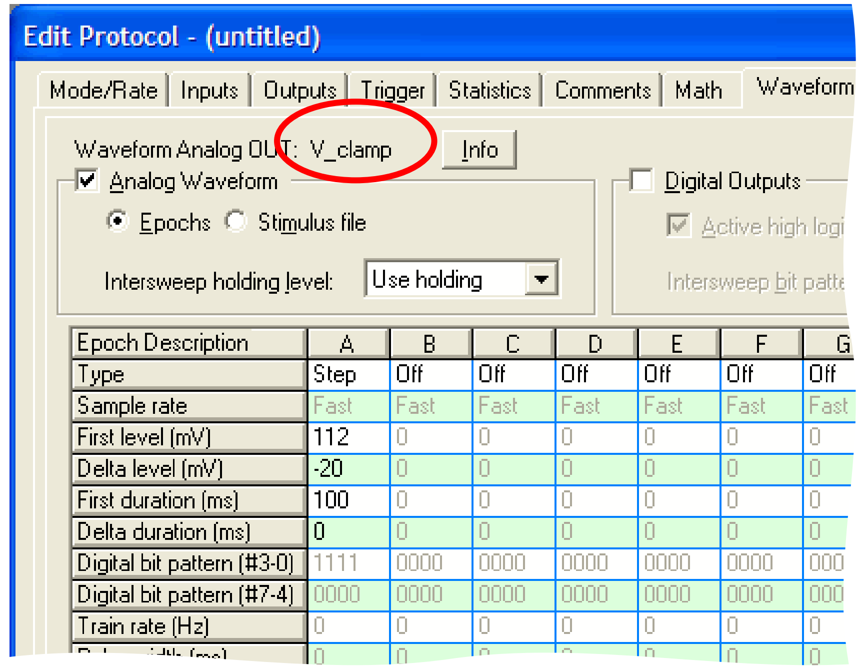
Axopatch Protocol 6

Now go to the Waveform tab, Channel #0 tab, where outputs are defined for ANALOG OUT #0.

A default waveform is already defined—we will delete this and create our own simple stimulus, but first familiarize yourself with some key settings on this tab.

The Analog Waveform check box enables analog command definition. Selecting Epochs means we define the waveform using the table in the middle of the tab. In this, the sweep can be divided into up to 50 sections (epochs) A–AX, and a waveform defined for each of these.

The Epoch Description table in the Waveform tab includes cut and paste functionality.



Note that the confirmation that “V_clamp” is the signal carrying the output waveform. Click the Info button to see the V_clamp vital statistics. Note too that in the Epoch Description table, First level and Delta level have the correct units (mV) for “V_clamp”.

Axopatch Protocol 7

Now to the definition of our waveform.

We will configure an output with a simple step, increasing the amplitude of the step with each sweep.

In column A of the epoch description table, keep "Step" in the Type row, but click in the "First level" row and type in –50. This sets the output level for epoch A in the first sweep of the run. Our entry of –50 mV maintains the holding level we set on the Outputs tab.

Epoch Description	A	B
Type	Step	Off
Sample rate	Fast	Fast
First level (mV)	-50	0
Delta level (mV)	0	0
First duration (ms)	100	0
Delta duration (ms)	0	0
Digital bit pattern (#3-0)	1111	0000
Digital bit pattern (#7-4)	0000	0000
Train rate (Hz)	0	0
Pulse width (ms)	0	0

Click in the next row (Delta level) and type in 0. This keeps the first level setting for subsequent sweeps—i.e. epoch A is maintained at –50 mV for each of the 10 sweeps in the trial.

Axopatch Protocol 8

Now to set the period for epoch A.

Click in the First duration row, and type in 50 for a 50 ms duration.

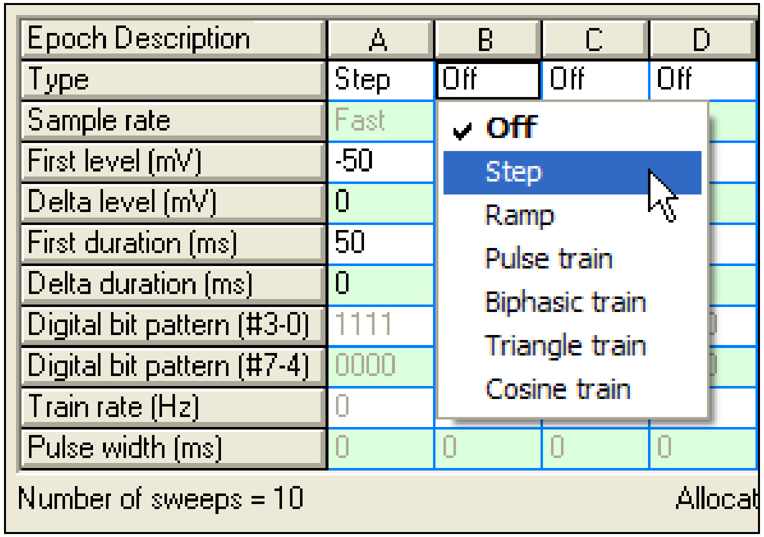
Press Enter to see this reported below the table.

This completes epoch A. Now we configure the step, in epoch B.

Epoch Description	A	B	C	
Type	Step	Off	Off	O
Sample rate	Fast	Fast	Fast	F
First level (mV)	-50	0	0	0
Delta level (mV)	0	0	0	0
First duration (ms)	50	0	0	0
Delta duration (ms)	0	0	0	0
Digital bit pattern (#3-0)	1111	0000	0000	0
Digital bit pattern (#7-4)	0000	0000	0000	0
Train rate (Hz)	0	0	0	0
Pulse width (ms)	0	0	0	0

Number of sweeps = 10

Stimulus File... First duration 50.00 ms (500 samples)



Click in the Type row in column B (currently set to "Off").

Select "Step" from the popup menu.

Axopatch Protocol 9

Set the level for the first sweep at –100 mV.

For this epoch, because we want an incrementing step level from sweep to sweep, we enter a delta level. Click on the Delta level cell and type in 20. This forces the step level up 20 mV with each successive sweep.

We have 10 sweeps starting at –100 mV, so the final sweep will have a step level of 80 mV, reported below the table.

Now set the First duration, at 100 milliseconds.

Again, this is reported below the table, in milliseconds as well as in samples.

We will not set a delta duration, which would alter the length of the epoch from sweep to sweep, so this completes our waveform definition for the voltage clamp protocol.

Epoch Description	A	B	C	D
Type	Step	Step	Off	Off
Sample rate	Fast	Fast	Fast	Fast
First level (mV)	-50	-100	0	0
Delta level (mV)	0	20	0	0
First duration (ms)	50	100	0	0
Delta duration (ms)	0	0	0	0
Digital bit pattern (#3-0)	1111	0000	0000	0000
Digital bit pattern (#7-4)	0000	0000	0000	0000
Train rate (Hz)	0	0	0	0
Pulse width (ms)	0	0	0	0

Number of sweeps = 10 Allocat

Stimulus File...

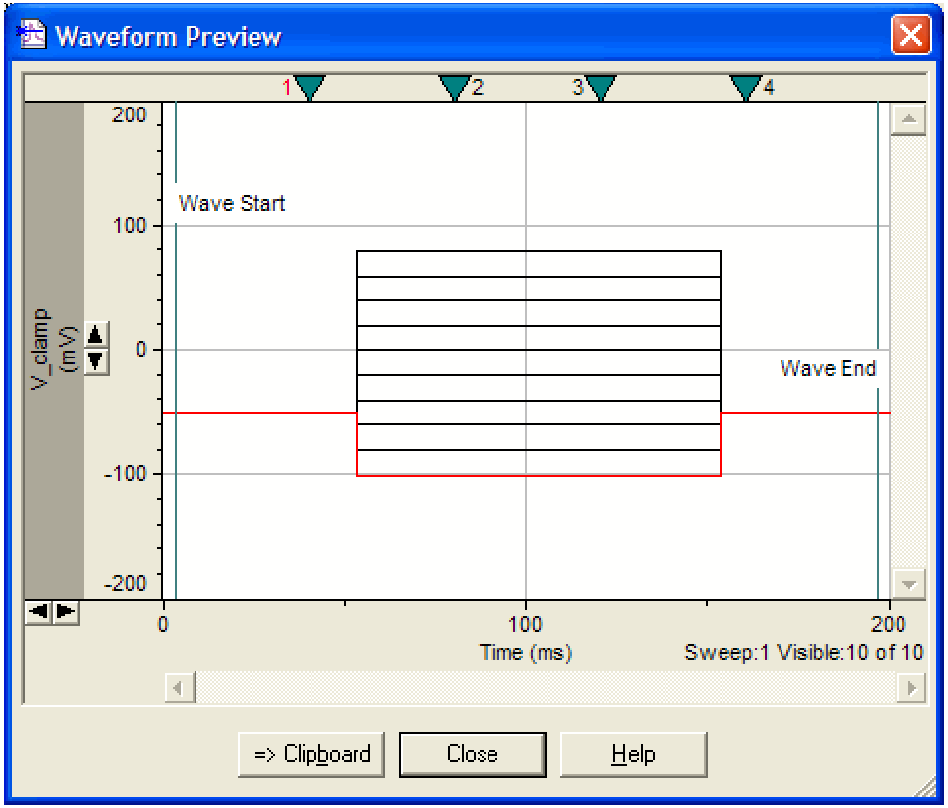
Final level 80.00 mV
First duration 100.00 ms (1000 samples)

Axopatch Protocol 10

Press the Update Preview button in the bottom right corner of the protocol editor.

This opens the Waveform Preview window shown at right, where you can see a graphical representation of the waveform you have defined.

This window can be kept open while you experiment with different epoch settings—press the Update button whenever you want to update the display.



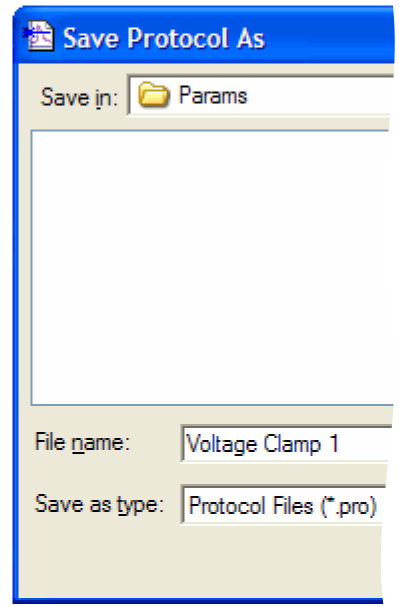
Note: The Waveform Preview opens with panes for all analog output channels—right-click in the upper pane and select Maximize Signal from the popup menu to get the display shown above.

Axopatch Protocol 11

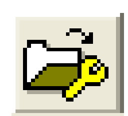
We have completed the setup of the voltage clamp protocol—close the protocol editor with the OK button.

The new protocol is loaded, still labeled "(untitled)", and we could acquire data under it if we wanted, but it is not saved for future use.

Go to Save Protocol As in the Acquire menu. This opens a standard file-saving dialog. Name the protocol "Voltage Clamp 1", and click Save.



The protocol is now saved and can be loaded whenever we want, with the Open Protocol command in the Acquire menu, or toolbutton:



MOLECULAR DEVICES

Axopatch Protocol 12

Setup of the current clamp protocol follows similar lines to that for the voltage clamp protocol.

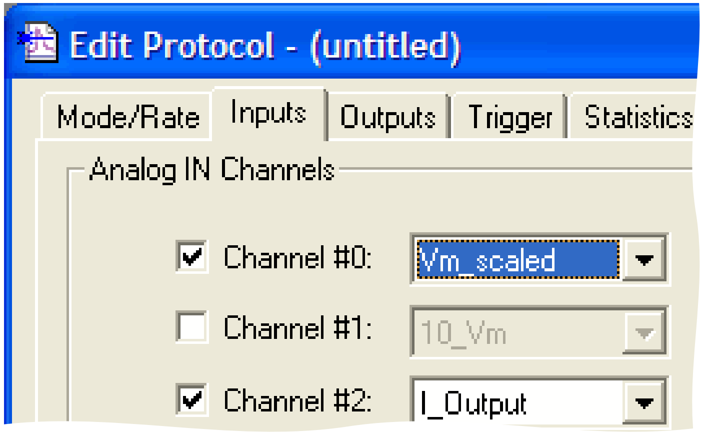
Open the protocol editor again with New Protocol, in the Acquire menu.

We will again accept the default settings in the Mode/Rate tab, so go straight to the Inputs tab.

This time select “Vm_scaled” for Channel #0.

Recall that the second, current-monitoring signal that we want to read in current clamp (“I_Output”) is set up for the connection from the I OUTPUT port on the amplifier to digitizer channel Analog IN #2 (Connections).

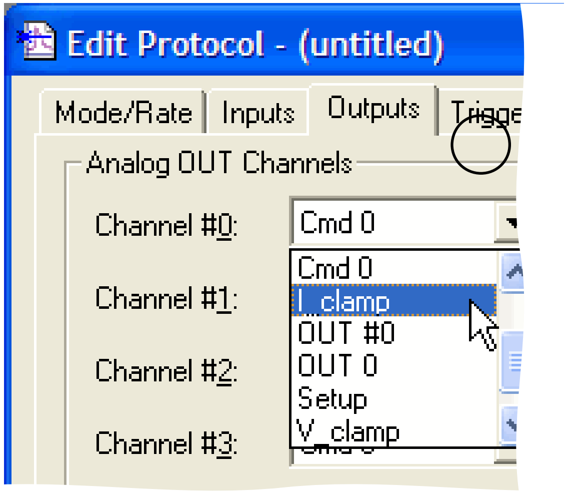
Check Channel #2 and select “I_Output”.



Axopatch Protocol 13

On the Outputs tab, select the current clamp command signal we configured for Analog OUT channel #0: “I_clamp”.

Leave the holding level at the default zero setting for current clamp.



	A	B	C	D	E
Type	Step	Ramp	Step	Trngl	Step
Sample rate	Fast	Fast	Fast	Fast	Fast
First level (nA)	-1	1	-0.5	1	0
Delta level (nA)	1	0	0	0	0
First duration (ms)	40	40	0.1	100	5
Delta duration (ms)	0	0	0	0	0
Digital bit pattern (#3-0)	1111	0000	0000	0000	0000
Digital bit pattern (#7-4)	0000	0000	0000	0000	0000
Train rate (Hz)	0	0	0	25	0
Pulse width (ms)	0	0	0	1	0

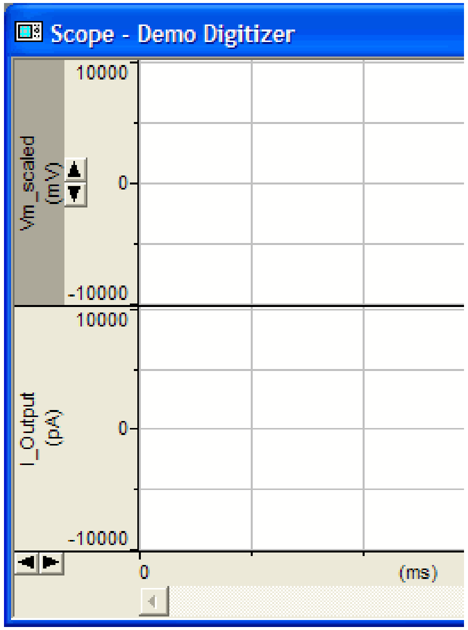
Create your own command waveform on the Waveform Channel #0 tab.

Experiment with the different waveform options, viewing these in the Waveform Preview window.

Axopatch Protocol 14

Finally, close the protocol editor by pressing OK, and save the protocol (Save Protocol As in the Acquire menu), calling it "Current Clamp 1".

You will see on exit that the scope window is set up in preparation to receive the two input signals configured for this protocol.

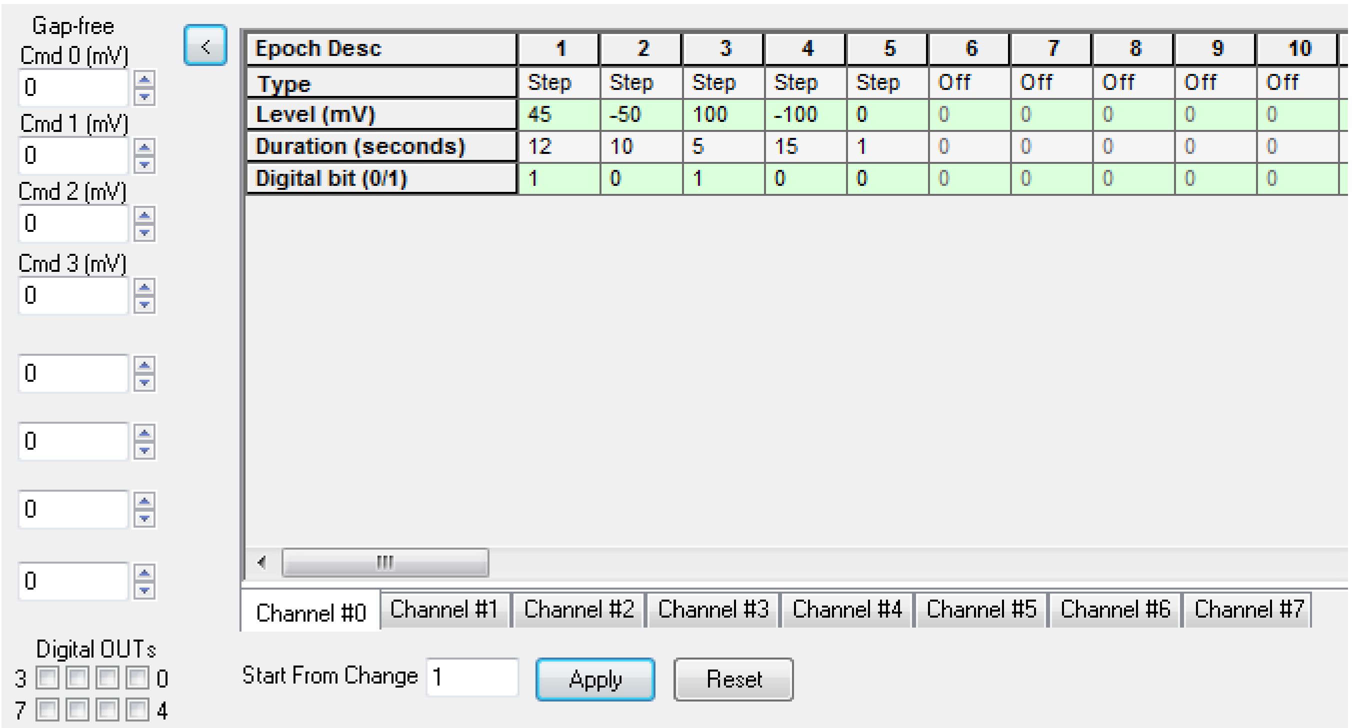


To change from current clamp to voltage clamp you need only load the appropriate protocol, then start acquisition. Alternatively, you can link each protocol to a sequencing key, so that you only have to press one toolbutton, or use one keyboard combination, to load each protocol. The sequencing keys setup dialog is in the Configure menu.

For more detailed information, consult the Clampex online Help.

Axopatch Protocol 15

When you use Gap-free mode in the Real Time Controls panel, open the pre-programming dialog by clicking the **<** button. You can pre-program voltage level and holding duration values for each channel, as well as turning the digital bit on or off. You can pre-program up to 50 epochs. You can also manually change values during a recording.



Epoch Desc	1	2	3	4	5	6	7	8	9	10
Type	Step	Step	Step	Step	Step	Off	Off	Off	Off	Off
Level (mV)	45	-50	100	-100	0	0	0	0	0	0
Duration (seconds)	12	10	5	15	1	0	0	0	0	0
Digital bit (0/1)	1	0	1	0	0	0	0	0	0	0

Finish

This completes the Axopatch section of the guide.

MultiClamp Sequence

This sequence describes how to set up two distinct data-acquisition “protocols” for use in whole-cell recording with a MultiClamp 700B.

Once we have created the protocols, as an optional final step, we will integrate these with the MultiClamp’s mode telegraph so that Clampex automatically loads the appropriate protocol when you shift between current and voltage clamp in MultiClamp Commander.

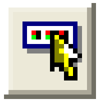
Move through the sequence page by page, or skip sections with the links below—but note that the discussion assumes the setup from earlier sections:

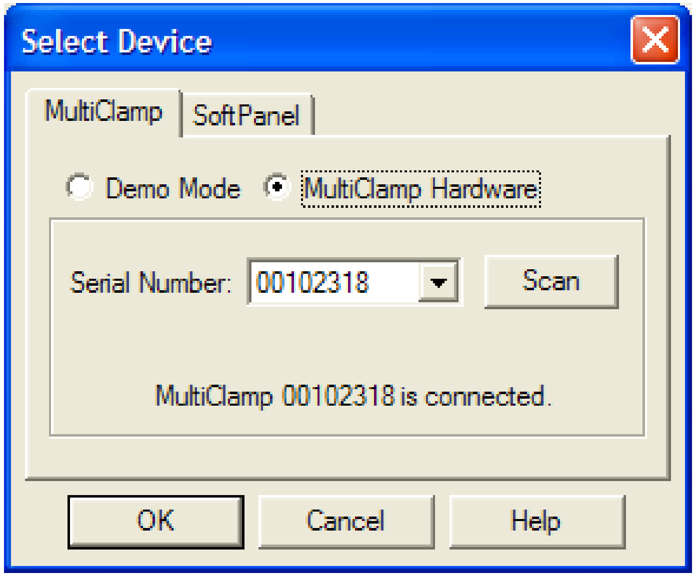
- Digitizer–Amplifier Connections
- Create Signals
- Configure Sequencing Keys
- Configure Telegraphs
- Configure Protocols

Connect MultiClamp

If you have not already done so, switch on your MultiClamp 700B and open MultiClamp Commander. If Commander opens in demo mode (reported in the title bar), you will need to connect the amplifier to the software.

In Commander, press the Select Device toolbutton:





Select “MultiClamp Hardware”.

700B: Press the Scan button—Commander displays the amplifier serial number when the amplifier is found.

Click OK to exit.

MultiClamp

Digitizer–Amplifier Connections

In this section we put in the cabling between the digitizer and MultiClamp. We will use just one MultiClamp channel (i.e. headstage) in this configuration.

MultiClamp Connections 1

We want the following signals:

Voltage Clamp

Digitizer Inputs

- Membrane current—primary output
- Membrane potential—secondary output

Digitizer Output

- Command potential

Current Clamp

Digitizer Inputs

- Membrane potential—primary output
- Membrane current—secondary output

Digitizer Output

- Command current

MultiClamp Connections 2

Clampex allows for more than one signal to be sent, at different times, on each channel (the relationship between signals and channels is more fully explained in the Create Signals section).

Because we are never in current clamp and voltage clamp at the same time, signals associated with these modes can share channels.

Specifically, the following signals can share channels:

- the primary input signals for current and voltage clamp
- the secondary input signals for current and voltage clamp
- the command signals for current and voltage clamp

The six signals from the previous slide, then, require only three digitizer-to-amplifier connections, as shown on the next slide.

MultiClamp Connections 3

700B (Channel 1)	COMMAND	PRIMARY OUTPUT	SECONDARY OUTPUT
Digitizer	ANALOG OUT 0	ANALOG IN 0	ANALOG IN 1

Finish

With the three cables connected, we are ready to configure Clampex, starting with telegraphs.

MultiClamp

Configure Telegraphs

MultiClamp 700B telegraphs are software messages sent from MultiClamp Commander to Clampex, registering key amplifier settings.

As well as simply reporting the settings in Clampex, the telegraphs are integrated into Clampex so that the greater proportion of signal setup is done automatically (as we will soon see).

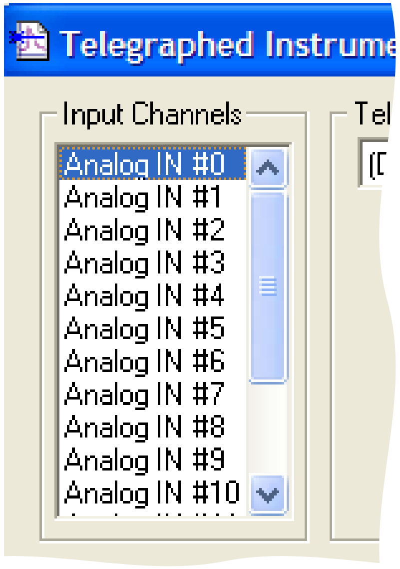
The most important telegraph remains the gain telegraph, used to automatically rescale the Clampex Scope window as gains settings are changed, and to ensure recorded data files are correctly scaled. Lowpass filter and whole-cell capacitance compensation settings are reported in the Real Time Controls and written into recorded file headers—as is the output gain.

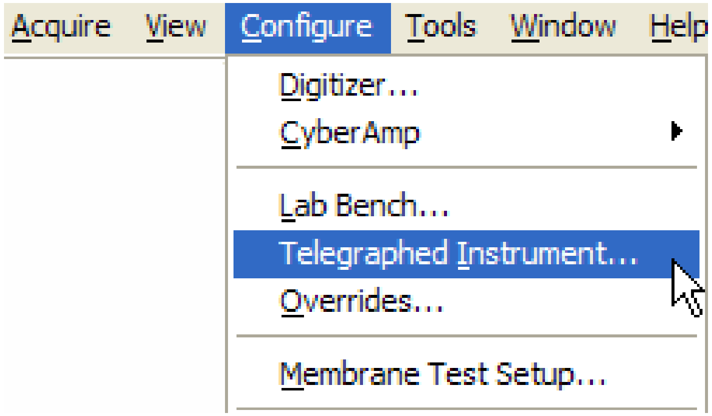
In addition, the MultiClamp has telegraphs for amplifier mode, and for the units and scale factors for command and acquisition signals. We will use these telegraphs in our setup in the following slides.

MOLECULAR DEVICES

MultiClamp Telegraphs 1

Open Telegraphed Instrument from the Clampex Configure menu.

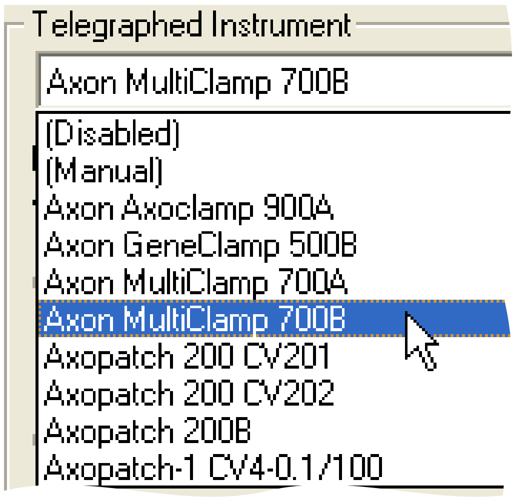




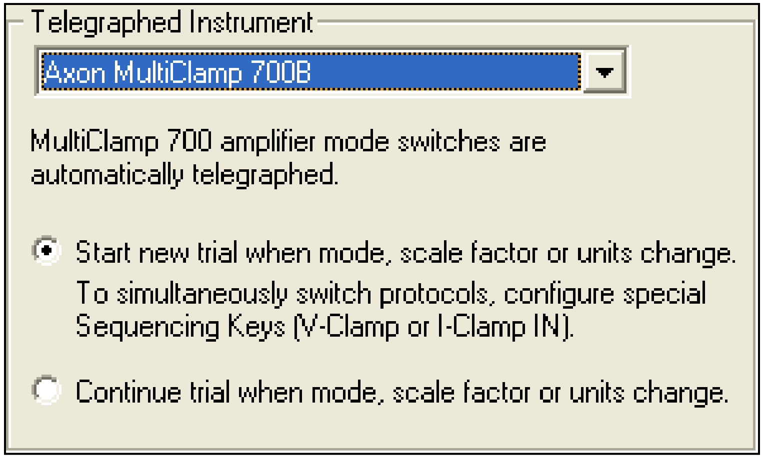
All telegraphs must be configured for a specific digitizer input channel. We will first enable telegraphs for the channel receiving the amplifier primary output. We have connected the MultiClamp Primary Output to Analog IN #0 on the Digidata (Connections), so select this from the Input Channels list.

MultiClamp Telegraphs 2

Select Axon MultiClamp 700B from the Telegraphed Instrument list.



When you have made this selection note the options with respect to linking protocols to amplifier modes—we will use this functionality later in the guide ([Configure Sequencing Keys]).

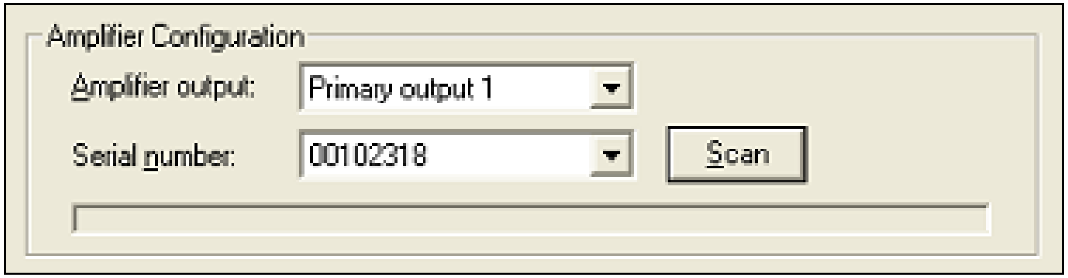


MultiClamp Telegraphs 3

The first enabled section on the dialog is Amplifier Configuration.

Identify the amplifier channel (i.e. headstage) and signal type for the selected digitizer channel:

"Primary output 1"

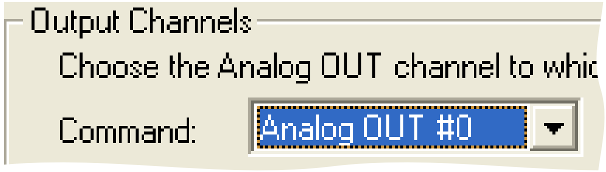


Next click Scan—the MultiClamp serial number is shown when the amplifier is found.

MultiClamp Telegraphs 4

MultiClamp telegraphs scale factors for command signals as well as for its output signals. Enable Clampex to receive these telegraphs in the bottom Output Channels section.

The digitizer input we are configuring receives output from headstage 1 (i.e. amplifier channel 1). The command signal for this headstage is fed from digitizer output Analog OUT #0 (in fact, the only command signal we have connected: Connections). Select Analog OUT #0 in the Command field.

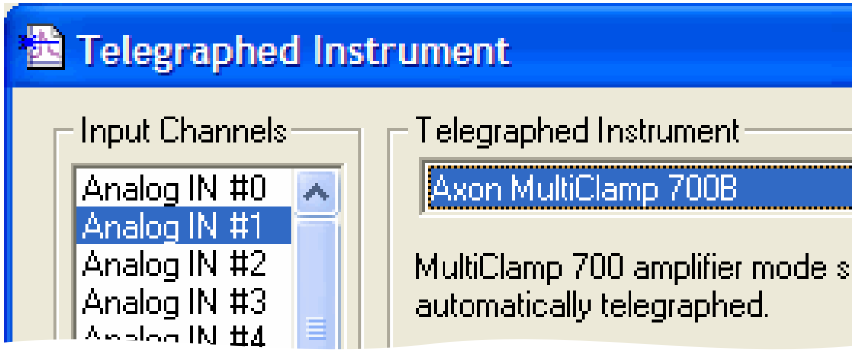


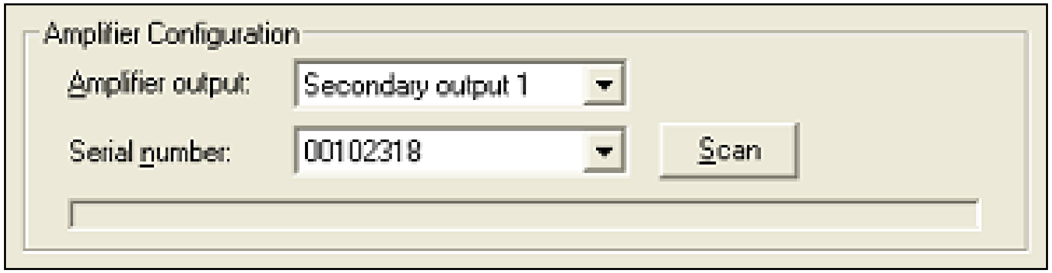
This completes telegraph setup for the primary signal. Now for the secondary signal.

MultiClamp Telegraphs 5

The MultiClamp Secondary Output BNC is connected to digitizer channel Analog IN #1 (Connections).

Select this channel, and MultiClamp 700B.



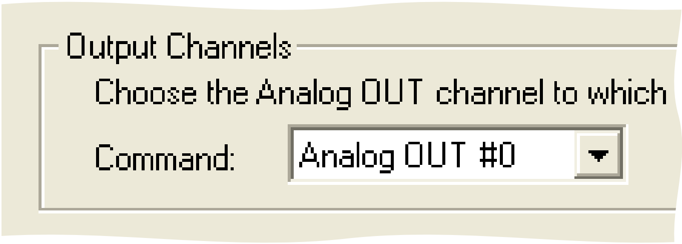


In Amplifier Configuration select “Secondary output 1”.

Enter amplifier identification details as before.

MultiClamp Telegraphs 6

The digitizer command has not changed for this MultiClamp channel, so again select
Analog OUT #0
as the command source.



Finish

This completes telegraph setup. We have configured Clampex to receive telegraphs for both the primary and secondary amplifier output signals, and for the command signal as well. Click OK to close the Telegraphed Instrument dialog.

We now go to the Lab Bench for signal configuration.

MultiClamp

Create Signals

In this section we name the signals we require, assigning these to input and output channels.

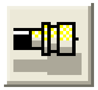
Before starting it is important to be clear on what signals and channels are:

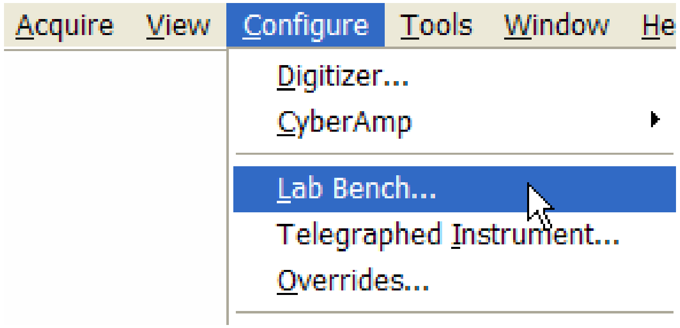
- **Signal:** a set of name, unit, scale factor and offset, by means of which the voltage inputs and outputs at the digitizer are represented in Clampex as the parameter being read at, or delivered to, the preparation.
- **Channel:** a cable connection to the digitizer, identified by the name of the BNC port where connection is made, e.g. Analog IN #0, Digital OUT #2.

As already noted, analog channels can be configured for different signals at different times, which is what we do in this section.

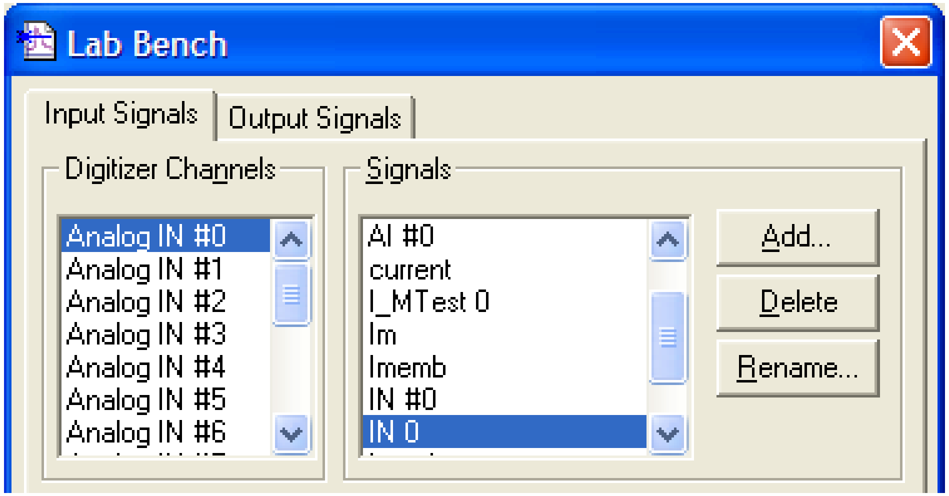
MultiClamp Signals 1

Open the Lab Bench from the Configure menu or use the toolbutton:

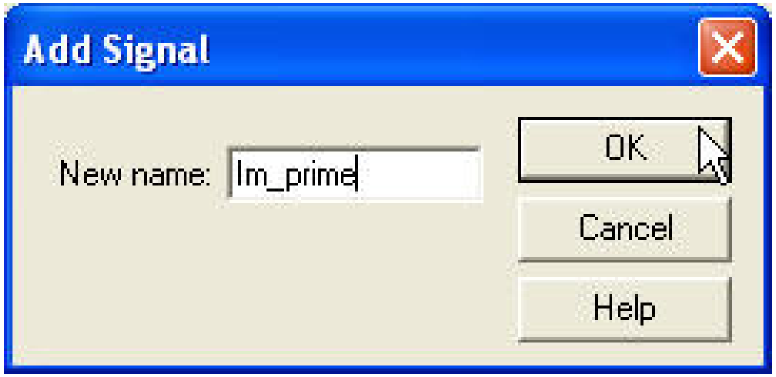




The Lab Bench opens with the Input Signals tab on top, and digitizer channel Analog IN #0 selected. We have the amplifier's primary output connected to this channel, so we need to create two signals—one each for voltage and current clamp—for this channel.



MultiClamp Signals 2

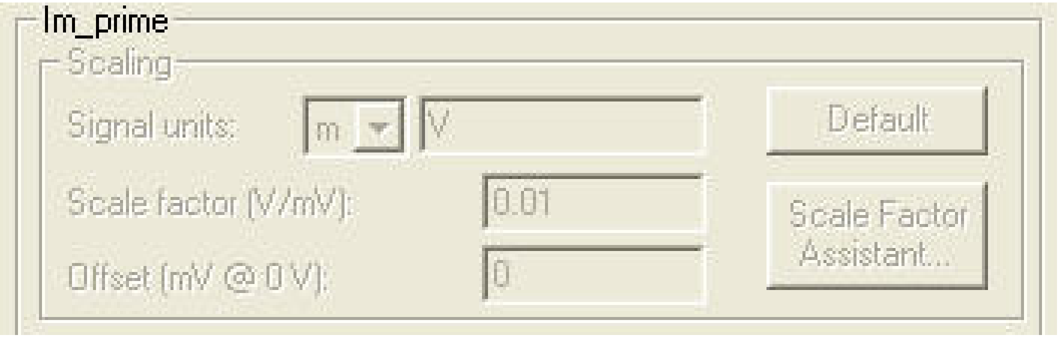


Click the Add button in the Signals section, opening the Add Signal dialog.

Type in “Im_prime”—the name we will give the scaled membrane current signal for voltage clamp, on headstage 1, which will on the primary output.

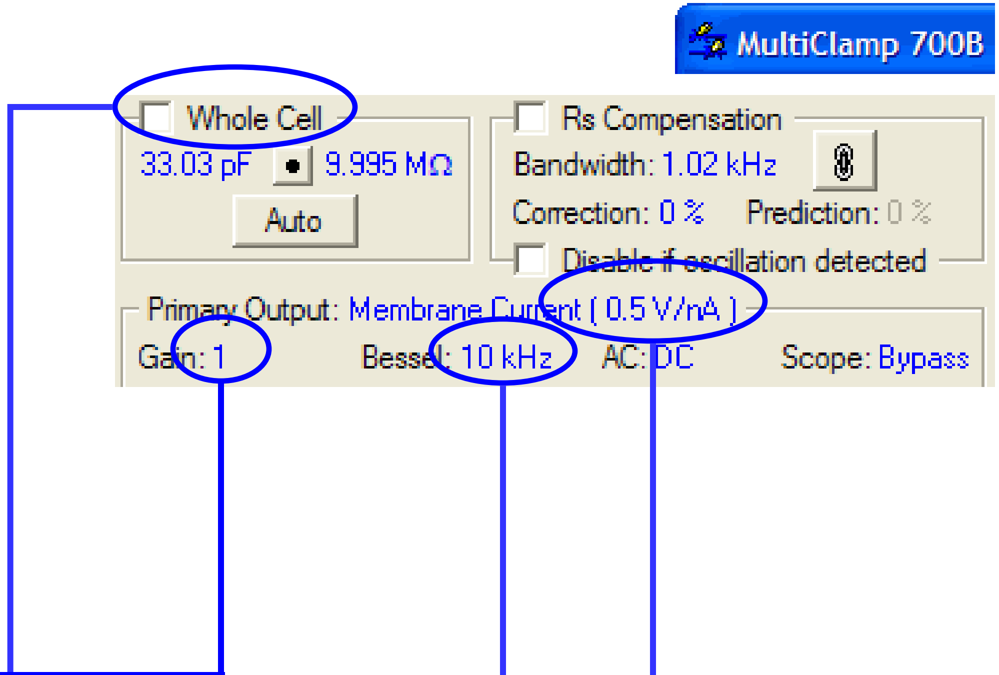
Press OK. With the new signal selected in the Signals list, the rest of the tab shows options and settings for that signal.

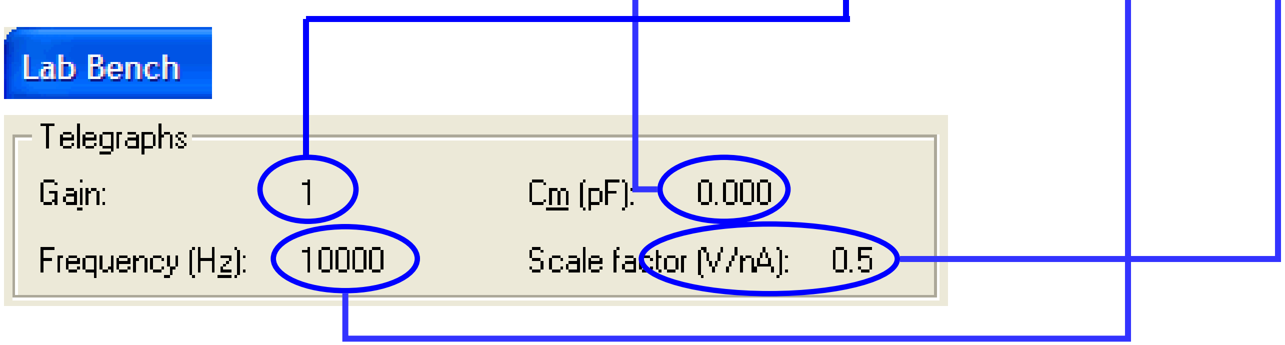
Note that the entire Scaling section is grayed, as it is not used. This is because signal scaling is now under the control of the telegraphs we set up in the last section. Do not worry if the units and scale here are incorrect–they are overridden.



MultiClamp Signals 3

The telegraphs are reported at the bottom of the Lab Bench. The screenshots show the telegraphs in the Lab Bench with Commander at default settings.



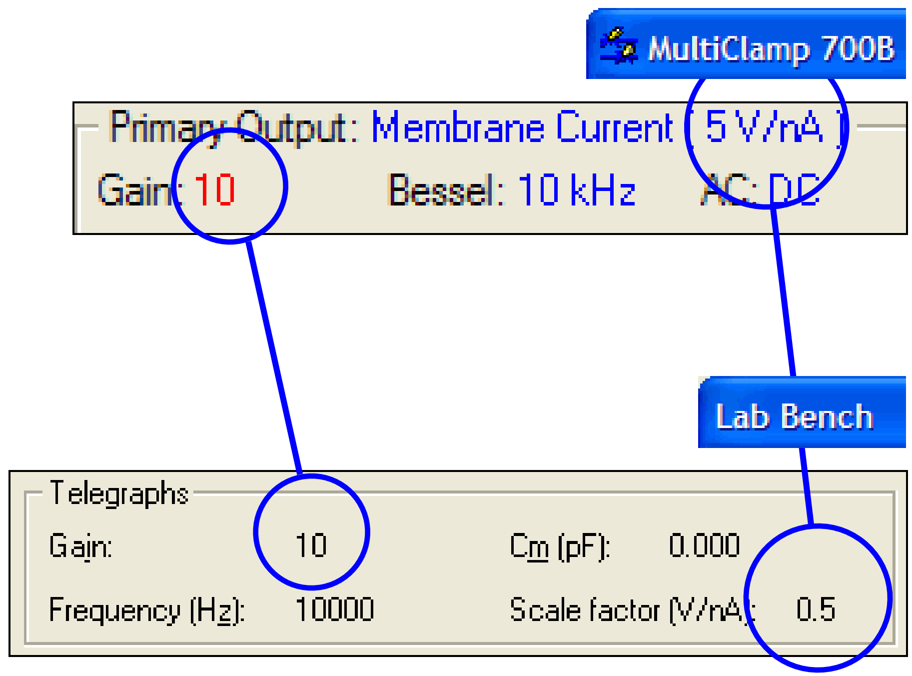


MultiClamp Signals 4

Change settings in Commander and see the telegraphs update in the Lab Bench. The filter, gain, and capacitance compensation telegraphs are also reported in the Real Time Controls.

Note that the scale factor reported in the Lab Bench does not change as you alter the output gain. Clampex reports the unity gain scale factor, i.e. the scale factor for an output gain of one.

Of course, the scale factor applied to the signal takes the gain into account—e.g. in these screenshots, Clampex will apply a scale factor of 10 x 0.5 V/nA = 5 V/nA, as reported in Commander.

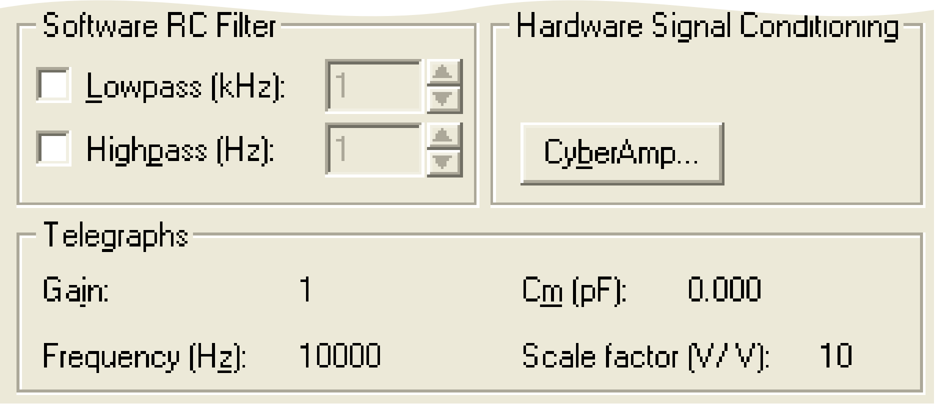


MultiClamp Signals 5

This completes the creation of our first signal. All we actually did was to create a signal name. Following that, with MultiClamp telegraphing enabled, the remainder of the signal configuration was handled automatically.

Before we proceed to the next signal, note the possibility of additional signal filtering in Clampex.

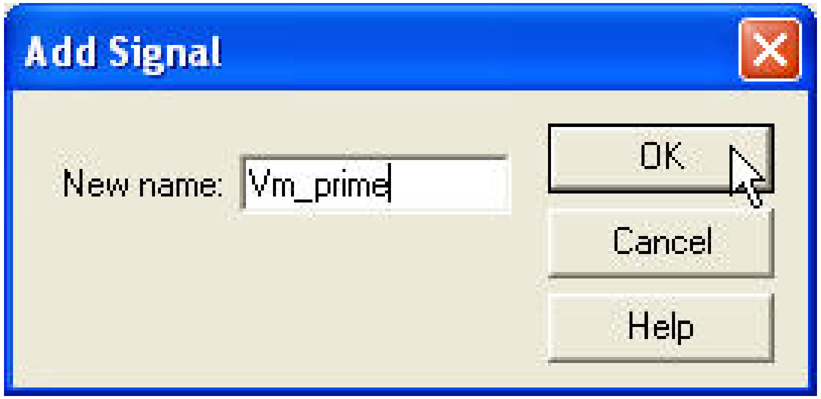
The Hardware Signal Conditioning section has configuration options for Axon Instruments' CyberAmp signal conditioner.



MultiClamp Signals 6

Now to the next signal— the amplifier primary output signal for reading membrane potential in current clamp.

We are using the same digitizer channel for both the current and voltage clamp primary output signals, so still with digitizer channel Analog IN #0 selected, press the Add button. This time type “Vm_prime” for the name of the new signal.

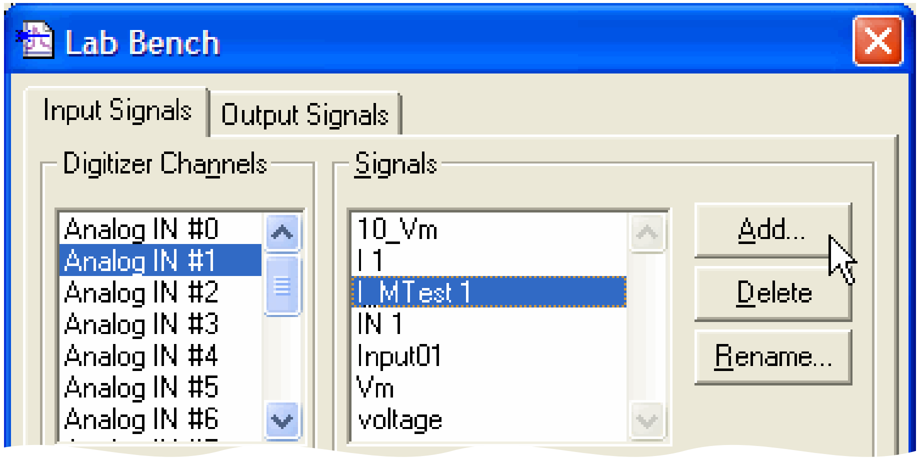


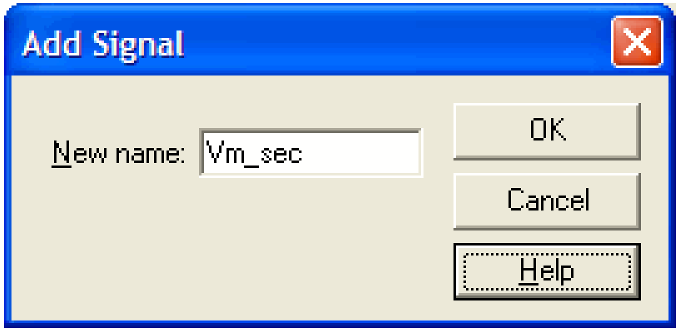
Again, because we have enabled telegraphs for the channel that this signal is associated with, signal units and scale factor are set automatically from MultiClamp Commander. When you change Commander to current clamp mode and membrane potential is measured, Clampex will update appropriately, if the telegraphs were configured to start a new trial.

MultiClamp Signals 7

Now we create current and voltage clamp signals for the amplifier secondary output.

We have the secondary output connected to Analog IN #1 (Connections). Select this as the digitizer channel, and then press the Add button.





Type in "Vm_sec", for the signal we will use to monitor membrane potential in voltage clamp.

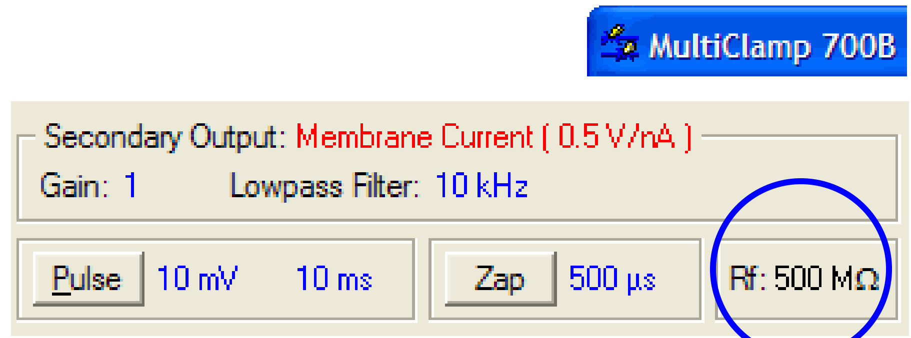
MultiClamp Signals 8

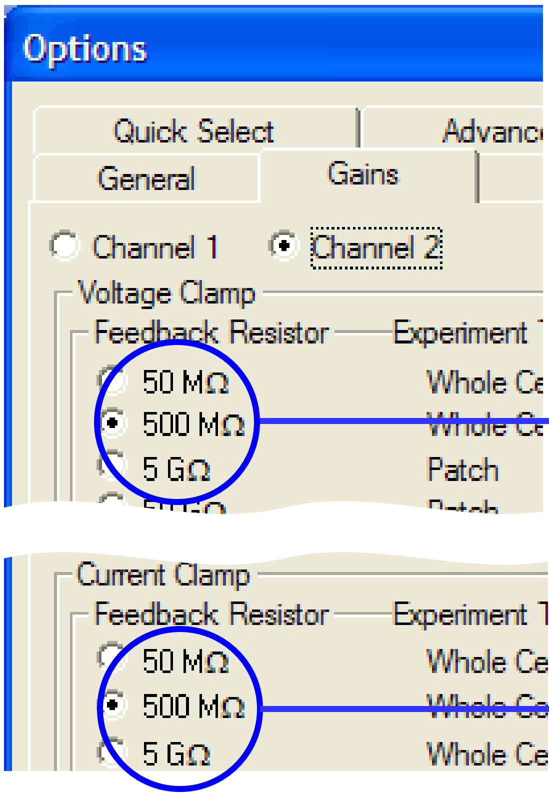
Now for the other secondary output signal from the MultiClamp, on digitizer channel Analog IN #1. This will monitor membrane current in current clamp.

Add “Im_sec” in the Add Signal dialog, as for previous signals.

MultiClamp Signals 9

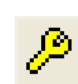
Signal Im_sec will be used when Commander is in current clamp with “Membrane Current” as the secondary output signal.





Note that the scale factor for current-reading output signals is affected by the choice of headstage resistor.

This can be adjusted in Commander’s Options dialog:



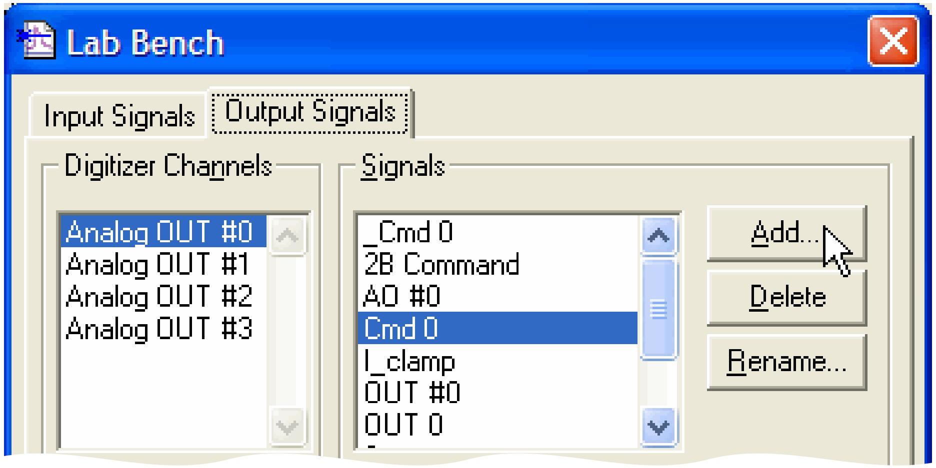
in the Gains tab, Feedback Resistor sections.

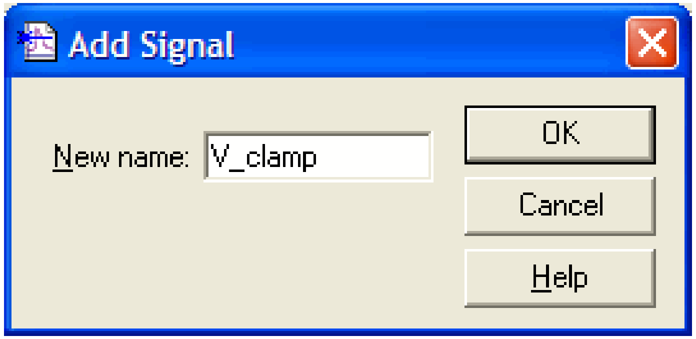
We will use the default 500 MΩ setting.

MultiClamp Signals 10

Now we create signals for the command waveforms.

Go to the Output Signals tab in the Lab Bench. Analog OUT #0 is selected. This is the channel we have connected for both voltage and current clamp commands (Connections).

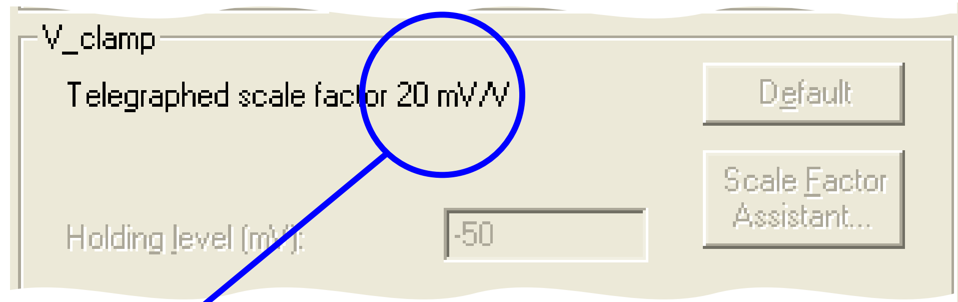


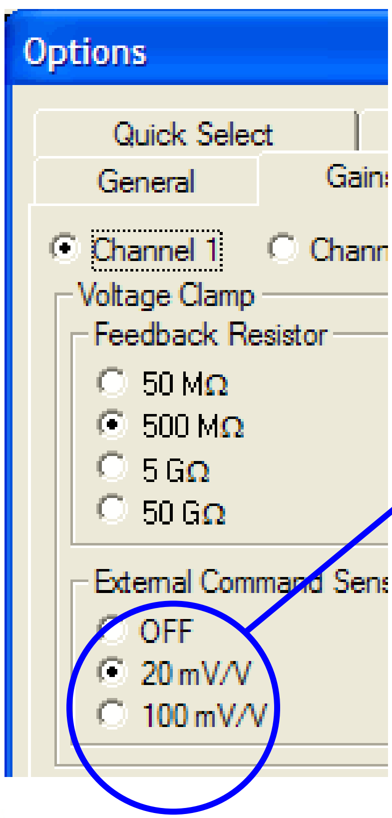


Press the Add button in the Signals section, and type “V_clamp” into the Add Signal dialog—for the command signal for voltage clamp.

MultiClamp Signals 11

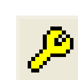
We enabled telegraphs for Analog OUT #0 in association with both Analog IN #0 and Analog IN #1, so the signal units and scale factor are simply reported from Commander.





MultiClamp can output command signals in voltage clamp at two scale factors—20 mV/V and 100 mV/V.

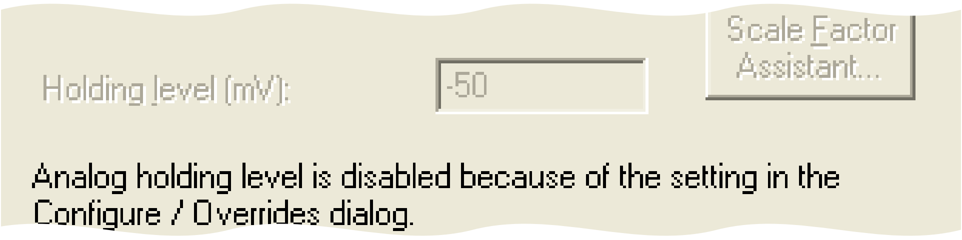
Select the command scale factor from the Commander Options dialog:

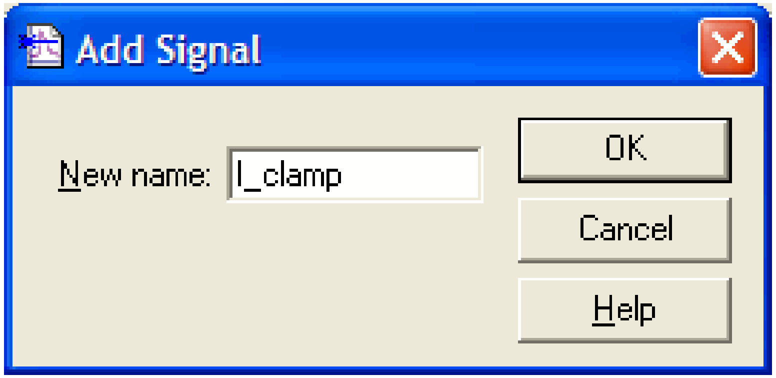


We will use the 20 mV/V setting.

MultiClamp Signals 12

Under default settings the command signal holding level cannot be set in the Lab Bench. We do not need to worry about the holding level reported in the field because we will set this when we incorporate the signal into a protocol.



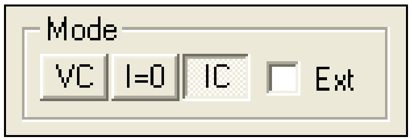


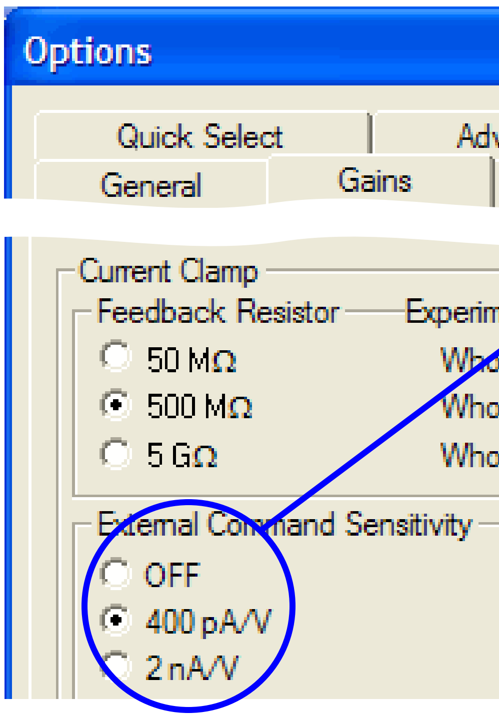
It only remains to create the current clamp command signal.

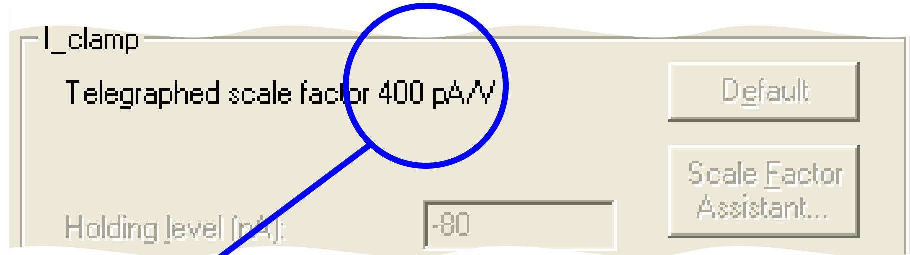
Click Add again, calling the new signal “I_clamp” .

MultiClamp Signals 13

Set the amplifier mode in Commander to current clamp:







- In the Lab Bench, the scale factor units change to express current.

  Again, Commander has two scaling settings for the command signal, 400 pA/V and 2 nA/V, selected in the Options dialog Gains tab.

  Check that you have 400 pA/V selected.

MultiClamp Signals 14

Finish

In this section we played with settings in Commander in order to see how the telegraphs work, but in the normal course of events you only need to add appropriately named signals in the Lab Bench, ensuring these are associated with the correct digitizer channels. Then, each time you run a protocol with one of the signals, Clampex uses the units and scale factors telegraphed from Commander at that time.

We created six signals:

Voltage clamp

- Im_prime
- Vm_sec
- V_clamp

Current clamp

- Vm_prime
- Im_sec
- I_clamp

This completes the creation and configuration of all our signals. We now proceed to the creation of protocols, where these signals are built into a broader set of acquisition parameters.

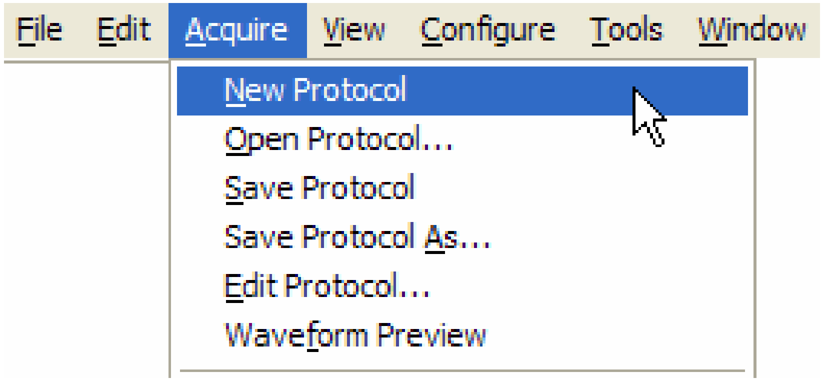
Configure Protocols

Protocols in Clampex are complete sets of acquisition parameters, including options for command waveforms and preliminary data analysis. Particular signals, defined in the Lab Bench, are specified for each protocol.

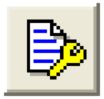
In this section we create two simple protocols, one each for current and voltage clamp, incorporating the signals we have just defined.

MultiClamp Protocols 1

Open the Protocol Editor by selecting New Protocol in the Acquire menu.



Note: If a previously saved protocol is not loaded in Clampex, it uses a place-holder protocol, labeled "(untitled)". If this is currently loaded you can open the editor to create a new protocol by selecting Edit Protocol, or by clicking the toolbutton:



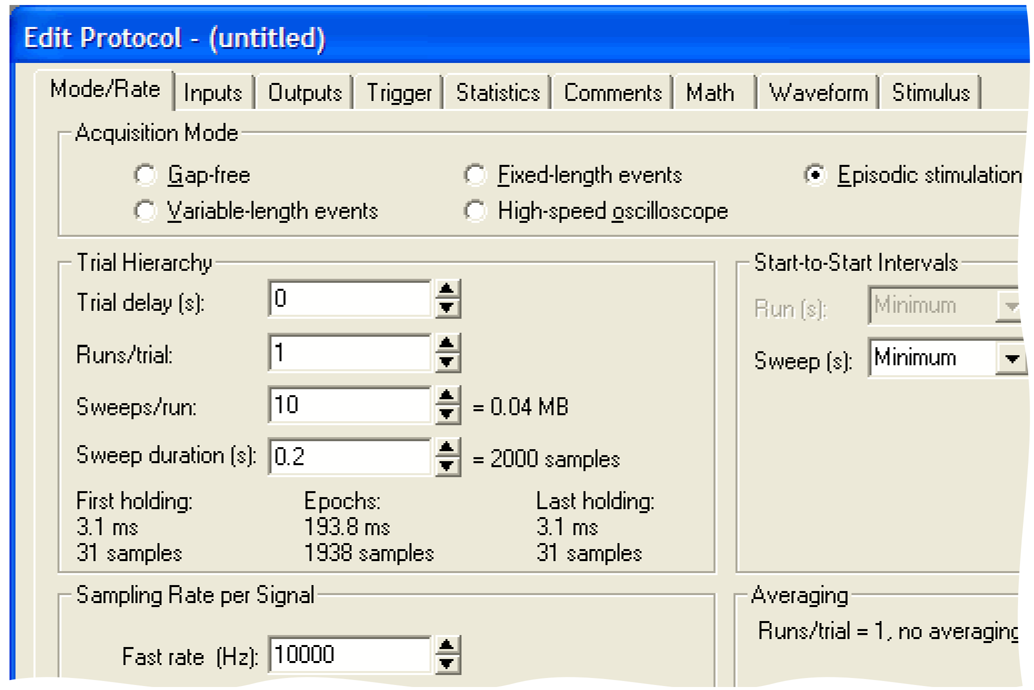
The currently loaded protocol is reported in the status bar at the bottom of the main Clampex window.

We will begin by setting up the protocol for voltage clamp.

MultiClamp Protocols 2

The front tab of the protocol editor has controls for, amongst other things, acquisition mode, sampling rate, and trial hierarchy.

The default acquisition mode is episodic stimulation—the only mode that allows a command waveform to be generated. We want to generate a command, so leave this setting. In fact, all the default settings on this tab can be left as they are, but take time to note key parameters such as the sampling rate (10 kHz), the number of samples per sweep, and the number of sweeps per run.



The sweep start-to-start interval is set at Minimum, so each new sweep starts as soon as the previous one is finished.

MOLECULAR DEVICES

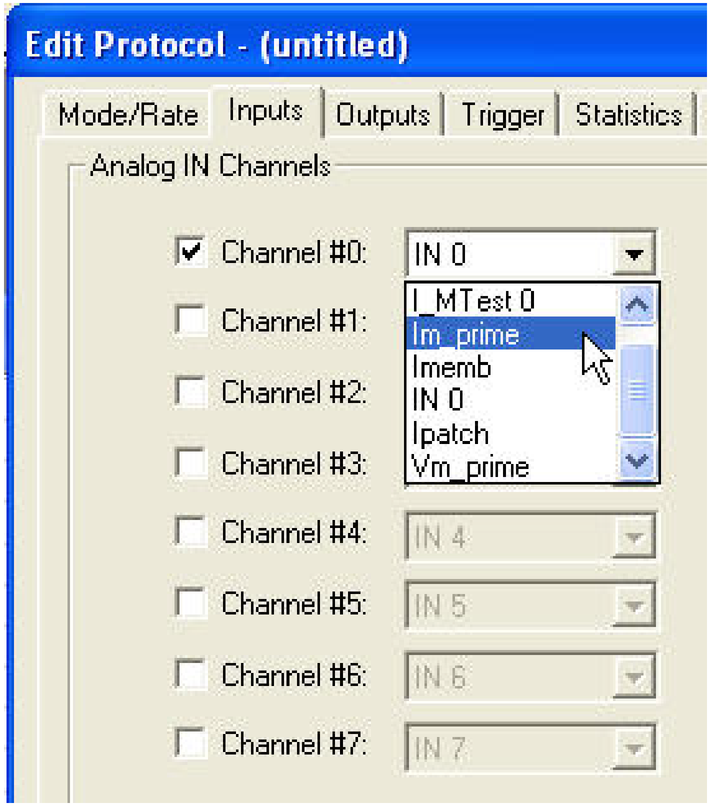
MultiClamp Protocols 3

Next go to the Inputs tab.

Here you select digitizer input channels for the protocol, as well as the signals that you want to be conveyed on these.

For voltage clamp, we want two input signals—one scaled signal for membrane current, and a second signal to monitor membrane voltage. We created these in the Lab Bench—“Im_prime” and “Vm_sec”—associating them with digitizer IN channels 0 and 1. Now we incorporate them into the voltage protocol.

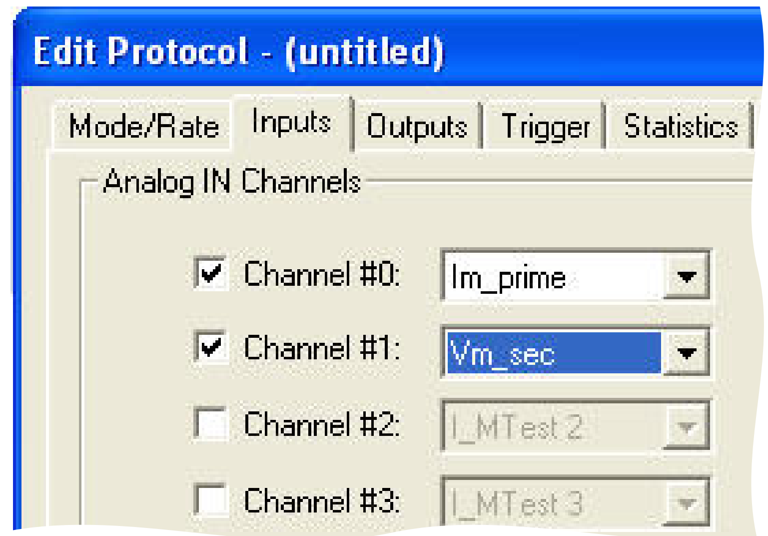
Channel #0 should be already checked. Open the list box beside it and select “Im_prime”.

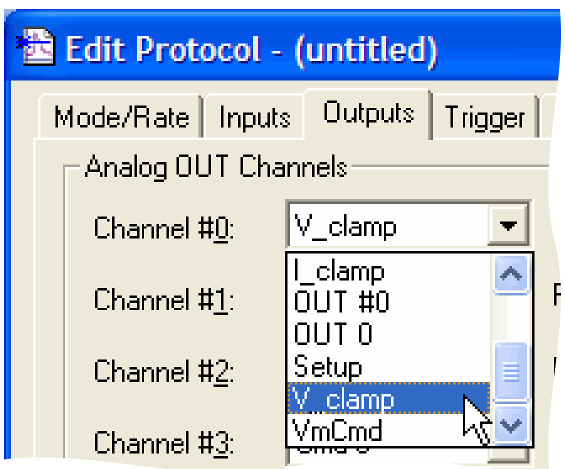


MultiClamp Protocols 4

Then check Channel #1 and select "Vm_sec".

This completes the Inputs tab.





Next, go to the Outputs tab.

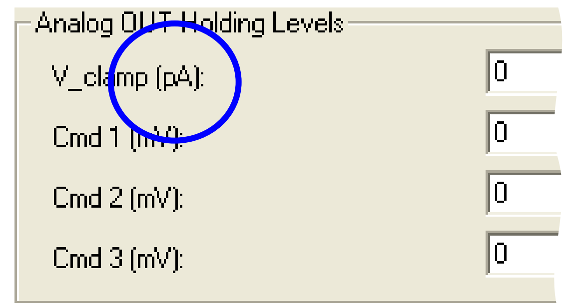
We created the signal "V_clamp" to deliver the voltage clamp command waveform, on digitizer output channel #0.

Select "V_clamp" from the Channel #0 list box.

MultiClamp Protocols 5

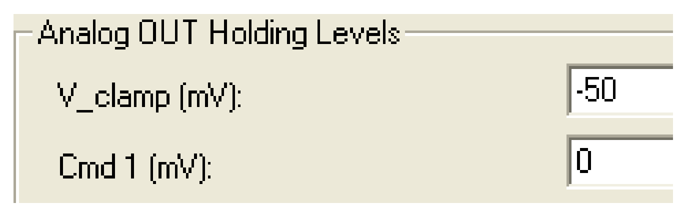
This tab is also used to set the holding level for the signal. There may be some confusion over the signal units for this, due to telegraphs.

With telegraphs enabled, Clampex shows command signal units appropriate for the amplifier mode, so if the MultiClamp was in Current Clamp mode (IC) when you opened the protocol editor, "V_clamp" will be shown with units for current.



This is no cause for alarm, since "V_clamp" will only be used in voltage clamp, and Clampex will be telegraphed the correct units at that time. It does mean that in order to set a holding level we need to recall the units used for the command signal in voltage clamp. These were millivolts. Alternatively, close the protocol editor with the OK button, switch to Voltage Clamp mode (VC) in Commander, and reopen the protocol editor. "V_ clamp" now shows the units that will be used when "V_clamp" is output—i.e. millivolts.

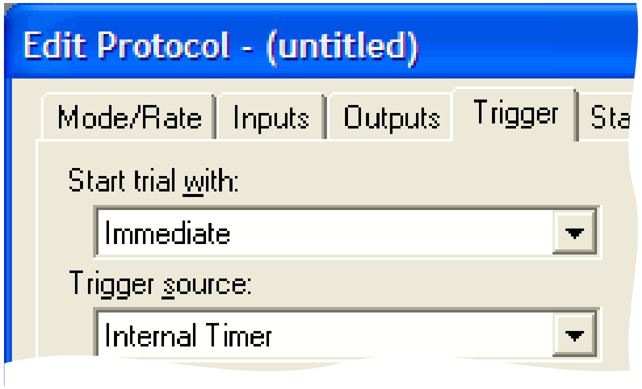
We want to set a holding level of –50 mV, so enter –50 in the holding level field.



MultiClamp Protocols 6

Although we will not make any changes for the purposes of our protocol, it is worth taking a quick look at the trigger settings.

Go to the Trigger tab.



Default settings give "Immediate" trial starts. This means Clampex is armed for data acquisition as soon as you select Record, or View Only, from the Acquire menu—or click the toolbuttons:

Record View Only

The default trigger source is "Internal Timer". This triggers the command waveform and data acquisition immediately after the trial is started, continuing through to the end of the trial automatically.

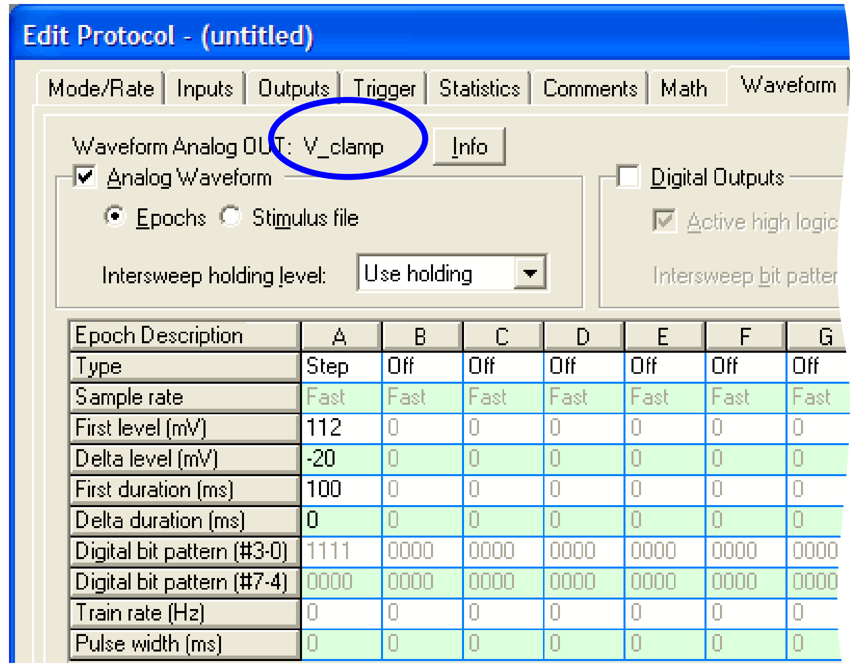
MultiClamp Protocols 7

Now go to the Waveform Channel #0 tab, where outputs are defined for digitizer output channel Analog OUT #0.

A default waveform is already defined—we will delete this and create our own simple stimulus, but first familiarize yourself with some key settings on this tab.

The Analog Waveform checkbox enables analog command definition. Selecting Epochs means we define the waveform using the table in the middle of the tab. In this, the sweep can be divided into up to 50 sections (epochs) A–AX, and a waveform defined for each of these.

The Epoch Description table in the Waveform tab includes cut and paste functionality.



Note the confirmation that “V_clamp” is the signal carrying the output waveform. The units shown for it—in the “Info” message box and in the epoch description table—are again derived from the amplifier mode telegraph from MultiClamp Commander.

MultiClamp Protocols 8

Now to the definition of our waveform.

We will configure an output with a simple step, increasing the amplitude of the step with each sweep.

In column A of the epoch description table, keep “Step” in the Type row, but click in the “First level” row and type in –50. This sets the output level for epoch A in the first sweep of the run. Our entry of –50 mV maintains the holding level.

Click in the next row (Delta level) and type in zero. This keeps the first level setting for subsequent sweeps—i.e. epoch A is maintained at –50 mV for each of the 10 sweeps in the trial.

Epoch Description	A	B
Type	Step	Off
Sample rate	Fast	Fast
First level (mV)	-50	0
Delta level (mV)	0	0
First duration (ms)	50	0
Delta duration (ms)	0	0
Digital bit pattern (#3-0)	1111	0000
Digital bit pattern (#7-4)	0000	0000
Train rate (Hz)	0	0
Pulse width (ms)	0	0

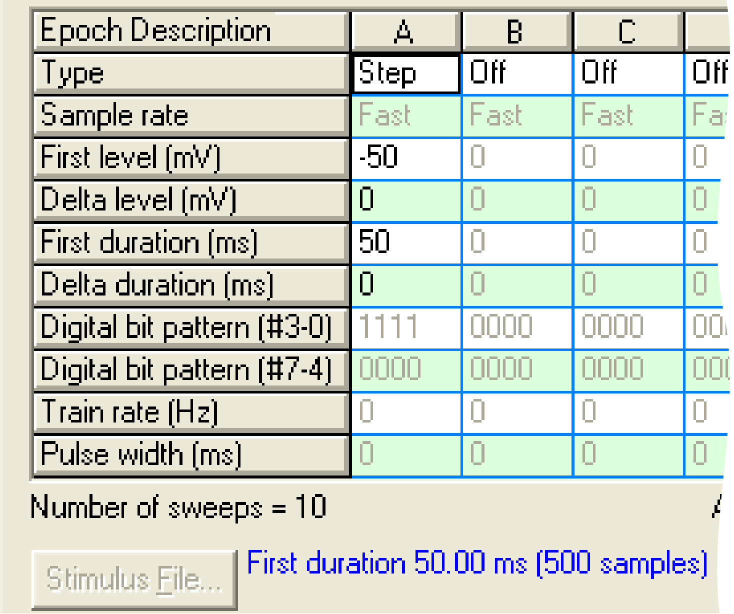
MultiClamp Protocols 9

Now to set the period for epoch A.

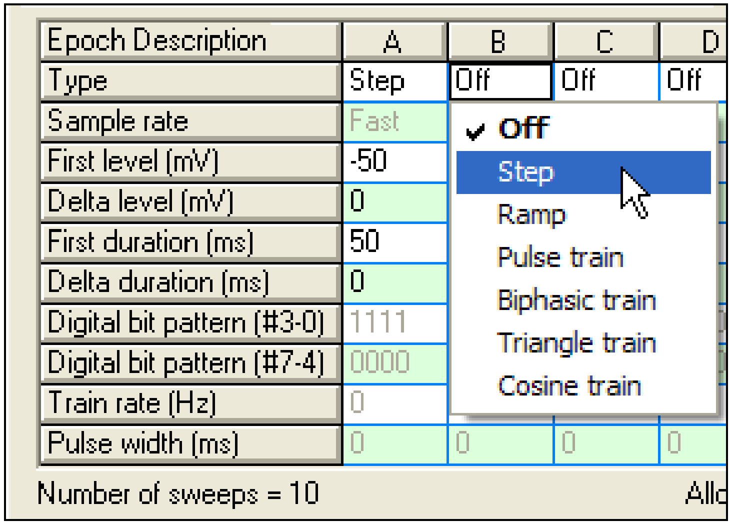
Click in the First duration row, and type in 50.

Our sampling interval is 10 kHz, so a 50 ms sample duration equates to 500 samples. Shift focus to a different cell in the table to see this reported below.

This completes epoch A. Now we configure the step, in epoch B.



Epoch Description	A	B	C	
Type	Step	Off	Off	Off
Sample rate	Fast	Fast	Fast	Fa
First level (mV)	-50	0	0	0
Delta level (mV)	0	0	0	0
First duration (ms)	50	0	0	0
Delta duration (ms)	0	0	0	0
Digital bit pattern (#3-0)	1111	0000	0000	00
Digital bit pattern (#7-4)	0000	0000	0000	00
Train rate (Hz)	0	0	0	0
Pulse width (ms)	0	0	0	0



Epoch Description	A	B	C	D
Type	Step	Off	Off	Off
Sample rate	Fast			
First level (mV)	-50			
Delta level (mV)	0			
First duration (ms)	50			
Delta duration (ms)	0			
Digital bit pattern (#3-0)	1111			
Digital bit pattern (#7-4)	0000			
Train rate (Hz)	0			
Pulse width (ms)	0	0	0	0

Click in the Type row in column B (currently set to “Off”).

Select “Step” from the popup menu.

MultiClamp Protocols 10

Set the level for the first sweep at –100 mV.

For this epoch, because we want an incrementing step level from sweep to sweep, we enter a delta level. Click on the Delta level cell and type in 20. This forces the step level up 20 mV with each successive sweep.

We have 10 sweeps starting at –100 mV, so the final sweep will have a step level of 80 mV, reported below the table.

Now set the duration, at 100 ms. Again, this is reported below the table, in milliseconds as well as in samples.

We will not set a delta duration, which would alter the length of the epoch from sweep to sweep, so this completes our waveform definition.

Epoch Description	A	B	C	D
Type	Step	Step	Off	Off
Sample rate	Fast	Fast	Fast	Fast
First level (mV)	-50	-100	0	0
Delta level (mV)	0	20	0	0
First duration (ms)	50	100	0	0
Delta duration (ms)	0	0	0	0
Digital bit pattern (#3-0)	1111	0000	0000	0000
Digital bit pattern (#7-4)	0000	0000	0000	0000
Train rate (Hz)	0	0	0	0
Pulse width (ms)	0	0	0	0

Number of sweeps = 10 Alloca

Stimulus File...

Final level 80.00 mV
First duration 100.00 ms (1000 samples)

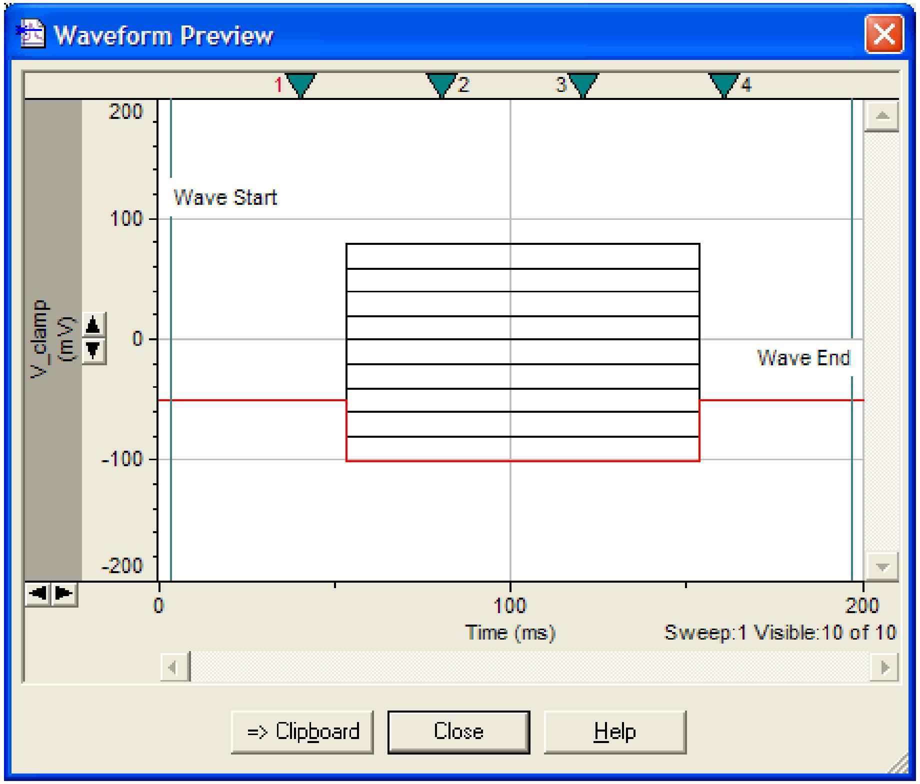
MOLECULAR DEVICES

MultiClamp Protocols 11

Press the Update Preview button in the bottom right corner of the protocol editor.

This opens the Waveform Preview window shown at right, where you can see a graphical representation of the waveform we have defined.

This window can be kept open while you experiment with different epoch settings—press the Update button whenever you want to update the display.



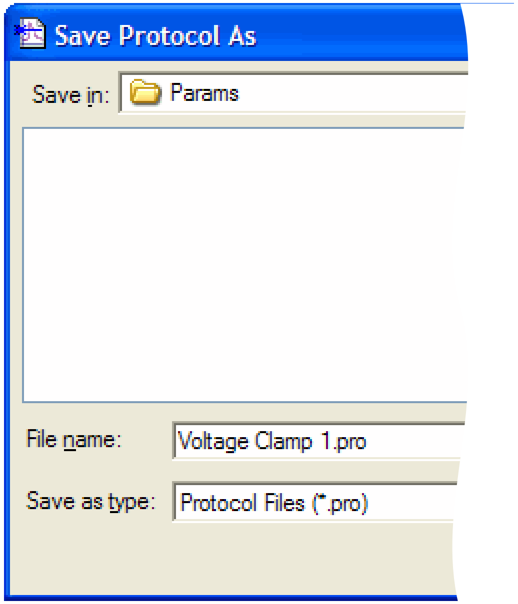
Note: The Waveform Preview opens with panes for all analog output channels—right-click in the upper pane and select Maximize Signal from the popup menu to get the display shown above.

MultiClamp Protocols 12

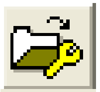
We have completed the setup of the voltage clamp protocol—close the protocol editor with the OK button.

The new protocol is loaded, still labeled "(untitled)", and we could acquire data under it if we wanted, but it is not saved for future use.

Go to Save Protocol As in the Acquire menu. This opens a standard file-saving dialog. Name the protocol "Voltage Clamp 1", and press the Save button.



The protocol is now saved and can be loaded whenever we want, with the Open Protocol command in the Acquire menu, or toolbutton:



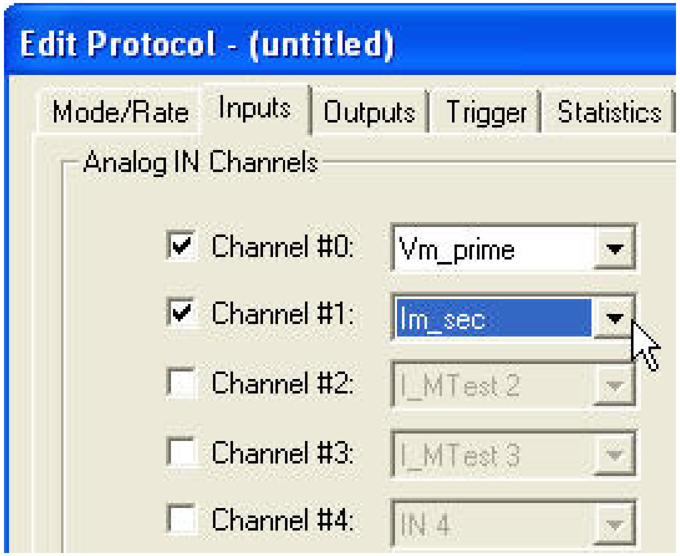
MultiClamp Protocols 13

Setup of the current clamp protocol follows similar lines to the protocol for voltage clamp.

Open the protocol editor again with New Protocol, in the Acquire menu.

We will again accept the default settings in the Mode/Rate tab, so go straight to the Inputs tab.

This time select "Vm_prime" for Channel #0, and "Im_sec" for Channel #1.



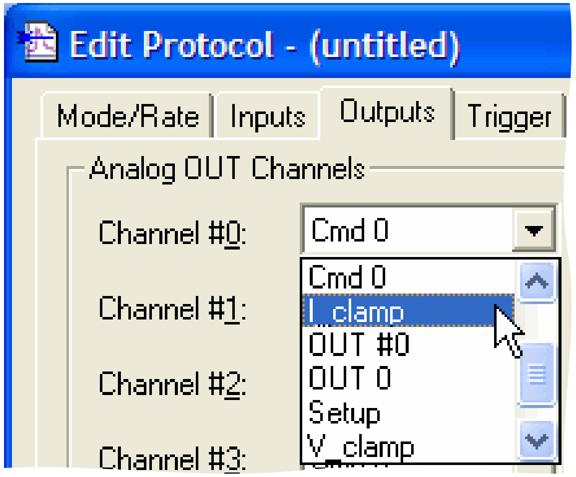
Note: Be sure the MultiClamp is in IC Mode to see the correct units when you come to configure the waveform.

MOLECULAR DEVICES

MultiClamp Protocols 14

On the Outputs tab, select the current clamp command signal we configured for digitizer Analog OUT channel #0: “I_clamp”.

Leave the holding level at the default zero setting for current clamp.



	A	B	C	D	E
Type	Step	Ramp	Step	Trngl	Step
Sample rate	Fast	Fast	Fast	Fast	Fast
First level (V)	-200	100	20	110	0
Delta level (V)	20	0	0	0	0
First duration (ms)	40	40	0.1	40	5
Delta duration (ms)	0	0	0	0	0
Digital bit pattern (#3-0)	1111	0000	0000	0000	0000
Digital bit pattern (#7-4)	0000	0000	0000	0000	0000
Train rate (Hz)	0	0	0	1	0
Pulse width (ms)	0	0	0	20	0

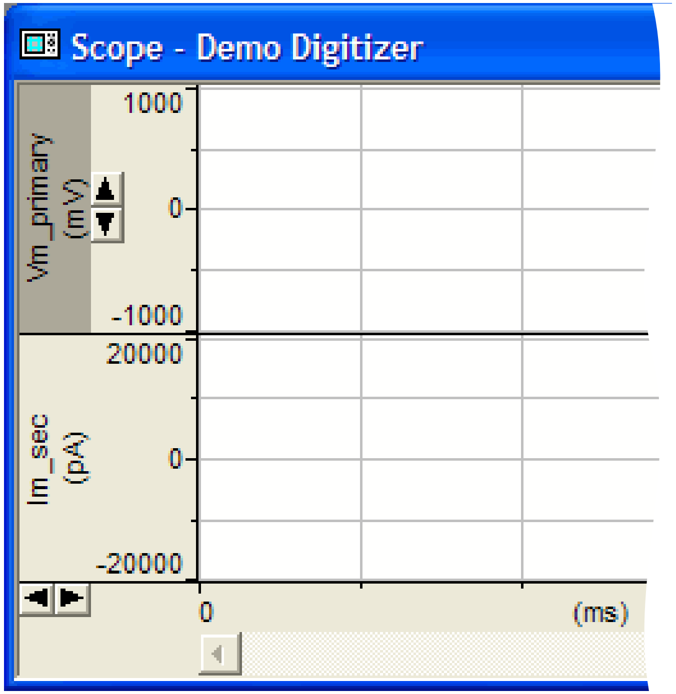
Create your own command waveform on the Channel #0 tab.

Experiment with the different waveform options viewing these in the Waveform Preview window.

MultiClamp Protocols 15

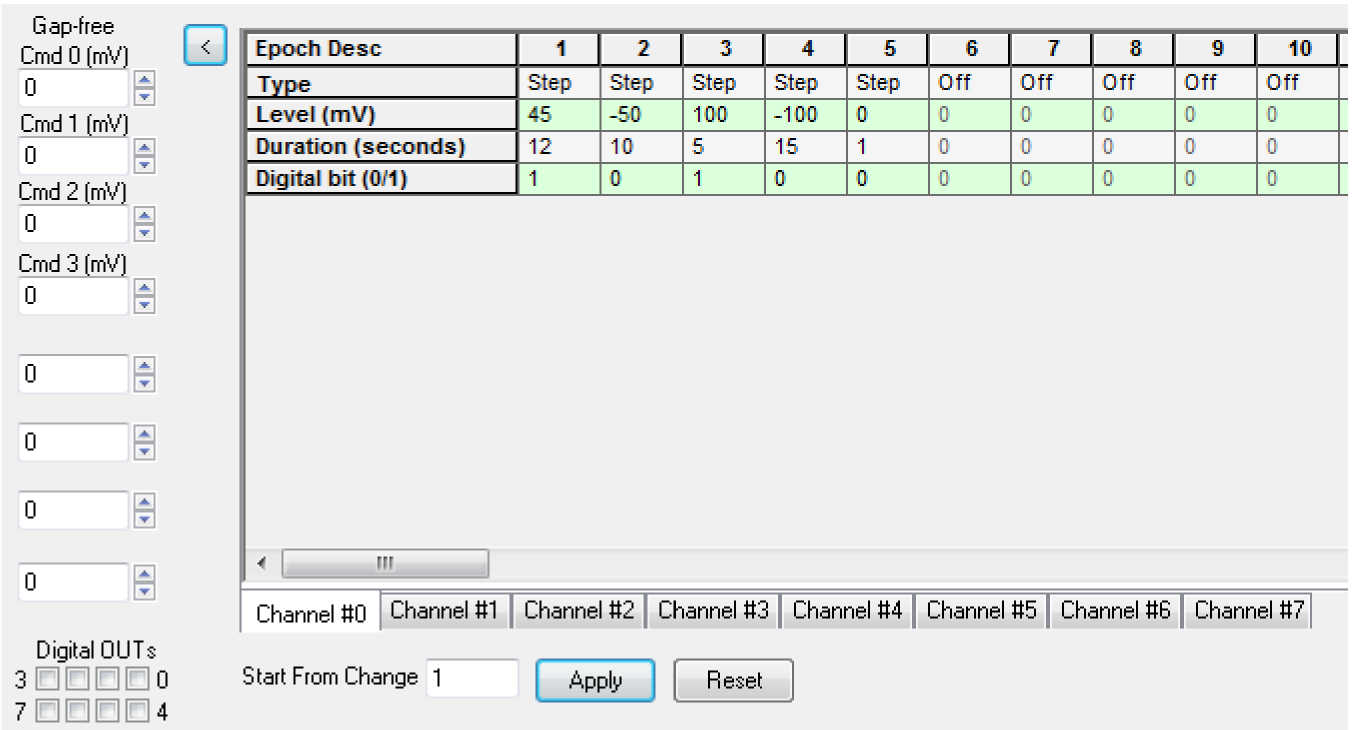
Finally, close the protocol editor by pressing OK, and save the protocol (Save Protocol As in the Acquire menu), calling it "Current Clamp 1".

You will see on exit that the Scope window is set up in preparation for the two input signals configured for this protocol, with units as currently telegraphed from MultiClamp.



MultiClamp Protocols 16

When you use Gap-free mode in the Real Time Controls panel, open the pre-programming dialog by clicking the **<** button. You can pre-program voltage level and holding duration values for each channel, as well as turning the digital bit on or off. You can pre-program up to 50 epochs. You can also manually change values during a recording.



Finish

This completes the protocol tutorial and completes this guide. However, as a final, optional step we link the protocols to MultiClamp Commander amplifier-mode telegraphs in the next section.

MultiClamp

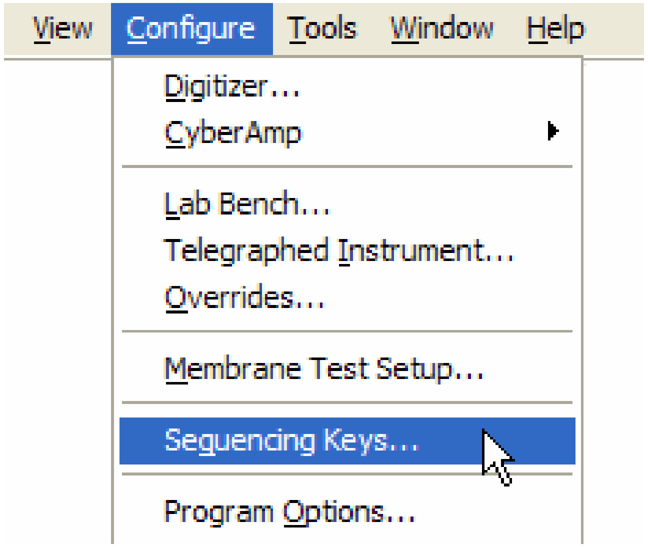
Configure Sequencing Keys

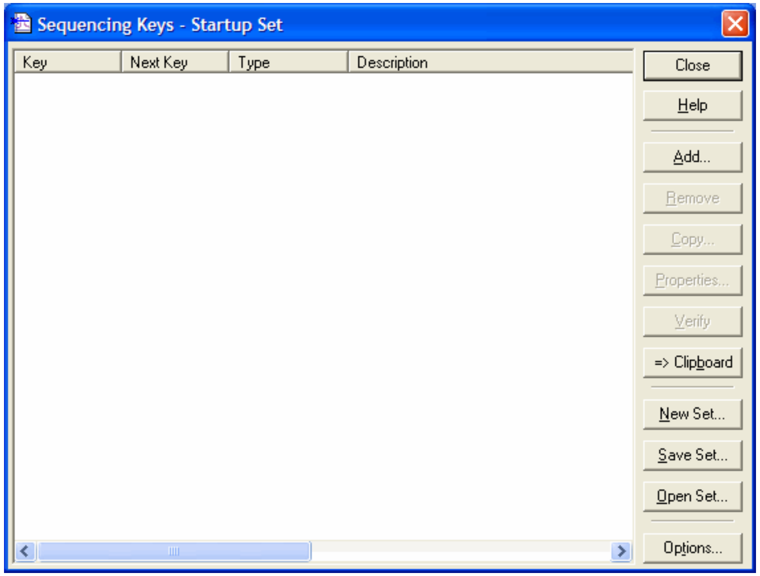
The amplifier-mode telegraphs sent by MultiClamp Commander can be used to automatically load protocols in Clampex. This means that you can have an appropriate protocol load and run, automatically, as soon as you change modes in Commander. This is done using sequencing keys.

In this section, as an optional final stage in the guide, we configure this linkage for the two protocols we have created.

MultiClamp Sequencing Keys 1

Open the Sequencing Keys dialog box from the Configure menu.



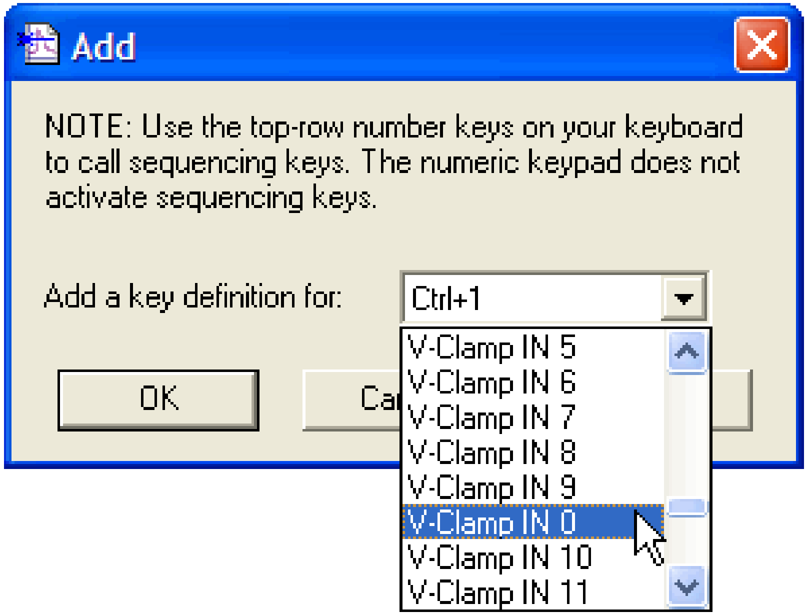


If the sequencing keys are at default settings, the dialog opens with an empty sequencing keys table, under the title "Startup Set".

MultiClamp Sequencing Keys 2

Press the Add button, and in the “Add a key definition for” list box scroll down to “V-Clamp IN 0”.

Select this sequencing key.



“IN 0” refers to Analog IN #0, the digitizer input channel we configured Clampex to receive MultiClamp scaled output telegraphs on.

“V-Clamp” means that the sequencing key we are about to configure will be triggered by a change to voltage clamp in the MultiClamp.

Click OK.

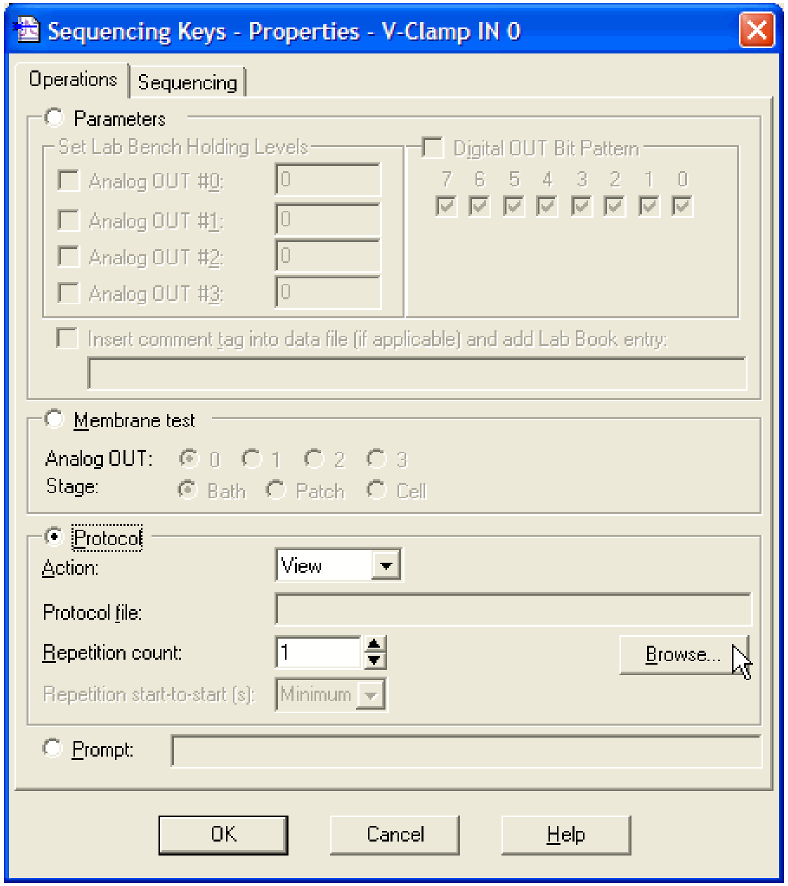
MOLECULAR DEVICES

MultiClamp Sequencing Keys 3

The Sequencing Keys Properties dialog opens with the Operations tab uppermost. Select the Protocol option.

In the Action field, for the purposes of demonstration, keep the “View” selection. This has the protocol run in View Only mode when it is called; in a real experiment you would choose the “Run” option.

Next we have to enter the protocol we want to run when the MultiClamp is in voltage clamp. Click Browse to open a file dialog.

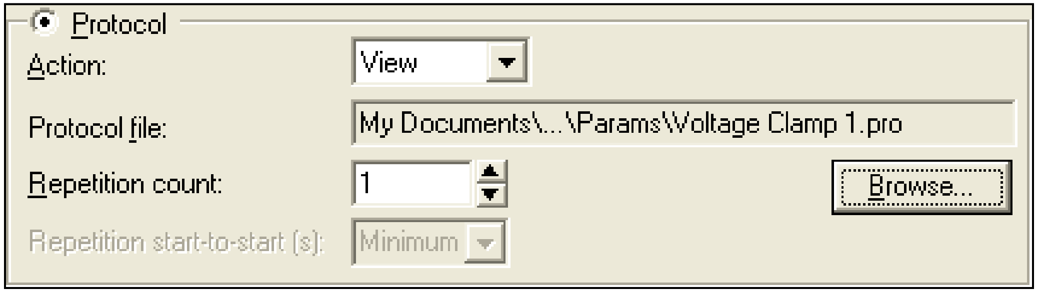


MultiClamp Sequencing Keys 4

The file dialog should open at the “Params” folder where we earlier saved the two protocols. We called the voltage clamp protocol “Voltage Clamp 1”. Select this now and click Open.

The protocol is reported in the protocol file field.

Leave the repetition count at one, to run the protocol just once when it is called.



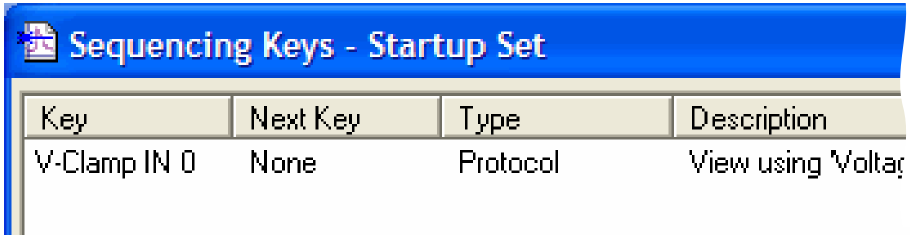
This completes the configuration of this sequencing key, however, before closing the Properties dialog have a look at the Sequencing tab. Use this tab to link the current key to a second one so that it runs after the current one is finished. You can create sequences of any number of operations using this functionality.

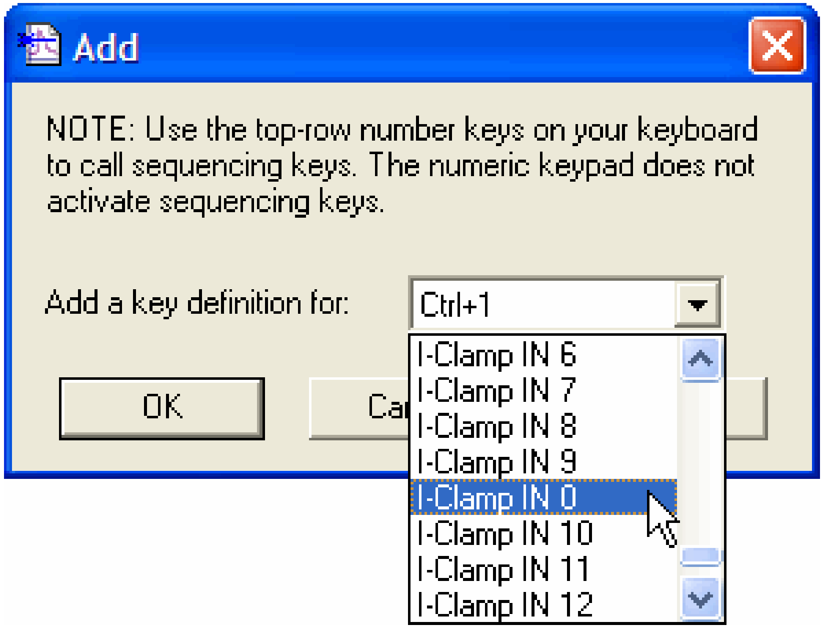
Click OK to return to the main Sequencing Keys dialog.

MultiClamp Sequencing Keys 5

Our first sequencing key appears in the table.

Check its details before pressing the Add button to add a second key, for current clamp.

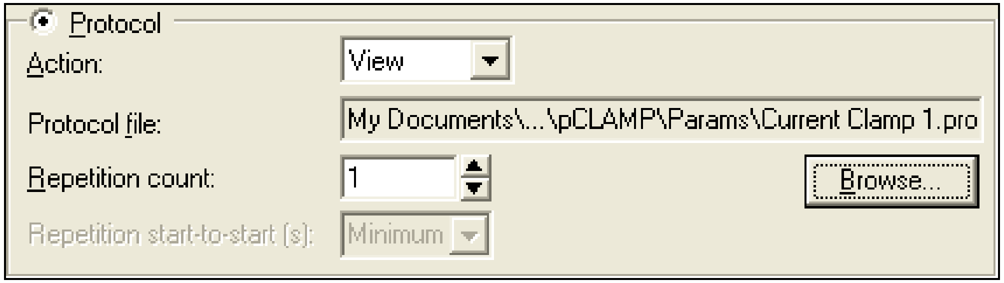




This time select “I-Clamp IN 0”. The input channel is the same, but we want this key to be triggered by a change to current clamp.

MultiClamp Sequencing Keys 6

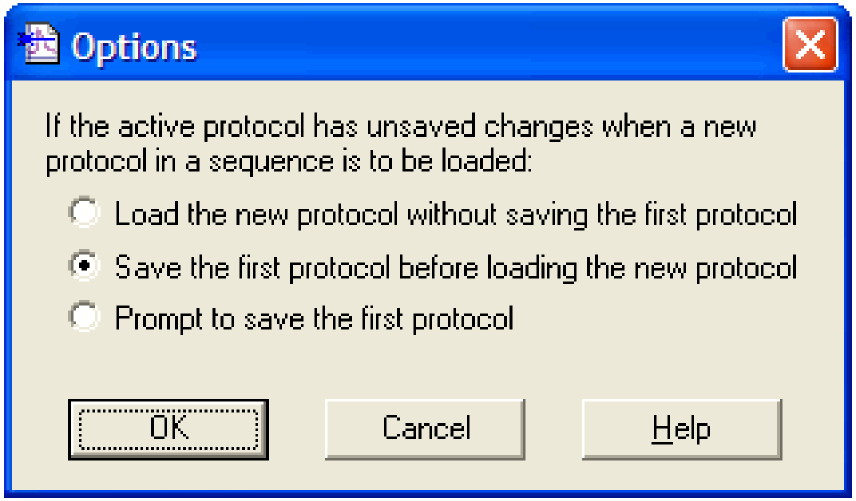
As before, select Protocol and press Browse, this time putting in place the protocol we configured for current clamp, "Current Clamp 1"



Close the Properties dialog, and check the details of the second sequencing key. It has been added at the top of the list, following the key order in the Add dialog list box.

MultiClamp Sequencing Keys 7

Finally, press the Options button, at the bottom of the Sequencing Keys command buttons. You are offered three options for saving protocols in a sequencing keys series.



Select the second option.

When you come to test the setup, this selection means that if you resize or rescale the Scope window while a protocol is running, your new window settings are automatically saved with the protocol when you switch over to the second protocol.

MultiClamp Sequencing Keys 8

Finish

This completes the setup of the sequencing keys—the sequencing key set is automatically saved when you close the dialog.

Test the Configuration

Connect the model cell to the Channel 1 headstage of the MultiClamp.

Make a final check on the I-Clamp and V-Clamp tabs in MultiClamp Commander to ensure you have the correct primary and secondary output signal types selected.

Now switch mode in Commander. The appropriate protocol should load and run in Clampex, displaying resultant data correctly labeled, and with the correct units, in the Scope window. You may need to scale the window to see the signals clearly.

Switch over to the alternate mode. Again, the appropriate protocol should load and run.

This completes the MultiClamp section of the guide. For more detailed information on any of the matters covered, use the online Help and consult the manual.

Axoclamp Sequence

This sequence describes how to set up two distinct data-acquisition “protocols” for use in whole-cell recording with an Axoclamp 900A.

Once we have created the protocols, as an optional final step, we will integrate these with the Axoclamp’s mode telegraph so that Clampex automatically loads the appropriate protocol when you shift between current and voltage clamp in Axoclamp Commander.

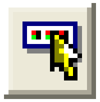
Move through the sequence page by page, or skip sections with the links below—but note that the discussion assumes the setup from earlier sections:

- Digitizer–Amplifier Connections
- Create Signals
- Configure Telegraphs
- Configure Protocols

Connect Axoclamp

If you have not already done so, switch on your Axoclamp 900A and open Axoclamp Commander. If Commander opens in demo mode (reported in the title bar), you will need to connect the amplifier to the software.

In Commander, press the Select Device toolbutton:



Select “Axoclamp Hardware”.

Click Scan—Commander displays the amplifier serial number when the amplifier is found.

Click **OK** to exit.

Digitizer–Amplifier Connections

In this section we put in the cabling between the digitizer and Axoclamp 900A.

Axoclamp Connections 1

The signals we want to record depend on the Axoclamp 900A mode.

Current Clamp modes (IC , I=0, DCC, and HVIC)

Digitizer Inputs

- Membrane potential
- Membrane current*

Digitizer Output

- Command current

Voltage Clamp (TEVC and dSEVC)

Digitizer Inputs

- Membrane current
- Membrane potential

Digitizer Output

- Command potential

**Note:* Current can be monitored on the CURRENT OUTPUT of each channel. This is optional.

Axoclamp Connections 2

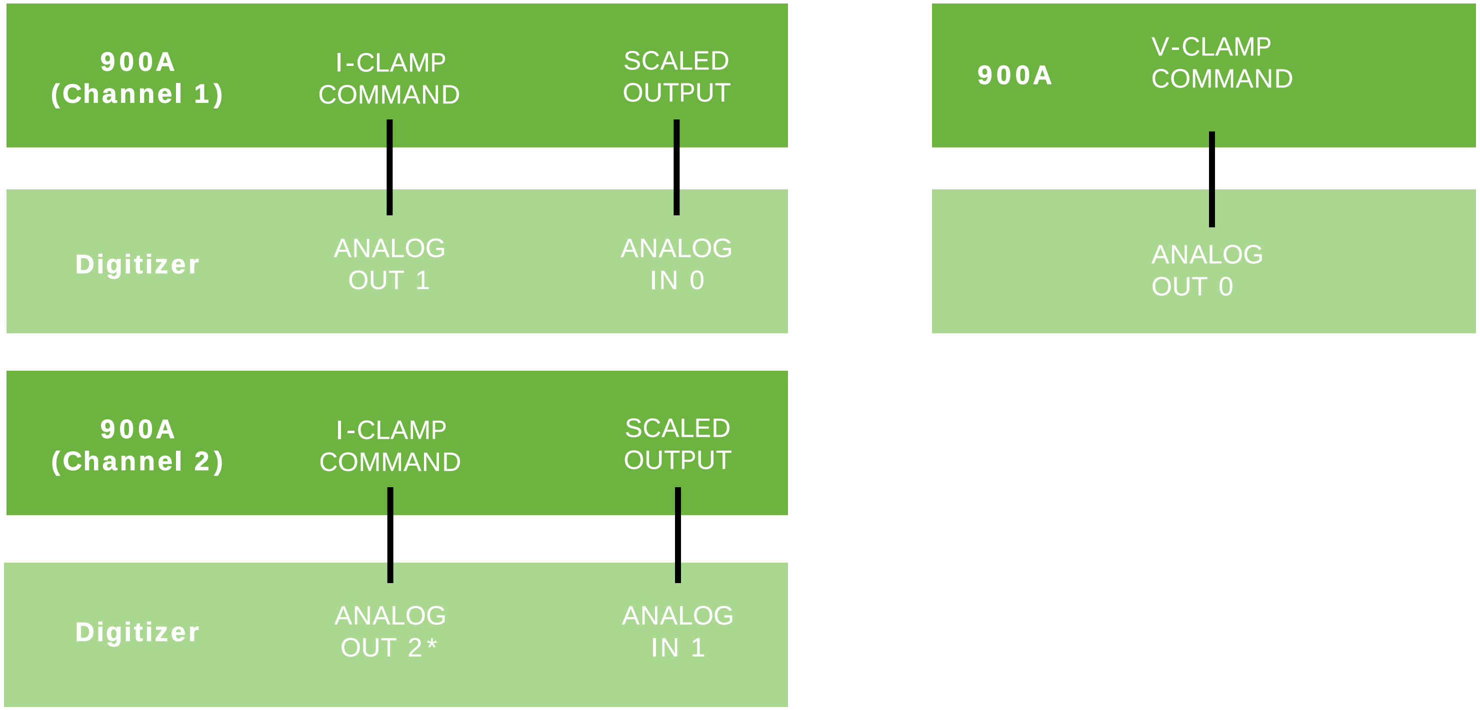
Clampex allows for more than one signal to be sent, at different times, on each channel (the relationship between signals and channels is more fully explained in the Create Signals section).

Because the Axoclamp 900A can only be in one mode at a given time, signals associated with these modes can share digitizer Analog IN and Analog OUT channels.

Which signals can share channels depends on how the Axoclamp is going to be used:

- For current clamp and TEVC, the input signals for current clamp (Vm) and voltage clamp (Im) on Channel 2.
- For current clamp and dSEVC, the input signals for current clamp (Vm) and voltage clamp (Im) on Channel 1.

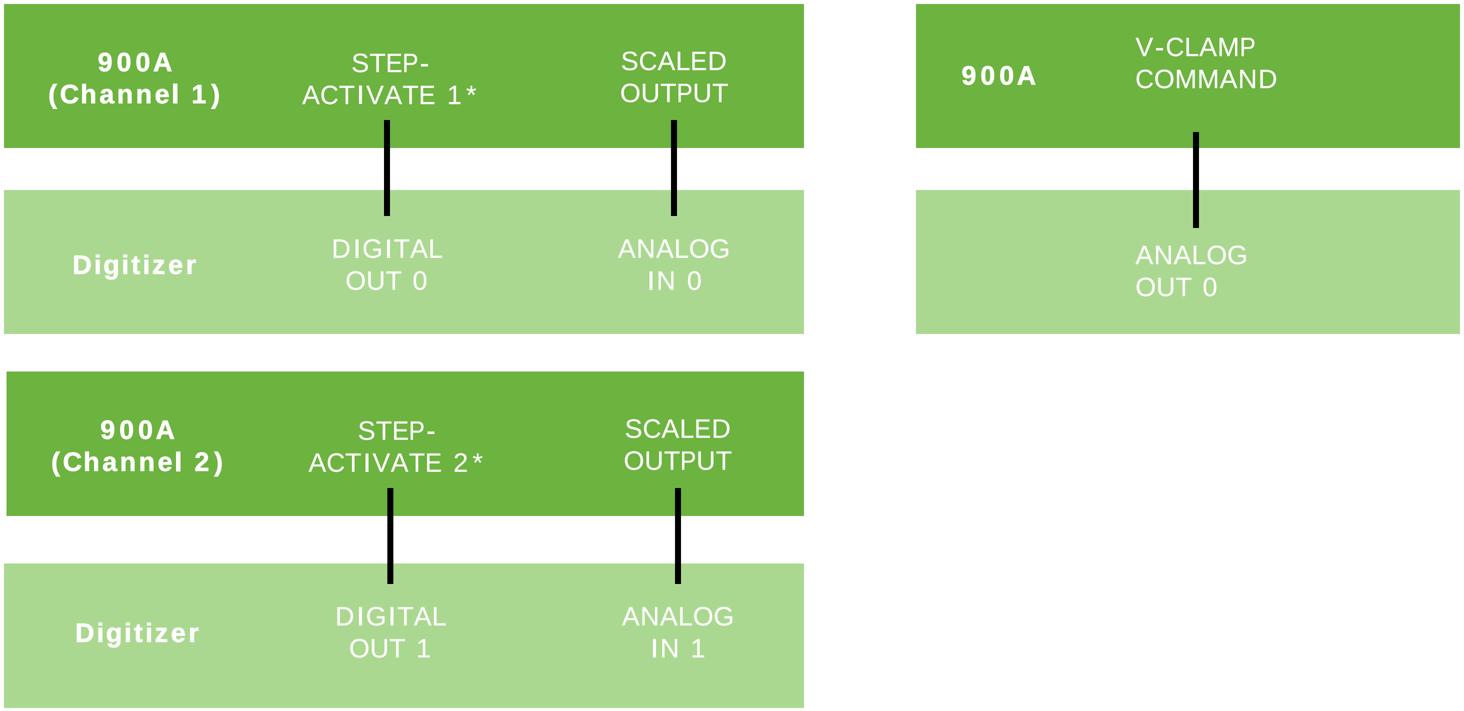
Axoclamp Connections 3



**Note:* To use both I-CLAMP COMMANDS and V-CLAMP COMMAND requires three Analog Outputs, as available on the Digidata 1440A. An alternative method when only two analog outputs are available is described in the next slide.

Axoclamp Connections 4

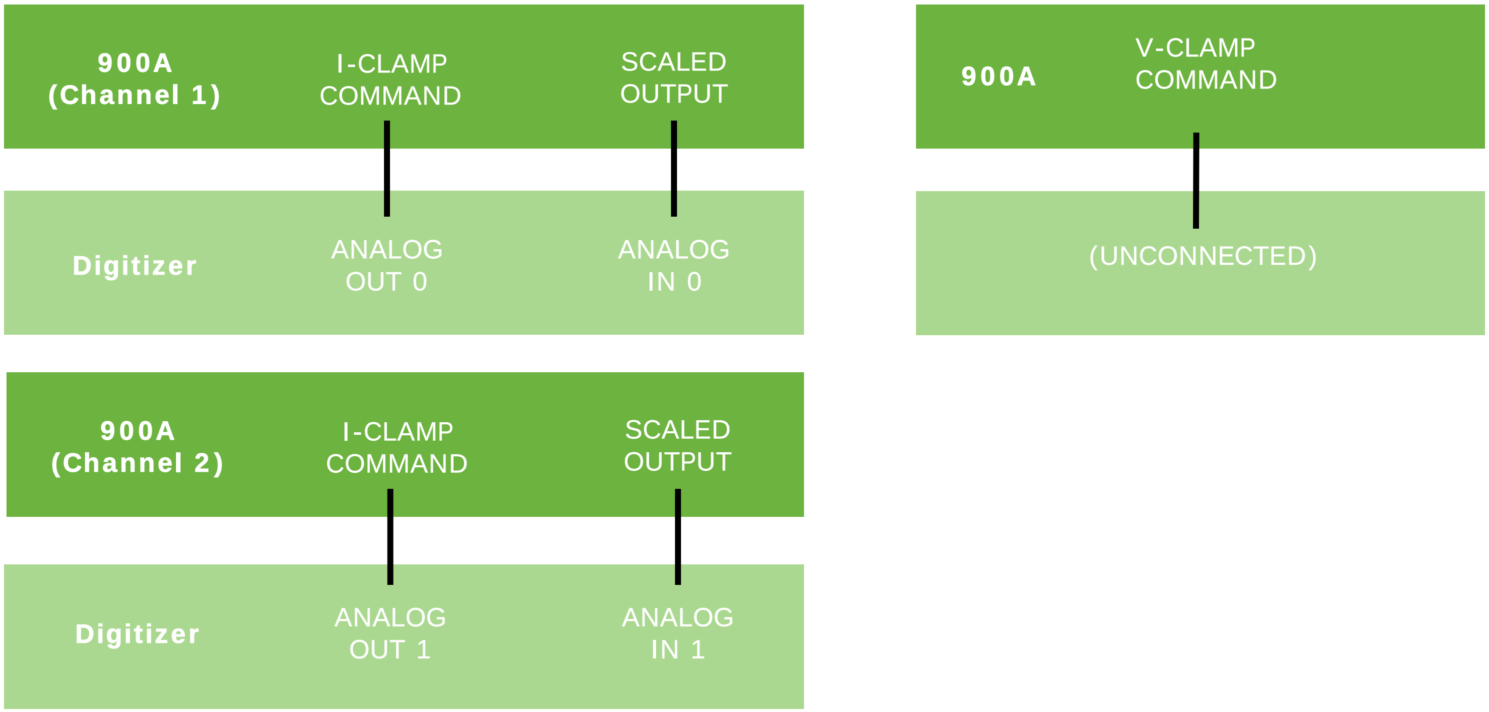
Alternative command connections



**Note:* STEP-ACTIVATE inputs are located on the rear of the unit.

Axoclamp Connections 5

Alternative command connections – no voltage clamp



Note: This set-up does not allow external commands in dSEVC or TEVC modes. It is only recommended if you do not plan to use either voltage clamp mode.

Axoclamp Connections 6

Optional: Current monitor outputs for IC modes.

If you want to monitor the current while in IC modes on both channels, you can use the dedicated CURRENT OUTPUT on each channel.

These outputs are not telegraphed to Clampex, so we will need to configure the scale factor manually in the Lab Bench.

Details on this configuration can be found later in Axoclamp Signals.

Axoclamp

Configure Telegraphs

Axoclamp 900A telegraphs are software messages sent from Axoclamp Commander to Clampex, registering key amplifier settings.

As well as simply reporting the settings in Clampex, the telegraphs are integrated into Clampex so that the greater proportion of signal setup is done automatically (as we will soon see).

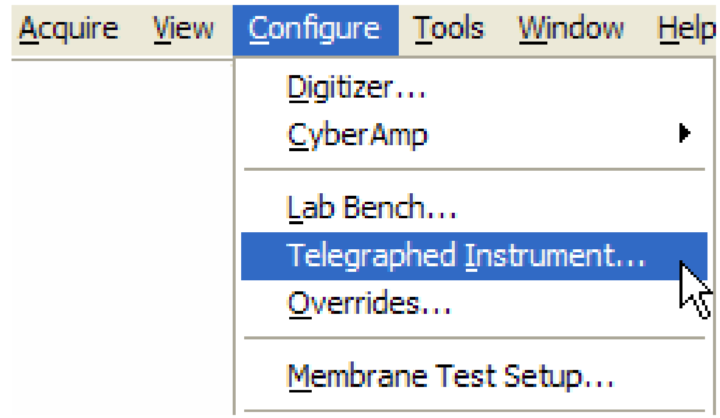
The most important telegraph remains the gain telegraph, used to automatically rescale the Clampex Scope window as gains settings are changed, and to ensure recorded data files are correctly scaled. The lowpass filter settings are also reported in the Real Time Controls and written into recorded file headers—as is the output gain.

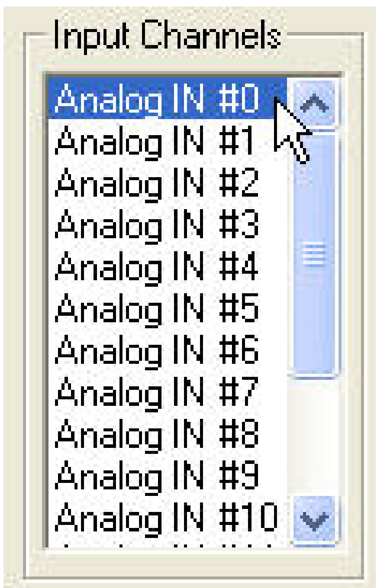
In addition, the Axoclamp has telegraphs for amplifier mode, and for the units and scale factors for command and acquisition signals. We will use these telegraphs in our setup in the following slides.

We will assume that a voltage clamp mode (dSEVC or TEVC) will be used, and the connections shown in Axoclamp Connections 3 are made. The changes necessary for other arrangements, *e.g.*, the connections in Axoclamp Connections 4 should be easy to make once the basic principles are understood.

Axoclamp Telegraphs 1

Open Telegraphed Instrument from the Clampex Configure menu.

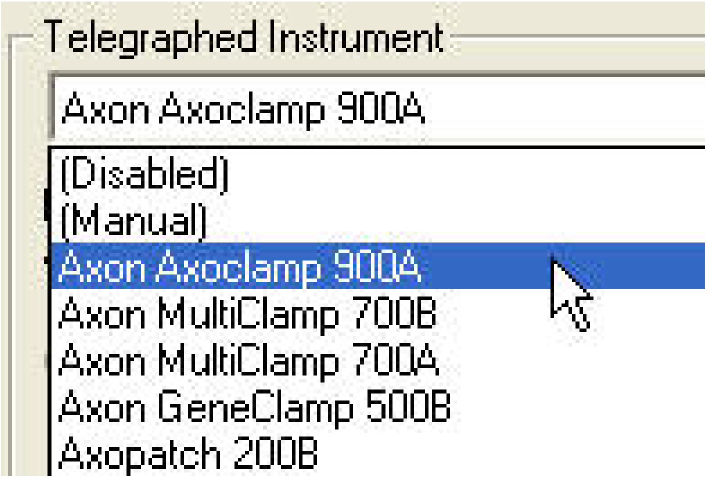




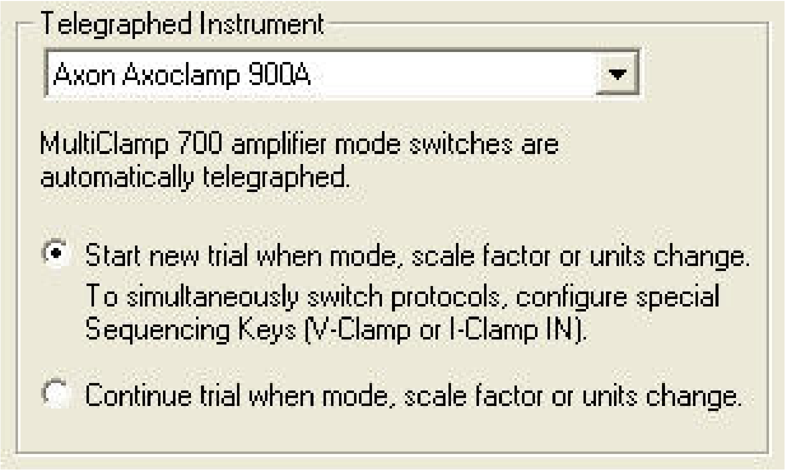
All telegraphs must be configured for a specific digitizer input channel. We will first enable telegraphs for the channel receiving the amplifier Scaled Output from Channel 1. We have connected the Axoclamp Scaled Output for Channel 1 to Analog IN #0 on the Digidata (Connections), so select this from the Input Channels list.

Axoclamp Telegraphs 2

Select Axon Axoclamp 900A from the Telegraphed Instrument list.



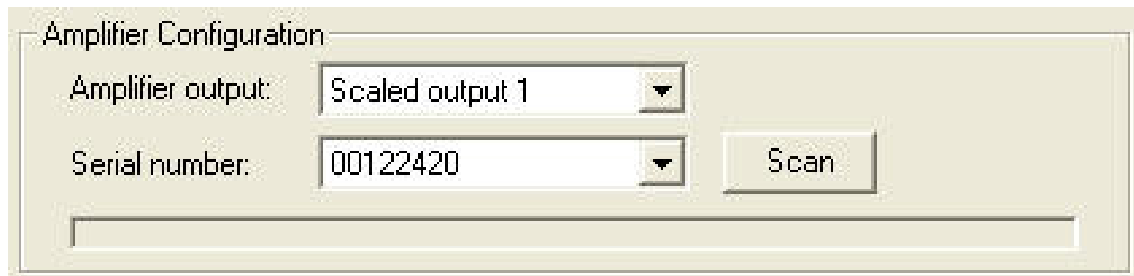
When you have made this selection note the options with respect to linking protocols to amplifier modes. You can learn more about this in the MultiClamp section on Sequencing Keys (Configure Sequencing Keys).



Axoclamp Telegraphs 3

The first enabled section on the dialog is Amplifier Configuration.

Identify the amplifier channel (i.e. headstage) and signal type for the selected digitizer channel (in this case, Analog In #0). In the previous slides we chose Scaled Output 1 to connect to Analog In #0

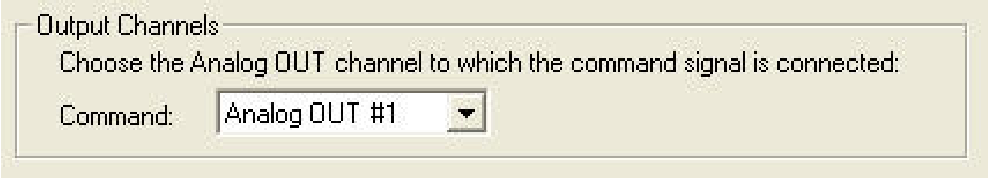


Next click Scan—the Axoclamp serial number is shown when the amplifier is found.

Axoclamp Telegraphs 4

Axoclamp telegraphs scale factors for command signals as well as for its output signals. Enable Clampex to receive these telegraphs in the bottom Output Channels section.

The digitizer input we are configuring receives output from headstage 1 (i.e. amplifier channel 1). The I-CLAMP COMMAND input for this headstage is fed from digitizer output Analog OUT #1 (see Connections; Analog Out #0 is connected to V-CLAMP COMMAND). Select Analog OUT #1 in the Command field*.

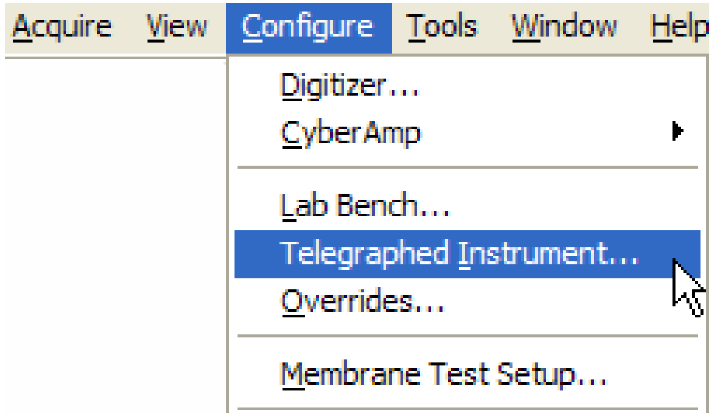


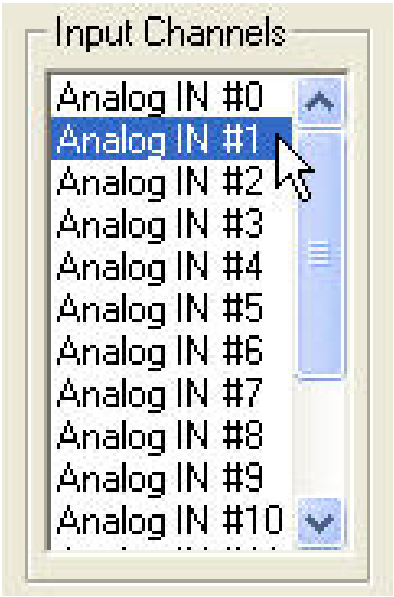
This completes telegraph setup for the Scaled Output of Axoclamp channel 1.
(The CURRENT OUTPUT is not telegraphed.)

*Note: If you are using the STEP-ACTIVATE inputs instead of I-CLAMP COMMAND, leave the Command field on (none).

Axoclamp Telegraphs 5

Open Telegraphed Instrument from the Clampex Configure menu.

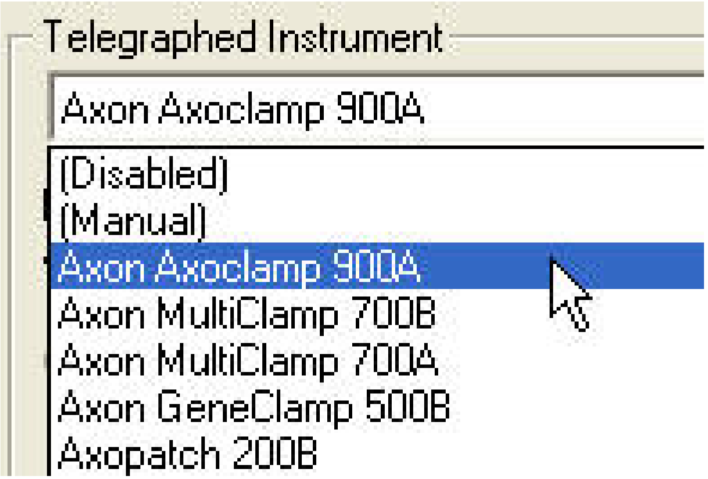




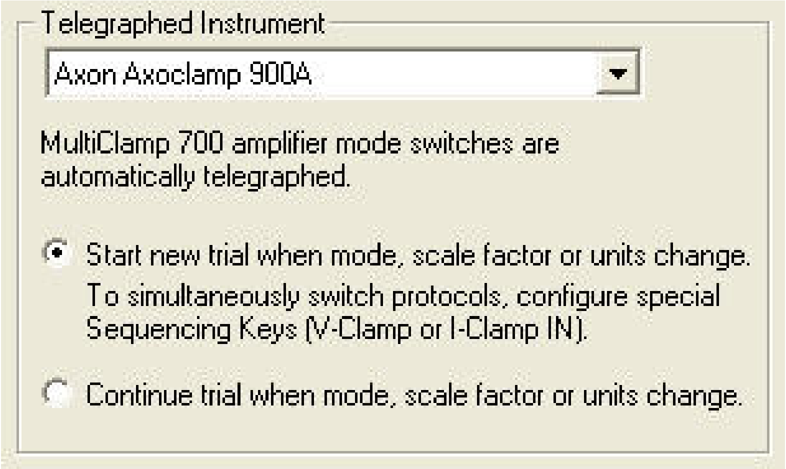
All telegraphs must be configured for a specific digitizer input channel. Now we will enable telegraphs for the channel receiving the Axoclamp Channel 2 Scaled Output. We have connected the Axoclamp Scaled Output for Channel 2 to Analog IN #1 on the Digidata (Connections), so select this from the Input Channels list.

Axoclamp Telegraphs 6

Select Axon Axoclamp 900A from the Telegraphed Instrument list.



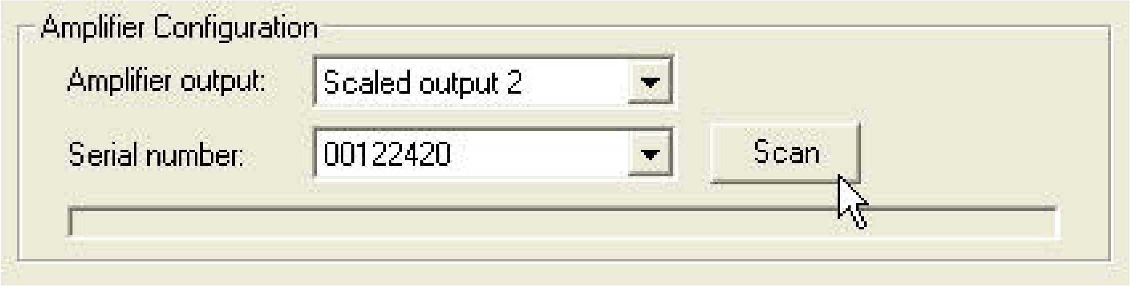
When you have made this selection note the options with respect to linking protocols to amplifier modes. You can learn more about this in the MultiClamp section on Sequencing Keys (Configure Sequencing Keys).



Axoclamp Telegraphs 7

The first enabled section on the dialog is Amplifier Configuration.

Identify the amplifier channel (i.e. headstage) and signal type for the selected digitizer channel (in this case, Analog In #1). In the previous slides we chose Scaled Output 2 to connect to Analog In #1

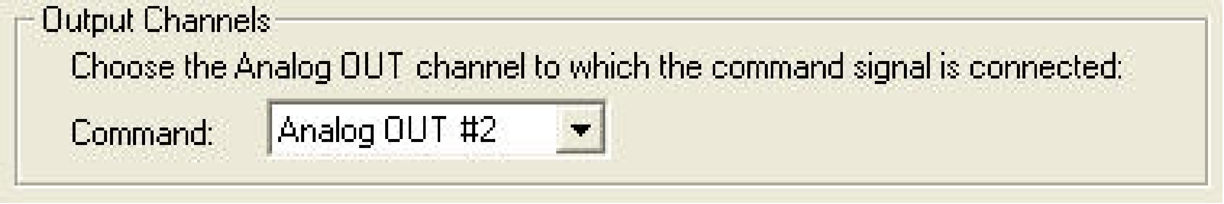


Next press the Scan button—the Axoclamp serial number is shown when the amplifier is found.

Axoclamp Telegraphs 8

Axoclamp telegraphs scale factors for command signals as well as for its output signals. Enable Clampex to receive these telegraphs in the bottom Output Channels section.

The digitizer input we are configuring receives output from headstage 2 (i.e. amplifier channel 2). The I-CLAMP COMMAND input for this headstage is fed from digitizer output Analog OUT #2 (see [Connections](#); Analog Out #0 is connect to V-CLAMP COMMAND). Select Analog OUT #2 in the Command field*.



This completes telegraph setup for the Scaled Output of Axoclamp Channel 2. (The CURRENT OUTPUT is not telegraphed. See [Axoclamp Connections 6](#) for notes about these outputs.)

****Note:*** If you are using the STEP-ACTIVATE inputs instead of I-CLAMP COMMAND, leave the Command field on (none).

MOLECULAR DEVICES

Axoclamp Telegraphs 9

Finish

This completes telegraph setup. We have configured Clampex to receive telegraphs as follows:

- SCALED OUTPUT Channel 1 to Analog IN #0
- SCALED OUTPUT Channel 2 to Analog IN #1
- I-CLAMP COMMAND Channel 1 to Analog OUT #1*
- I-CLAMP COMMAND Channel 2 to Analog OUT #2*
- V-CLAMP COMMAND is not telegraphed, although connected to Analog OUT #0.

We now go to the Lab Bench for signal configuration.

**Note:* Assuming connections as shown in Axoclamp Connections 3.

Axoclamp 900A

Create Signals

In this section we name the signals we require, assigning these to input and output channels.

Before starting it is important to be clear on what signals and channels are:

- **Signal:** a set of name, unit, scale factor and offset, by means of which the voltage inputs and outputs at the digitizer are represented in Clampex as the parameter being read at, or delivered to, the preparation.
- **Channel:** a cable connection to the digitizer, identified by the name of the BNC port where connection is made, e.g. Analog IN #0, Digital OUT #2.

As already noted, analog channels can be configured for different signals at different times, which is what we do in this section.

Axoclamp Signals 1

The configuration of the signals in the Lab Bench depends upon the modes in which you plan to use the Axoclamp 900A.

Select a mode from the menu below, and complete the configuration for that mode.

Then return to this menu for other modes you plan to use.

Axoclamp 900A Modes

IC (both channels)

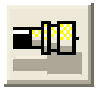
dSEVC

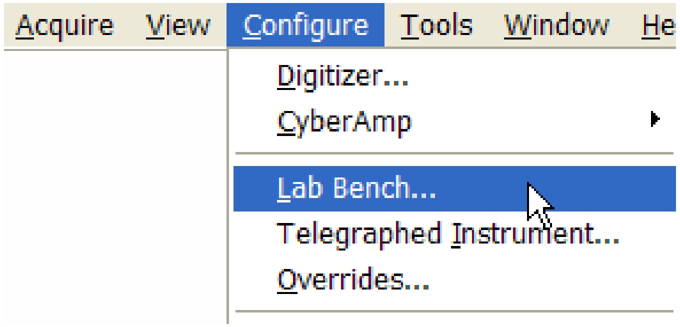
TEVC

Axoclamp Signals 2

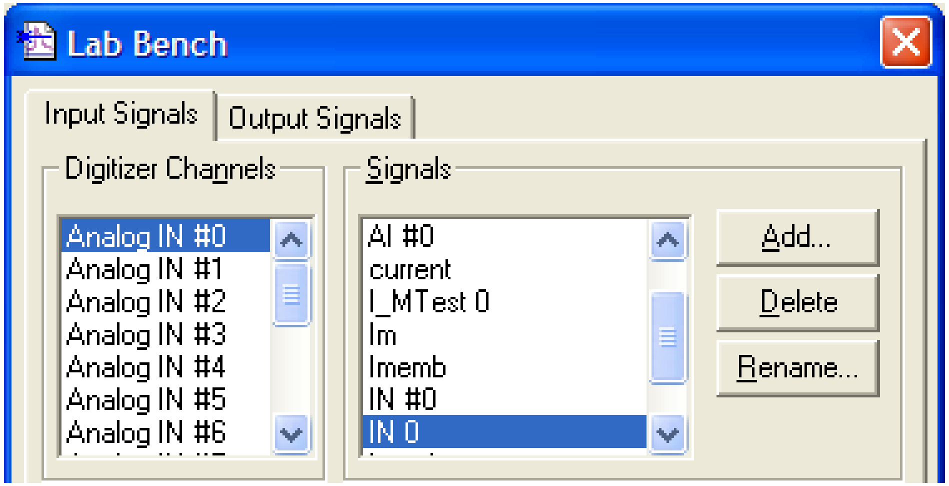
Current Clamp (both channels)

Open the Lab Bench from the Configure menu—or use the toolbutton:





The Lab Bench opens with the Input Signals tab on top, and digitizer channel Analog IN #0 selected. We have the Scaled Output of Channel 1 connected to this channel. In this mode the Scaled Output we are interested in is Membrane Potential, so we need to create an appropriate signal.



Axoclamp Signals 3

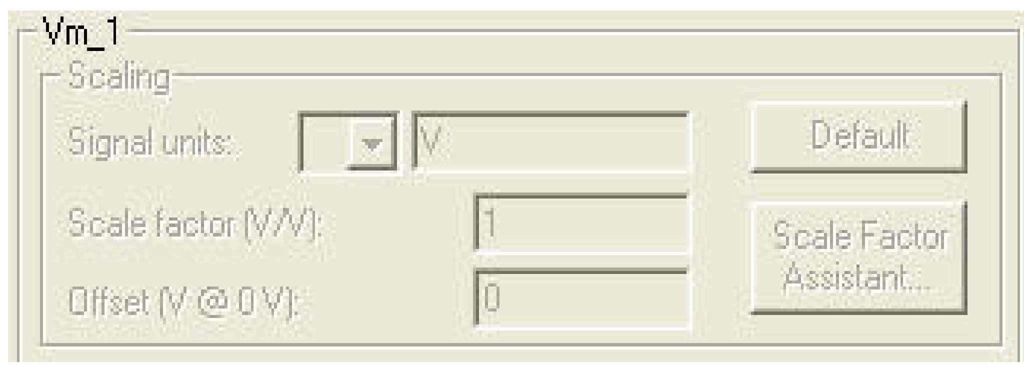
Current Clamp (both channels)

Click the Add button in the Signals section, opening the Add Signal dialog.

Type in “Vm_1”—the name we will give the scaled membrane potential signal for current clamp, on Channel 1.

Press OK. With the new signal selected in the Signals list, the rest of the tab shows options and settings for that signal.

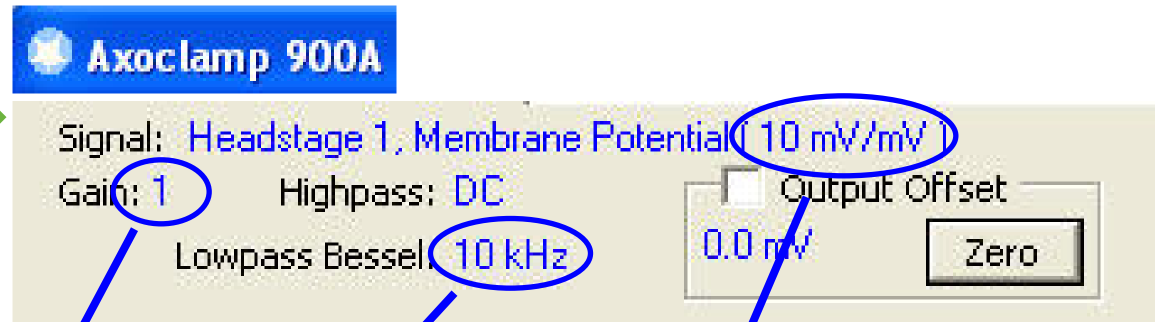
Note that the entire Scaling section is grayed, as it is not used. This is because signal scaling is now under the control of the telegraphs we set up in the last section. Do not worry if the units and scale here are incorrect– they are overridden.

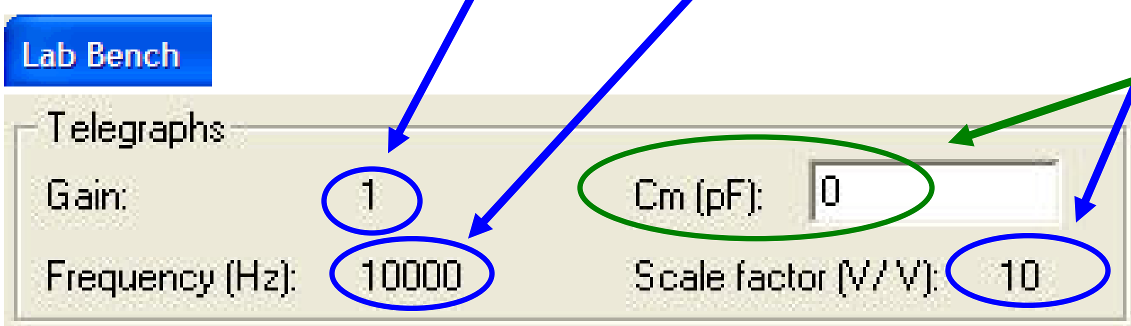


Axoclamp Signals 4

Current Clamp (both channels)

The telegraphs are reported at the bottom of the Lab Bench.
The screenshots show the telegraphs in the Lab Bench with Commander at default settings.





Axoclamp Commander 900A does not report whole-cell capacitance values, since it has no whole-cell capacitance compensation. You may enter a value if you know the capacitance.

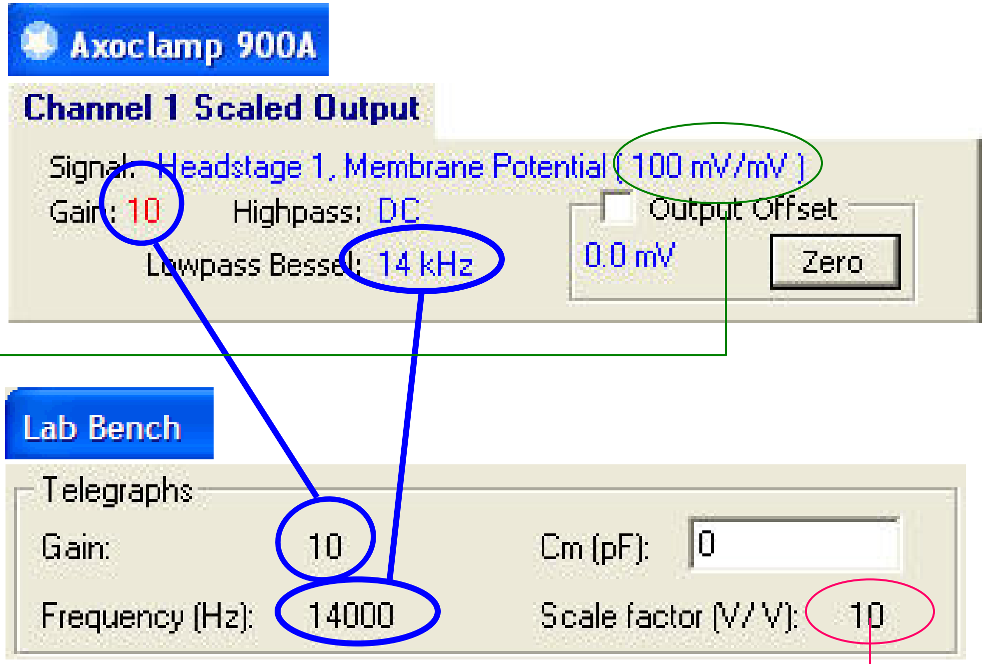
Axoclamp Signals 5

Current Clamp (both channels)

Change settings in Commander and see the telegraphs update in the Lab Bench. The filter, gain, and capacitance compensation telegraphs are also reported in the Real Time Controls.

Note that the scale factor reported in the Lab Bench does not change as you alter the output gain. Clampex reports the unity gain scale factor, i.e. the scale factor for an output gain of one (in this case, 10 mV/mV = 10 V/V).

Of course, the scale factor applied to the signal takes the gain into account— e.g. in these screenshots, Clampex will apply a scale factor of 10 x 10 mV/mV = 100 mV/mV, as reported in Commander.

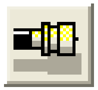


Axoclamp Signals 6

Current Clamp (both channels)

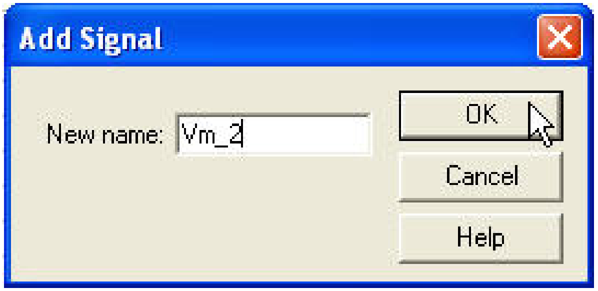
This completes the creation of our first signal. All we actually did was to create a signal name. Following that, with Axoclamp telegraphing enabled, the remainder of the signal configuration was handled automatically.

The signal we created was for Channel 1, in IC mode. To make a matching signal for Channel 2 the same steps are followed.



Open the Lab Bench

Select the digitizer channel, Analog IN #1



Add a new signal

This completes the configuration of the input signals used when the Axoclamp 900A channels are in current clamp mode.

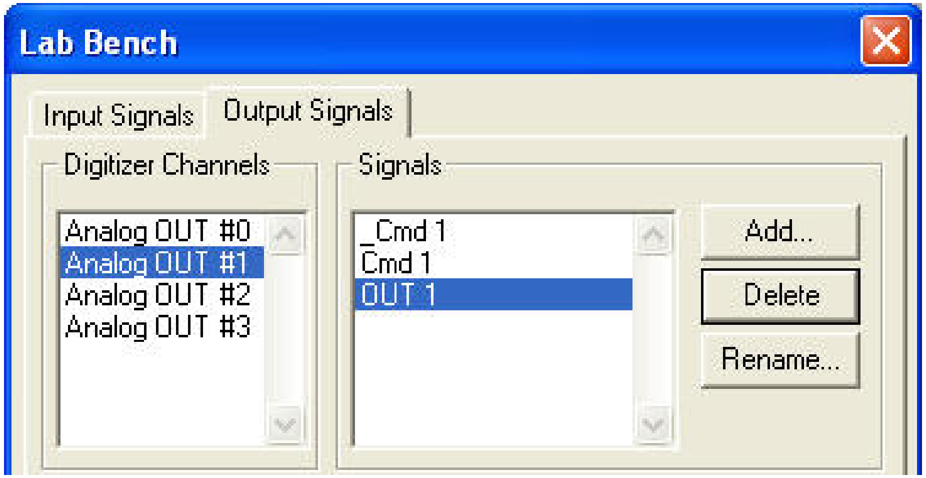
Axoclamp Signals 7

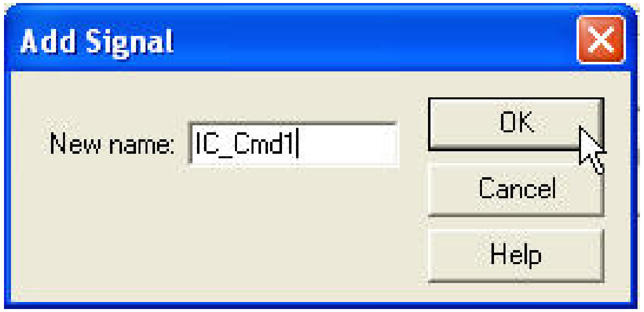
Current Clamp (both channels)

Now we create signals for the current command waveform on Channel 1.

This only applies if the Axoclamp 900A is connected as in Axoclamp Connections 3. If you are using the STEP-ACTIVATE inputs (Axoclamp Connections 4) to apply current commands, skip this part.

Go to the Output Signals tab in the Lab Bench. Select Analog OUT #1. This is the channel we have connected for current clamp commands to Channel 1* (Axoclamp Connections 3).





Press the Add button in the Signals section, and type "IC_Cmd1" into the Add Signal dialog—for the command signal for current clamp on Channel 1.

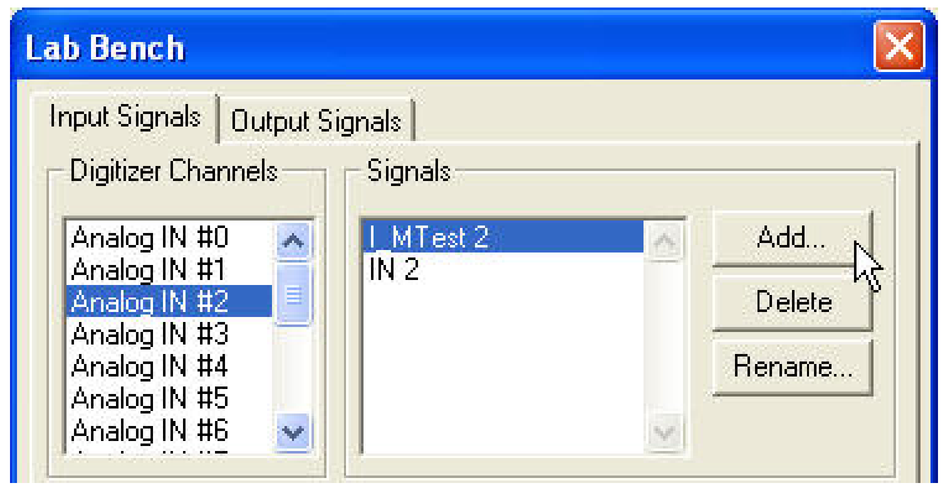
Axoclamp Signals 8

Current Clamp (both channels)

Now we create signals for the current command waveform on Channel 2.

This only applies if the Axoclamp 900A is connected as in Axoclamp Connections 3. If you are using the STEP-ACTIVATE inputs (Axoclamp Connections 4) to apply current commands, skip this part.

Go to the Output Signals tab in the Lab Bench. Select Analog OUT #2. This is the channel we have connected for current clamp commands to Channel 2* (Axoclamp Connections 3).



Press the Add button in the Signals section, and type “IC_Cmd2” into the Add Signal dialog—for the command signal for current clamp on Channel 2.

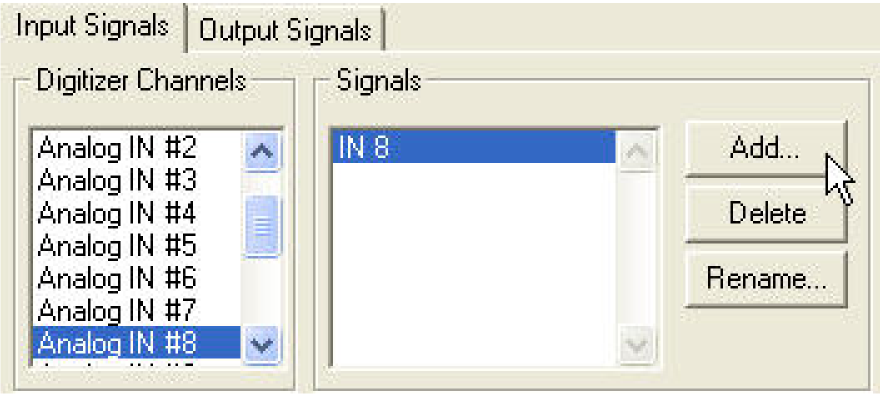
Axoclamp Signals 9

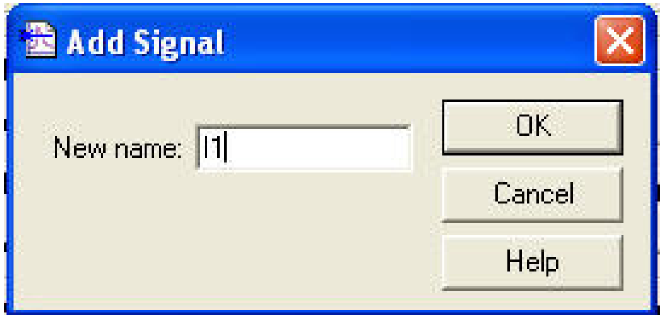
Current Clamp (both channels)

Now we create optional signals used to monitor the current while in current clamp.

Each channel has a dedicated CURRENT OUTPUT. Because its scale factor is not telegraphed to Clampex, we need to configure these signals manually in the Lab Bench.

Go to the Output Signals tab in the Lab Bench. Select Analog OUT #8. This is the channel we have connected to CURRENT OUTPUT on Channel 1 (Axoclamp Connections).





Press the Add button in the Signals section, and type “I1” into the Add Signal dialog—for the current on Channel 1.

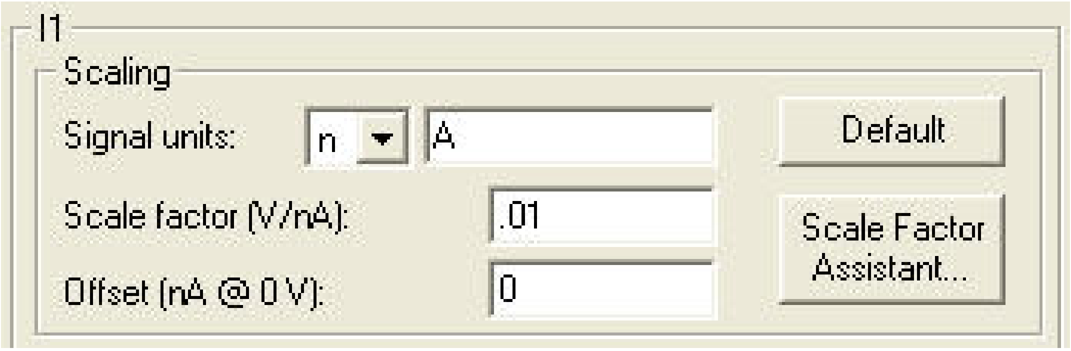
We’ll do the scale factor and units on the next slide.

Axoclamp Signals 10

Current Clamp (both channels)

Now we determine the scale factor and enter it.

First, select the units “nA” for this signal, by choosing “n” from the drop-down prefix list and typing “A” into the units field.



The Scale factor (V/nA) depends upon the headstage. The HS-9A and HS-2A headstages have an H value corresponding to the value of the feedback resistor in the headstage.

The value of H is printed on the headstage. It is the number following the small “x”. For example: HS-9A x**1** indicates H=1.

The Scale factor to enter in the Lab Bench is given by this formula:

Scale factor (V/nA) = 0.01 / H

Therefore, for H=1, the scale factor is 0.01; if H=10, the scale factor is 0.001, and so on.

You should now be able to repeat these steps to create a matching signal on Analog IN #9, to be used to monitor Channel 2 CURRENT OUTPUT.

Axoclamp Signals 11

Current Clamp (both channels)

Finished!

In this section we created signals for the operation of Channel 1 and Channel 2 in current clamp mode. These signals will work whether one or both channels is in current clamp mode.We created these signals:

Channel 1

Vm_1 for membrane potential from SCALED OUTPUT

IC_Cmd1 for current command waveforms to I-CLAMP COMMAND

I1 for membrane current from CURRENT OUTPUT (optional)

Channel 2

Vm_2 for membrane potential from SCALED OUTPUT

IC_Cmd2 for current command waveforms to I-CLAMP COMMAND

I2 for membrane current from CURRENT OUTPUT (optional)

As noted, the command signals may be created in alternative ways.
If you choose to use the STEP-ACTIVATE commands, then you need not create the IC_Cmd1 and IC_Cmd2 signals.

Click here to return to Modes Menu to create more signals for use in other modes (*e.g.*, TEVC, dSEVC,…)

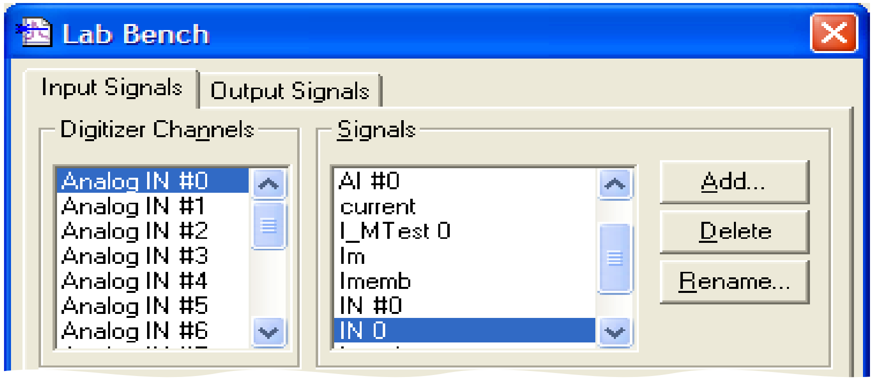
MOLECULAR DEVICES

Axoclamp Signals 12

dSEVC

Now we create signals for use when the Axoclamp 900A is in dSEVC mode. In this mode, only headstage 1 is active. However, both SCALED OUTPUTS may carry signals from headstage 1.
We will configure Channel 1 SCALED OUTPUT to be Membrane Current of headstage 1; while Channel 2 SCALED OUTPUT will be Membrane Potential, also of headstage 1.
We will then configure a voltage clamp command signal.

We have the SCALED OUTPUT of Channel 1 connected to Analog IN #0 (Connections). Select this as the digitizer channel, and then press the Add button.

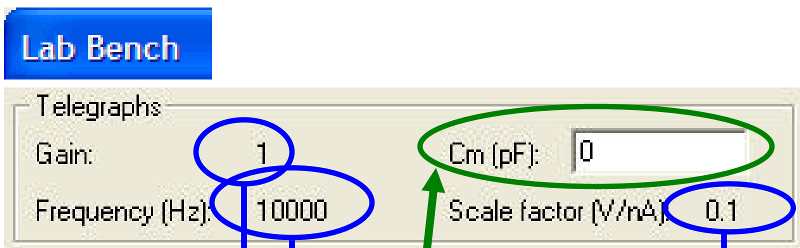


Type in "Im_dSEVC", for the signal we will use to monitor membrane current in dSEVC.

Axoclamp Signals 13

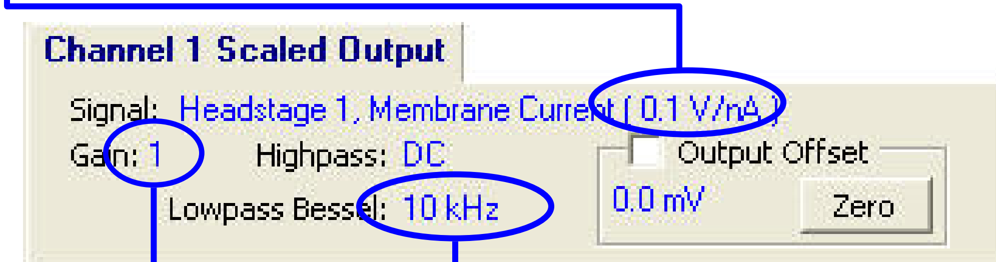
dSEVC

When a signal's parameters are telegraphed, the Scaling section on the Lab Bench is grayed. Don't worry if the grayed-out settings are incorrect; they are overridden.



The telegraphed parameters are reported at the bottom of the Lab Bench. The screenshots show the telegraphs in the Lab Bench with Channel 1 in dSEVC mode, at default settings.

Axoclamp Commander 900A does not report whole-cell capacitance values, since it has no whole-cell capacitance compensation. You may enter a value if you know the capacitance.

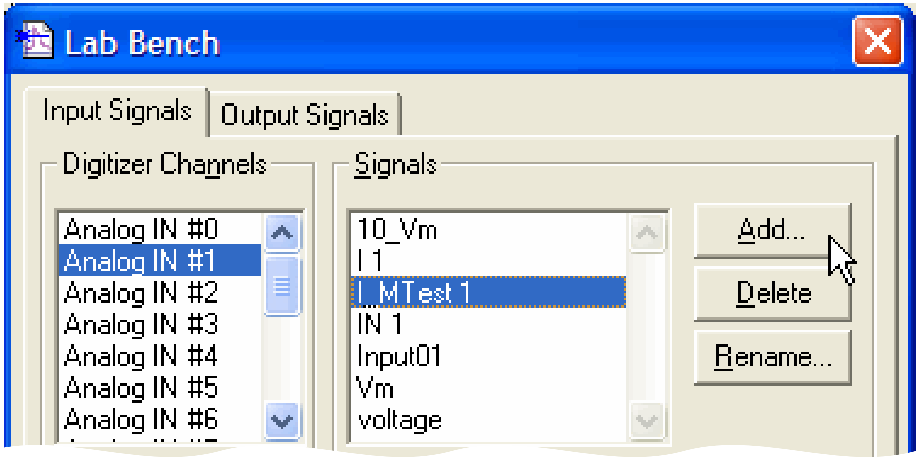


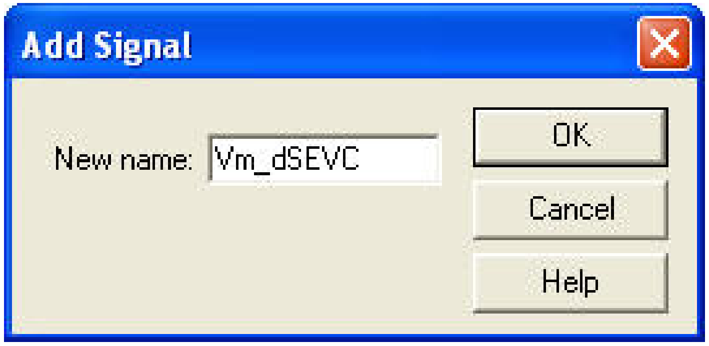
Note: *You may need to select "Headstage 1, Membrane Current" on the Axoclamp 900A Commander*

Axoclamp Signals 14

dSEVC

We have the SCALED OUTPUT of Channel 2 connected to Analog IN #1 (Connections). Select this as the digitizer channel, and then press the Add button.





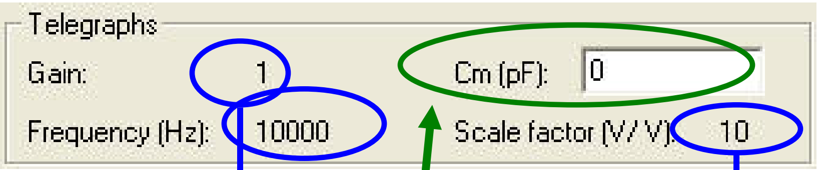
Type in “Vm_dSEVC”, for the signal we will use to monitor membrane potential in dSEVC.

Axoclamp Signals 15

dSEVC

In dSEVC mode, only headstage 1 is operating. Therefore we may select signals from headstage 1 to be carried on SCALED OUTPUT on Channel 1 and Channel 2.

In this case, we choose to monitor the "Headstage 1, Membrane Potential" on SCALED OUTPUT of Channel 2.



As before, the telegraphed parameters are reported at the bottom of the Lab Bench. The screenshots show the telegraphs in the Lab Bench with Channel 1 in dSEVC mode, at default settings.

Axoclamp Commander 900A does not report whole-cell capacitance values, since it has no whole-cell capacitance compensation. You may enter a value if you know the capacitance.

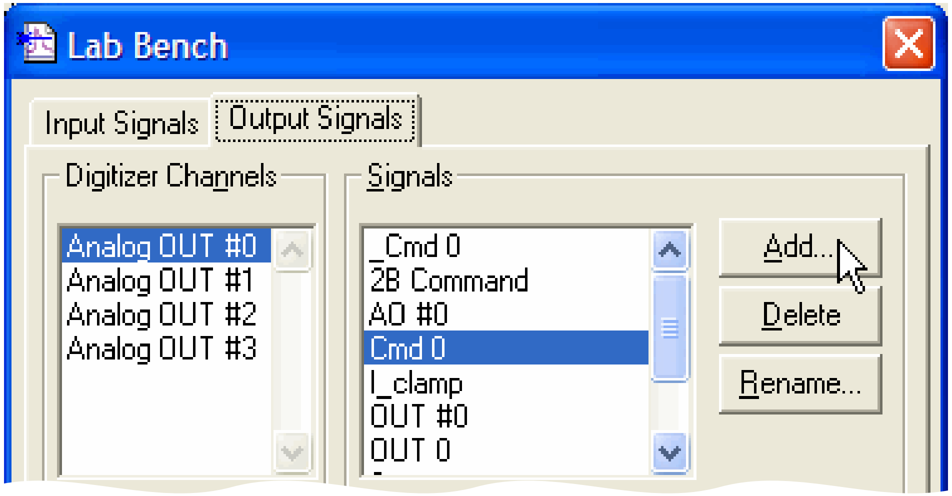
Note: *You may need to select "Headstage 1, Membrane Potential" on the Axoclamp 900A Commander*

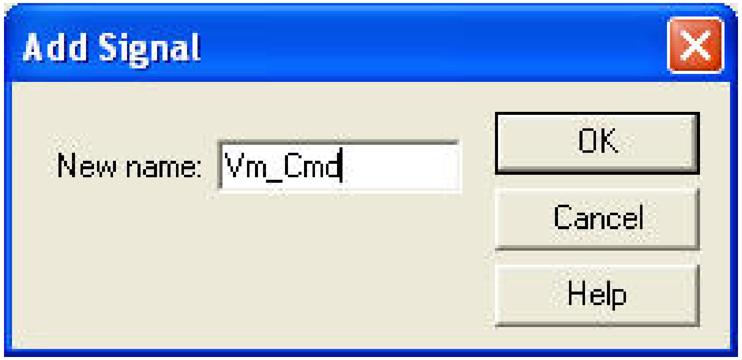
Axoclamp Signals 16

dSEVC

Now we create a signal for the voltage command waveform on while in dSEVC mode.

Go to the Output Signals tab in the Lab Bench. Select Analog OUT #0. This is the channel we have connected to V-CLAMP COMMAND (Axoclamp Connections 3).





Click Add in the Signals section, and type “Vm_Cmd” into the Add Signal dialog as the name of the new voltage command signal.

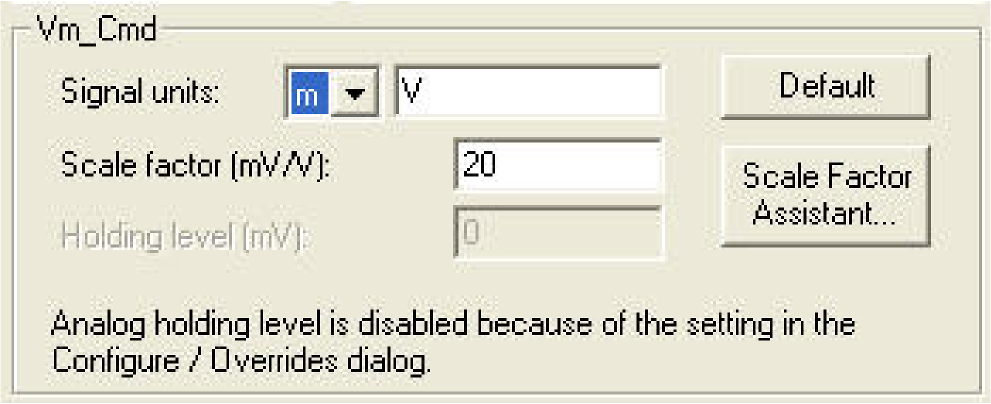
Axoclamp Signals 17

dSEVC

Now we need to set the units and scale factor for this signal.

Since it is a voltage clamp command, choose “m” and type “V”, as shown below. This tells Clampex that the units are “mV”.

For the Axoclamp 900A (and other Axon CNS instruments), the usual voltage clamp command scale factor is 20 mV/V.



The scale factor of 20 mV/V tells Clampex how to convert your desired command to the appropriate voltage to produce from Analog OUT.

For example, if your desired holding potential is –50 mV, Clampex converts this to –50 mV / (20 mV/V) = -2.5 V at the actual Digidata Analog OUT.

Axoclamp Signals 18

dSEVC

Finished!

In this section we created signals for the operation of Channel 1 in dSEVC mode. In this mode, headstage 2 does not operate and so SCALED OUTPUT on Channel 2 can be used to carry a signal from headstage 1.

We created three signals:

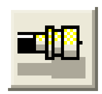
- **Im_dSEVC** for membrane current from Channel 1 SCALED OUTPUT
- **Vm_dSEVC** for membrane potential from Channel 2 SCALED OUTPUT (but measured at headstage 1)
- **VC_Cmd** for voltage command waveforms to V-CLAMP COMMAND

Click here to return to Modes Menu to create more signals for use in other modes (*e.g.*, TEVC, dSEVC,…)

Axoclamp Signals 19

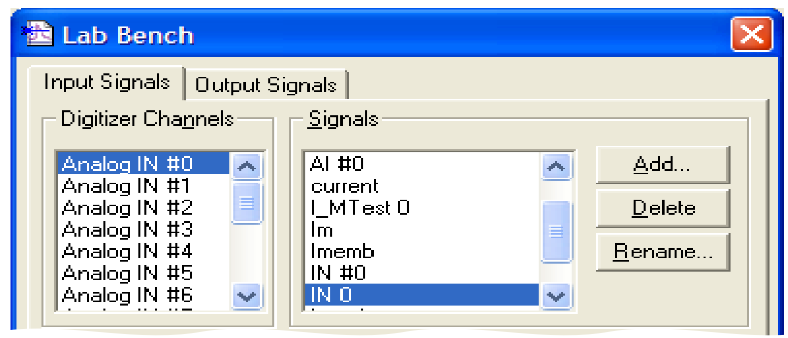
TEVC

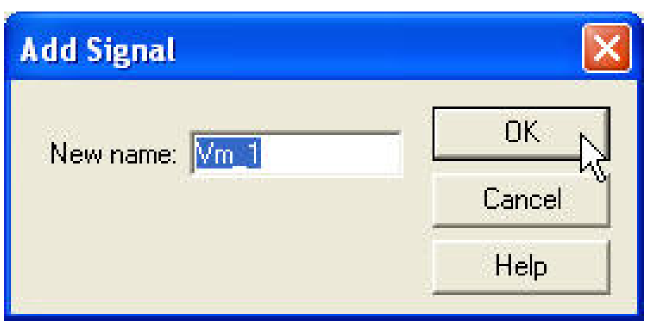
Now we create signals for use when the Axoclamp 900A is in TEVC mode. In this mode, headstage 1 is measures the membrane potential. Headstage 2 injects current.
Therefore, we will configure Channel 1 SCALED OUTPUT to be Membrane Potential of headstage 1. Channel 2 SCALED OUTPUT will be Membrane Current, measured on headstage 2.
We will then configure a voltage clamp command signal.



Open the Lab Bench

We have the SCALED OUTPUT of Channel 1 connected to Analog IN #0 (Connections). Select this as the digitizer channel, and then press the Add button.





Type in “Vm_1”, for the signal we will use to monitor membrane potential in TEVC.
Note: this is the same signal used when headstage 1 is in IC mode. If you have already created this signal, it is not necessary to do so again.

MOLECULAR DEVICES

Axoclamp Signals 20

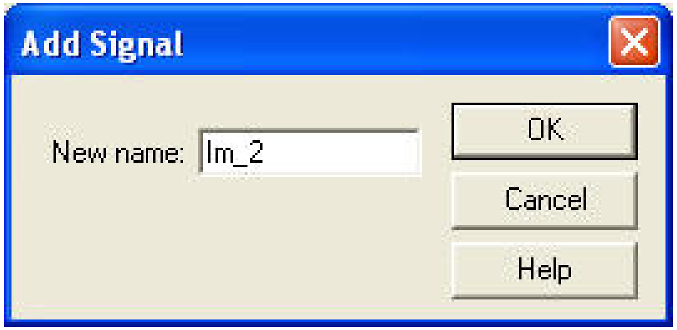
TEVC

This completes the creation of our first TEVC signal. All we actually did was to create a signal name. Following that, with Axoclamp telegraphing enabled, the remainder of the signal configuration was handled automatically.

The signal we created was for Channel 1, which measures Vm when the Axoclamp 900A is in TEVC mode.

Now we need to make a signal for Channel 2, which measures the membrane current. The same steps are followed.

From the Lab Bench, select the digitizer channel, Analog IN #1



Add a new signal

This completes the configuration of the input signals used when the Axoclamp 900A operates in TEVC mode. Now let's look at how the signals are telegraphed.

Axoclamp Signals 21

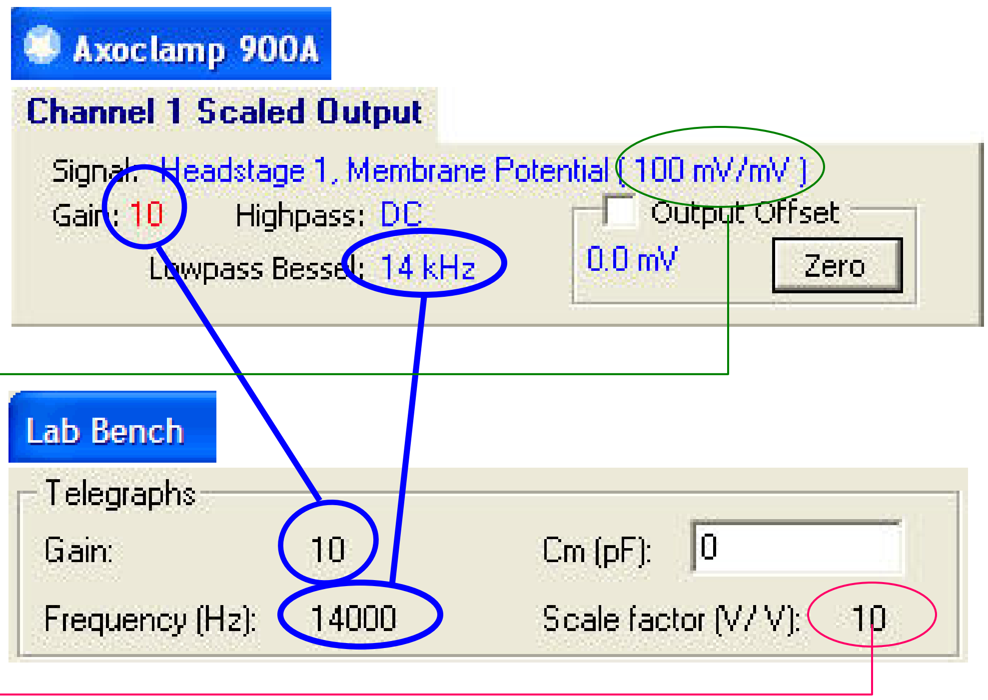
TEVC

Reset the Axoclamp 900A to defaults, a put a model cell (in CELL position) on the headstages in preparation for TEVC. Switch the instrument to TEVC mode. Don't worry about getting the best clamp– we just want to look at the operation of the telegraphs.

Open the Lab Bench, and change settings in Commander. You see the telegraphs update in the Lab Bench:

Note that the scale factor reported in the Lab Bench does not change as you alter the output gain. Clampex reports the unity gain scale factor, i.e. the scale factor for an output gain of one (in this case, 10 mV/mV = 10 V/V).

Of course, the scale factor applied to the signal takes the gain into account— e.g. in these screenshots, Clampex will apply a scale factor of 10 x 10 mV/mV = 100 mV/mV, as reported in Commander.

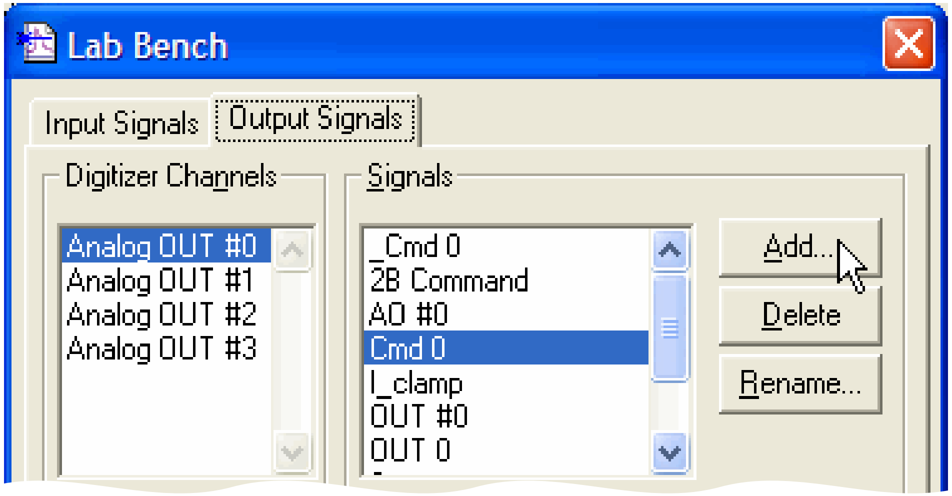


Axoclamp Signals 22

TEVC

Now we create a signal for the voltage command waveform on while in TEVC mode.

Go to the Output Signals tab in the Lab Bench. Select Analog OUT #0. This is the channel we have connected to V-CLAMP COMMAND (Axoclamp Connections 3).



Click Add in the Signals section, and type "Vm_Cmd" into the Add Signal dialog—the name of the new voltage command signal. Click OK.

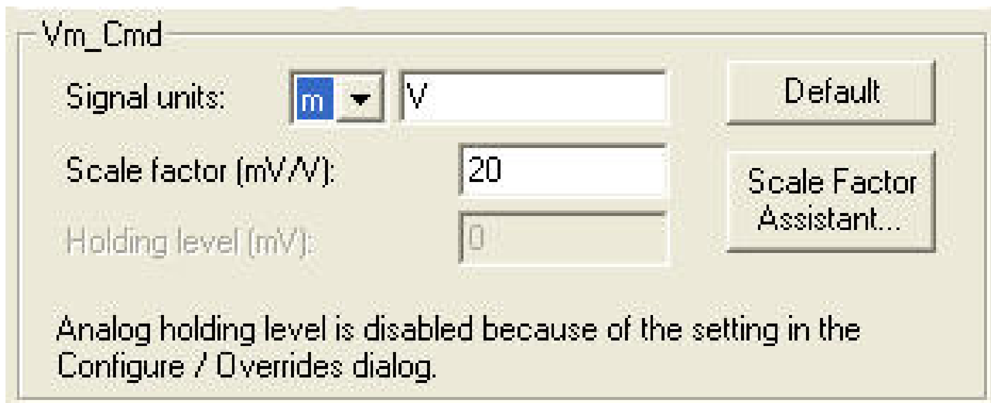
Axoclamp Signals 23

TEVC

Now we need to set the units and scale factor for this signal.

Since it is a voltage clamp command, choose “m” and type “V”, as shown below. This tells Clampex that the units are “mV”.

For the Axoclamp 900A (and other Axon CNS instruments), the usual voltage clamp command scale factor is 20 mV/V.



The scale factor of 20 mV/V tells Clampex how to convert your desired command to the appropriate voltage to produce from Analog OUT.

For example, if your desired holding potential is –50 mV, Clampex converts this to –50 mV / (20 mV/V) = -2.5 V at the actual Digidata Analog OUT.

Axoclamp Signals 24

TEVC

Finished!

In this section we created signals for experiments in TEVC mode. In this mode, headstage 1 measures Vm and SCALED OUTPUT on Channel 1 carries this signal. Headstage 2 measures Im and SCALED OUTPUT on Channel 2 carries this signal.

We created three signals:

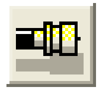
- **Im_2** for membrane current from Channel 2 SCALED OUTPUT
- **Vm_1** for membrane potential from Channel 1 SCALED OUTPUT
- **VC_Cmd** for voltage command waveforms to V-CLAMP COMMAND

Click here to return to Modes Menu to create more signals for use in other modes (*e.g.*, TEVC, dSEVC,…)

Axoclamp Signals 25

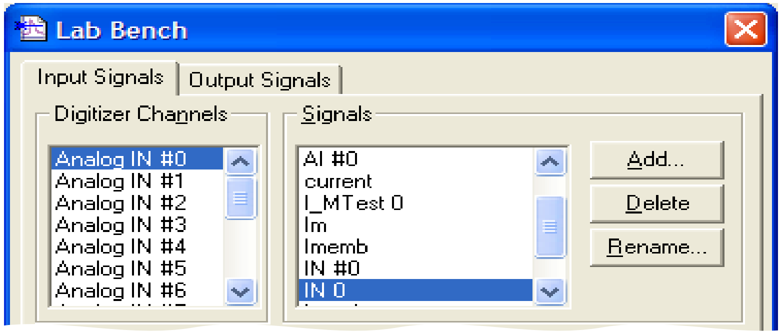
TEVC

Now we create signals for use when the Axoclamp 900A is in TEVC mode.
In this mode, headstage 1 is measures the membrane potential.
Headstage 2 injects current.
Therefore, we will configure Channel 1 SCALED OUTPUT to be Membrane Potential of headstage 1.
Channel 2 SCALED OUTPUT will be Membrane Current, measured on headstage 2.
We will then configure a voltage clamp command signal.



Open the Lab Bench

We have the SCALED OUTPUT of Channel 1 connected to Analog IN #0 (Connections). Select this as the digitizer channel, and then click Add.



Type in “Vm_1”, for the signal we will use to monitor membrane potential in TEVC.
Note: this is the same signal used when headstage 1 is in IC mode. If you have already created this signal, it is not necessary to do so again.

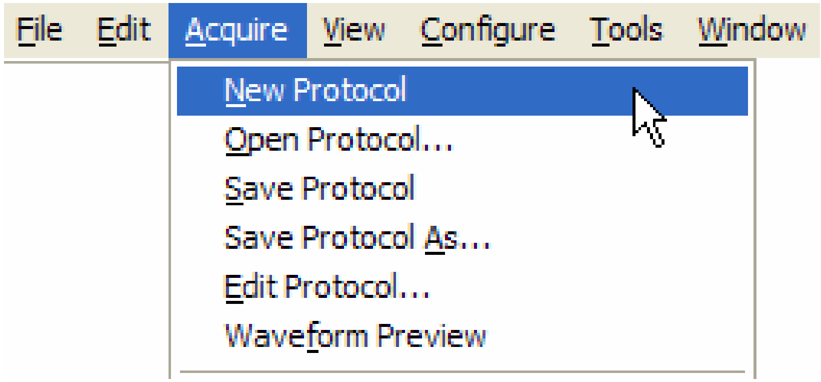
Configure Protocols

Protocols in Clampex are complete sets of acquisition parameters, including options for command waveforms and preliminary data analysis. Particular signals, defined in the Lab Bench, are specified for each protocol.

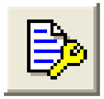
In this section we create two simple protocols, one each for current clamp and two-electrode voltage clamp, incorporating the signals we have just defined.

Axoclamp Protocols 1

Open the Protocol Editor by selecting New Protocol in the Acquire menu.



Note: If a previously saved protocol is not loaded in Clampex, it uses a place-holder protocol, labeled "(untitled)". If this is currently loaded you can open the editor to create a new protocol by selecting Edit Protocol, or by clicking the toolbutton:



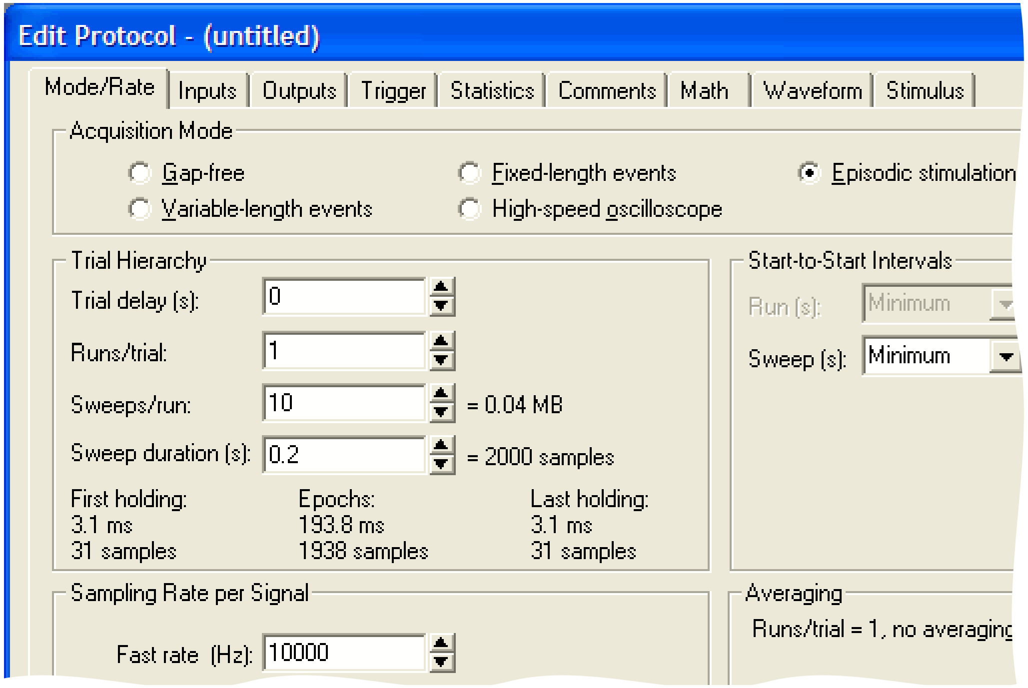
The currently loaded protocol is reported in the status bar at the bottom of the main Clampex window.

We will begin by setting up the protocol for voltage clamp (TEVC).

Axoclamp Protocols 2

The front tab of the protocol editor has controls for, amongst other things, acquisition mode, sampling rate, and trial hierarchy.

The default acquisition mode is episodic stimulation—the only mode that allows a command waveform to be generated. We want to generate a command, so leave this setting. In fact, all the default settings on this tab can be left as they are, but take time to note key parameters such as the sampling rate (10 kHz), the number of samples per sweep, and the number of sweeps per run.



The sweep start-to-start interval is set at Minimum, so each new sweep starts as soon as the previous one is finished.

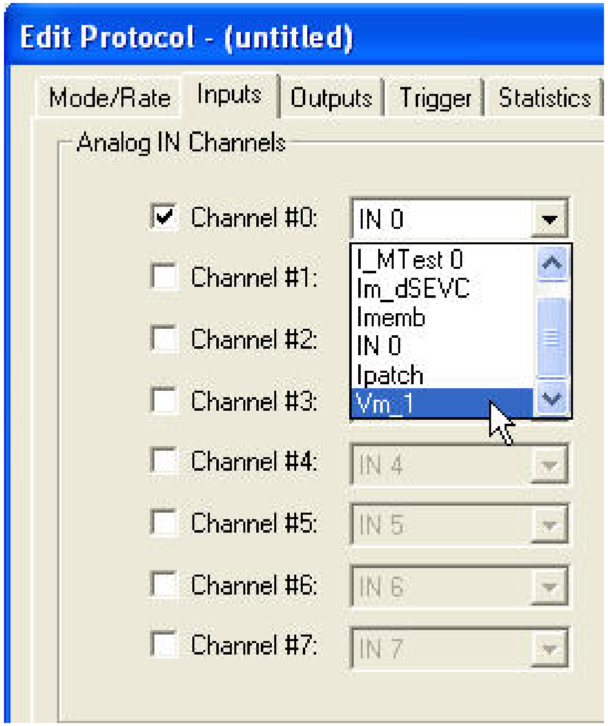
Axoclamp Protocols 3

Next go to the Inputs tab.

Here you select digitizer input channels for the protocol, as well as the signals that you want to be conveyed on these.

For TEVC, we want two input signals—one scaled signal for membrane current, and a second signal to monitor membrane voltage. We created these in the Lab Bench—“Vm_1” and “Im_2”—associating them with digitizer Analog IN channels 0 and 1, respectively (see Axoclamp Signals). Now we incorporate them into the voltage protocol.

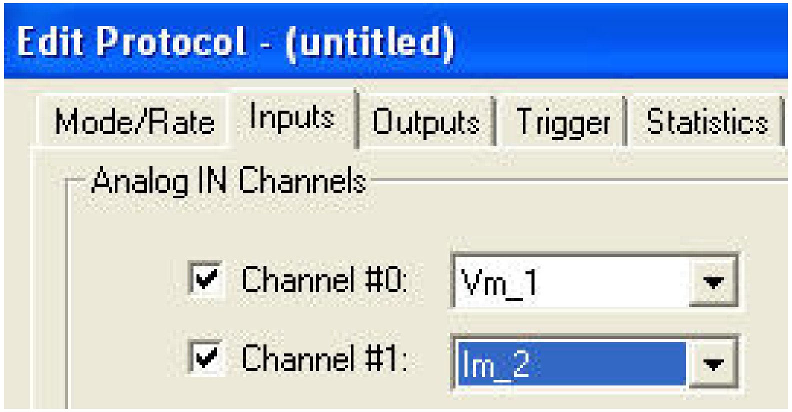
Channel #0 should be already checked. Open the list box beside it and select “Vm_1”.

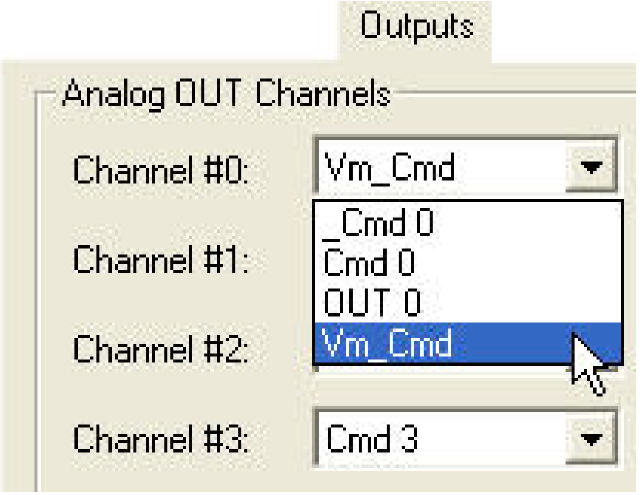


Axoclamp Protocols 4

Then check Channel #1 and select “Im_2”.

This completes the Inputs tab.





Next, go to the Outputs tab.

We created the signal “Vm_Cmd” to deliver the voltage clamp command waveform, on digitizer output channel #0 (Axoclamp Signals 22) .

Select “Vm_Cmd” from the Channel #0 list box.

Axoclamp Protocols 5

We have already configured the signal Vm_Cmd in the Lab Bench (Axoclamp Signals 23), so the units appear as we designated. Note that the scale factor is also indicated, along with the resulting range of available commands.

This tab is also used to set the holding level for the signal. Note that the units are as we designated in the Lab Bench

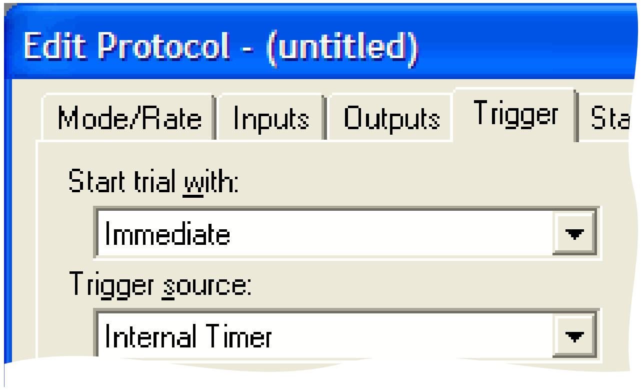
We want to set a holding level of –50 mV, so enter –50 in the holding level field.

MOLECULAR DEVICES

Axoclamp Protocols 6

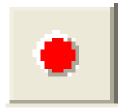
Although we will not make any changes for the purposes of our protocol, it is worth taking a quick look at the trigger settings.

Go to the Trigger tab.

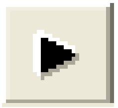


Default settings give “Immediate” trial starts. This means Clampex is armed for data acquisition as soon as you select Record, or View Only, from the Acquire menu—or click the toolbuttons:

Record



View Only



The default trigger source is “Internal Timer”. This triggers the command waveform and data acquisition immediately after the trial is started, continuing through to the end of the trial automatically.

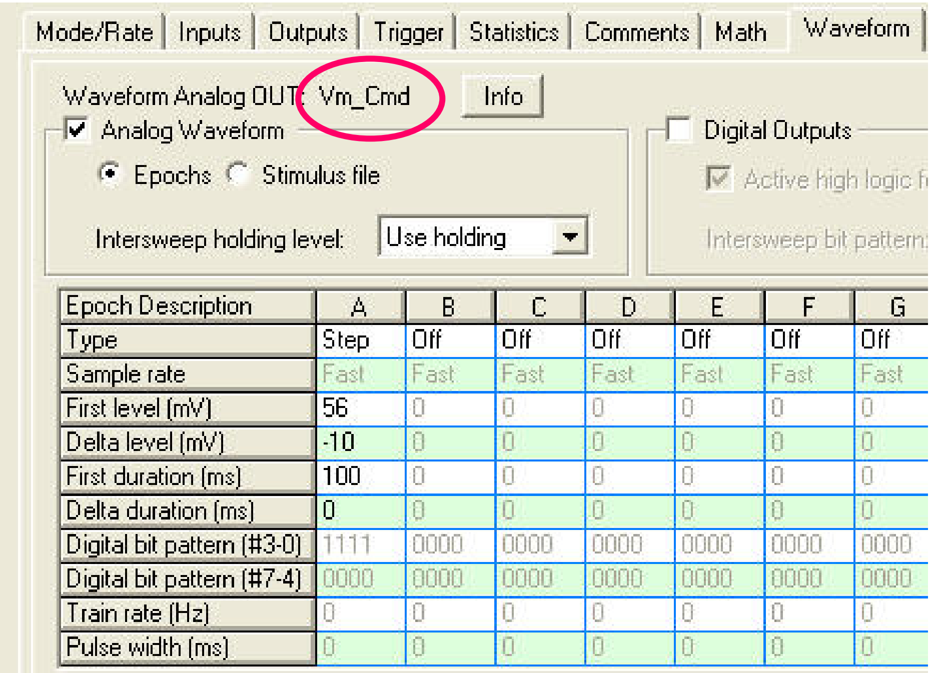
Axoclamp Protocols 7

Now go to the Waveform Channel #0 tab, where outputs are defined for digitizer output channel Analog OUT #0.

A default waveform is already defined—we will delete this and create our own simple stimulus, but first familiarize yourself with some key settings on this tab.

The Analog Waveform checkbox enables analog command definition. Selecting Epochs means we define the waveform using the table in the middle of the tab. In this, the sweep can be divided into up to 50 sections (epochs) A–AX, and a waveform defined for each of these.

The Epoch Description table in the Waveform tab includes cut and paste functionality.



Epoch Description	A	B	C	D	E	F	G
Type	Step	Off	Off	Off	Off	Off	Off
Sample rate	Fast	Fast	Fast	Fast	Fast	Fast	Fast
First level (mV)	56	0	0	0	0	0	0
Delta level (mV)	-10	0	0	0	0	0	0
First duration (ms)	100	0	0	0	0	0	0
Delta duration (ms)	0	0	0	0	0	0	0
Digital bit pattern (#3-0)	1111	0000	0000	0000	0000	0000	0000
Digital bit pattern (#7-4)	0000	0000	0000	0000	0000	0000	0000
Train rate (Hz)	0	0	0	0	0	0	0
Pulse width (ms)	0	0	0	0	0	0	0

The confirmation that “Vm_Cmd” is the signal carrying the output waveform. The units shown for it—in the “Info” message box and in the epoch description table—are again derived from the amplifier mode telegraph from Axoclamp Commander.

Axoclamp Protocols 8

Now to the definition of our waveform.

We will configure an output with a simple step, increasing the amplitude of the step with each sweep.

In column A of the epoch description table, keep “Step” in the Type row, but click in the “First level” row and type in –50. This sets the output level for epoch A in the first sweep of the run. Our entry of –50 mV maintains the holding level.

Click in the next row (Delta level) and type in zero. This keeps the first level setting for subsequent sweeps—i.e. epoch A is maintained at –50 mV for each of the 10 sweeps in the trial.

Epoch Description	A	B
Type	Step	Off
Sample rate	Fast	Fast
First level (mV)	-50	0
Delta level (mV)	0	0
First duration (ms)	50	0
Delta duration (ms)	0	0
Digital bit pattern (#3-0)	1111	0000
Digital bit pattern (#7-4)	0000	0000
Train rate (Hz)	0	0
Pulse width (ms)	0	0

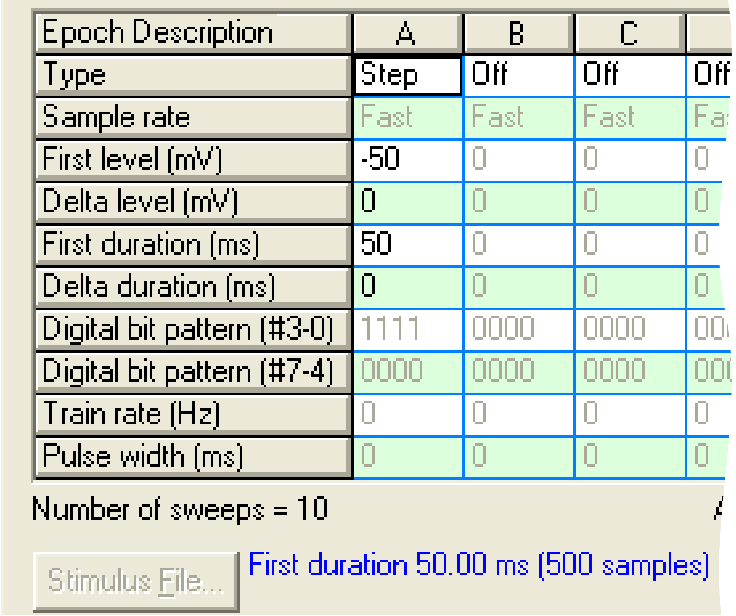
Axoclamp Protocols 9

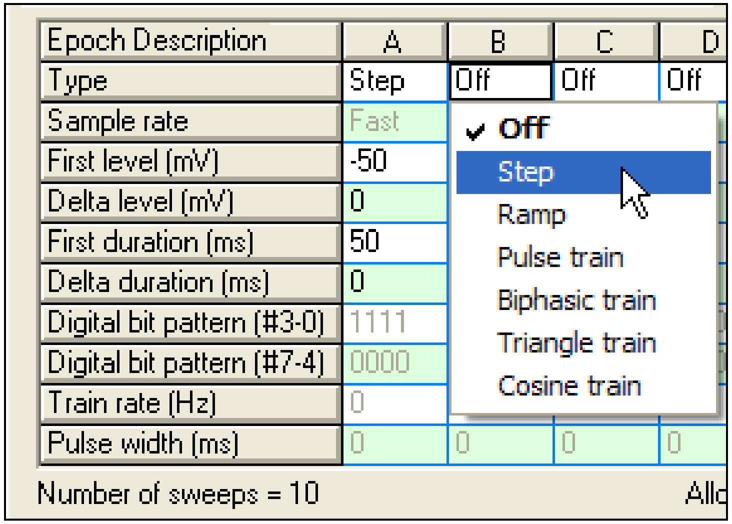
Now to set the period for epoch A.

Click in the First duration row, and type in 50.

Our sampling interval is 10 kHz, so a 50 ms sample duration equates to 500 samples. Shift focus to a different cell in the table to see this reported below.

This completes epoch A. Now we configure the step, in epoch B.





Click in the Type row in column B (currently set to “Off”).

Select “Step” from the popup menu.

Axoclamp Protocols 10

Set the level for the first sweep at –100 mV.

For this epoch, because we want an incrementing step level from sweep to sweep, we enter a delta level. Click on the Delta level cell and type in 20. This forces the step level up 20 mV with each successive sweep.

We have 10 sweeps starting at –100 mV, so the final sweep will have a step level of 80 mV, reported below the table.

Now set the duration, at 100 ms. Again, this is reported below the table, in milliseconds as well as in samples.

We will not set a delta duration, which would alter the length of the epoch from sweep to sweep, so this completes our waveform definition.

Epoch Description	A	B	C	D
Type	Step	Step	Off	Off
Sample rate	Fast	Fast	Fast	Fast
First level (mV)	-50	-100	0	0
Delta level (mV)	0	20	0	0
First duration (ms)	50	100	0	0
Delta duration (ms)	0	0	0	0
Digital bit pattern (#3-0)	1111	0000	0000	0000
Digital bit pattern (#7-4)	0000	0000	0000	0000
Train rate (Hz)	0	0	0	0
Pulse width (ms)	0	0	0	0

Number of sweeps = 10 Alloca

Stimulus File...

Final level 80.00 mV
First duration 100.00 ms (1000 samples)

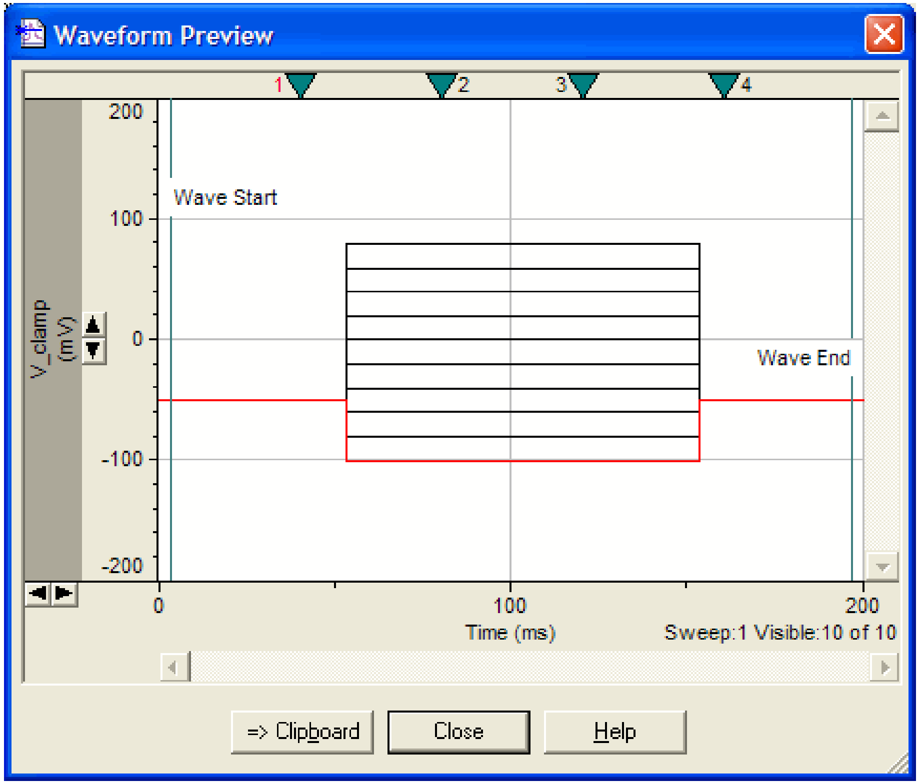
MOLECULAR DEVICES

Axoclamp Protocols 11

Press the Update Preview button in the bottom right corner of the protocol editor.

This opens the Waveform Preview window shown at right, where you can see a graphical representation of the waveform we have defined.

This window can be kept open while you experiment with different epoch settings—press the Update button whenever you want to update the display.

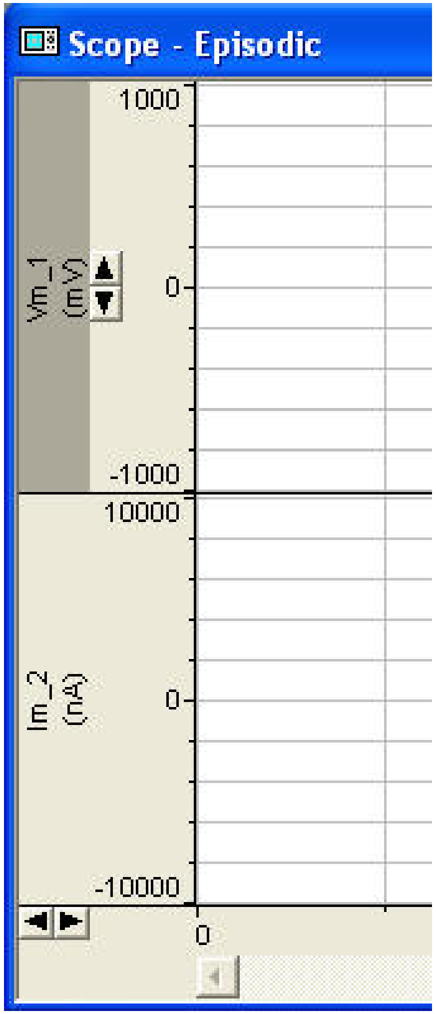


Note: The Waveform Preview opens with panes for all analog output channels—right-click in the upper pane and select Maximize Signal from the popup menu to get the display shown above.

Axoclamp Protocols 12

Finally, close the protocol editor by clicking OK.

You will see on exit that the Scope window is set up in preparation for the two input signals configured for this protocol, with units as currently telegraphed from the Axoclamp 900A. Here the Axoclamp has been put into TEVC mode.

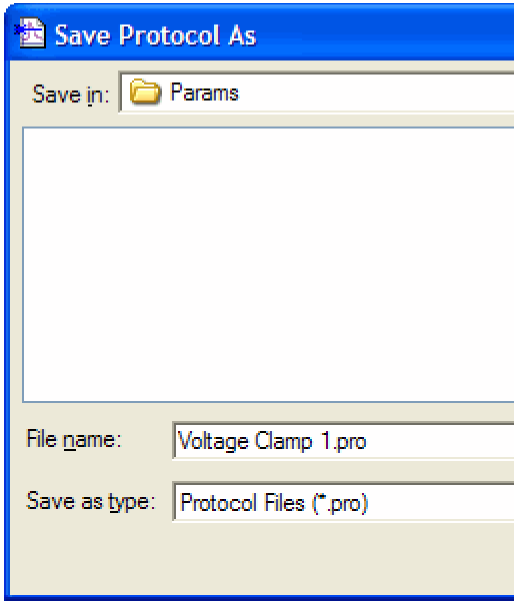


Axoclamp Protocols 13

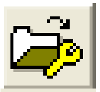
We have completed the setup of the voltage clamp protocol—close the protocol editor with the OK button.

The new protocol is loaded, still labeled "(untitled)", and we could acquire data under it if we wanted, but it is not saved for future use.

Go to Save Protocol As in the Acquire menu. This opens a standard file-saving dialog. Name the protocol "Voltage Clamp 1", and press the Save button.



The protocol is now saved and can be loaded whenever we want, with the Open Protocol command in the Acquire menu, or toolbutton:



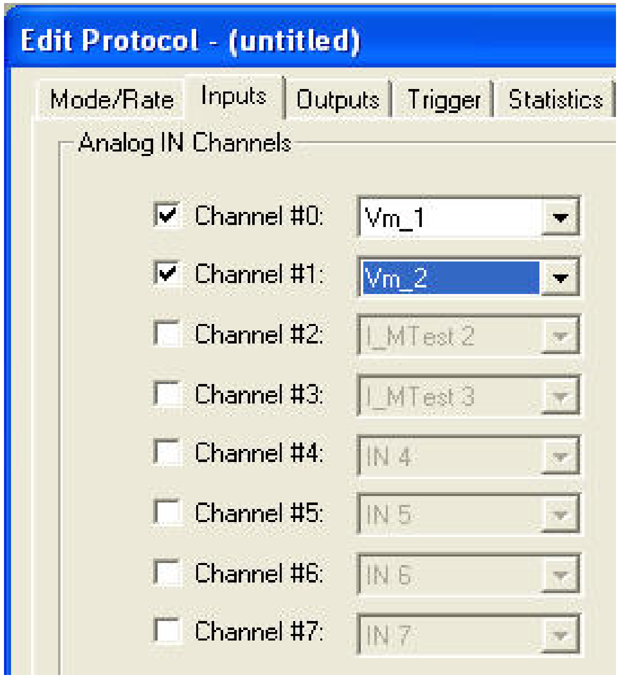
Axoclamp Protocols 14

Setup of the current clamp protocol follows similar lines to the protocol for voltage clamp.

Open the protocol editor again with New Protocol, in the Acquire menu.

We will again accept the default settings in the Mode/Rate tab, so go straight to the Inputs tab.

This time select "Vm_1" for Channel #0, and "Vm_2" for Channel #1.

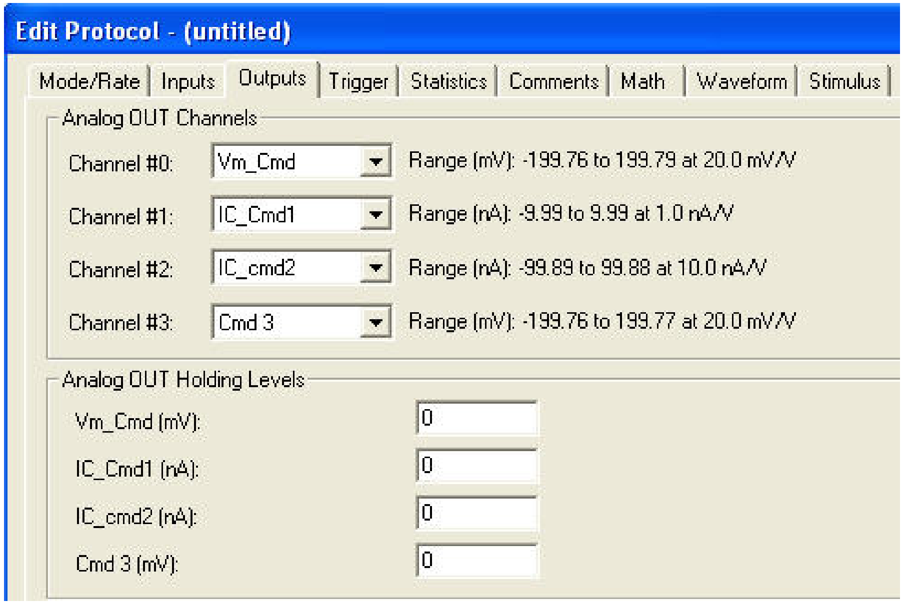


Note: Be sure both channels of the Axoclamp are in IC Mode to see the correct units when you configure the waveform.

Axoclamp Protocols 15

On the Outputs tab, select the current -clamp command signal we configured for digitizer Analog OUT #1: “IC_Cmd1”. Similarly, select “IC_Cmd2” on Analog OUT #2.*

Leave the holding level at the default zero setting for current clamp.



****Note:*** This applies only for the Digidata 1440A with four analog outputs. With a Digidata 1320-series, you may configure OUT #0 for VC, and STEP-ACTIVATE to apply command steps in current clamp; see slide Axoclamp Connections 4.

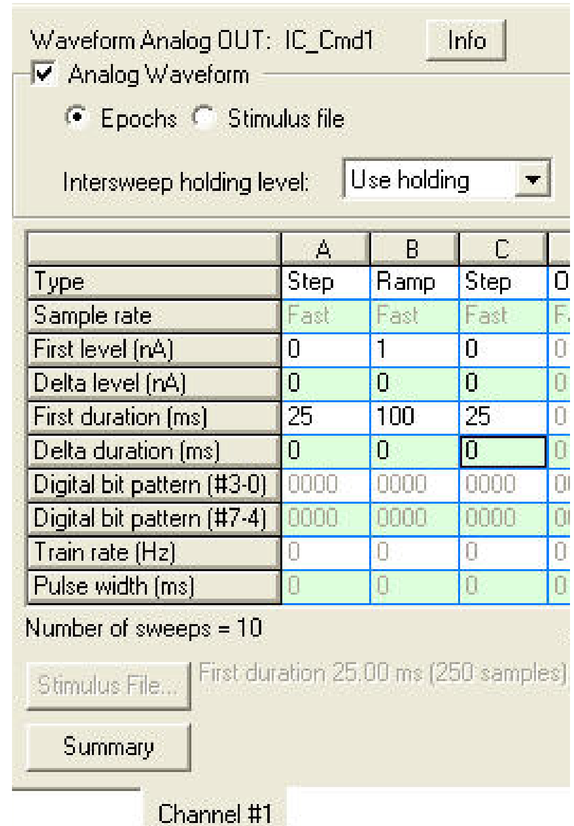
For details on using the digital outputs to trigger STEP-ACTIVATE, see Axoclamp Protocols 18.

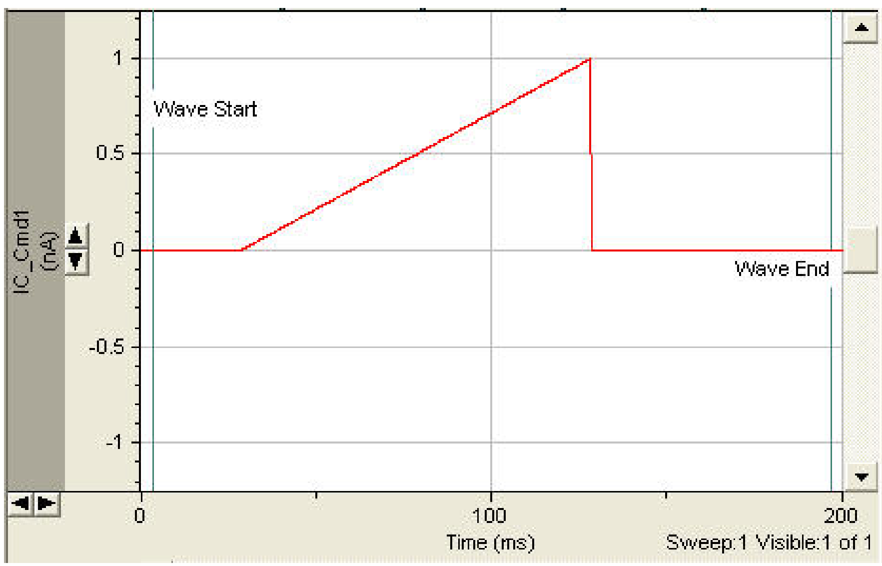
If you do not plan to use a voltage clamp mode, you may of course use OUT #0 and OUT #1 for the two current commands.

Axoclamp Protocols 16

Create your own command waveform on the Channel #1 tab, in this example.

Experiment with the different waveform options, and display them in the Waveform Preview.

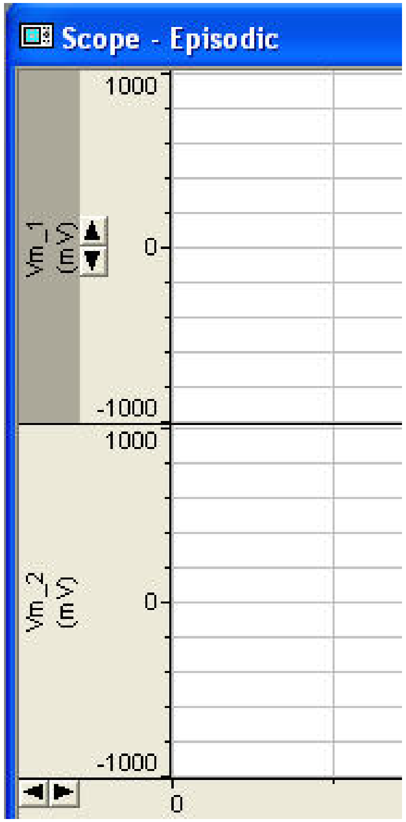




Axoclamp Protocols 17

Finally, close the protocol editor by pressing OK, and save the protocol (Save Protocol As in the Acquire menu), calling it "Current Clamp 1".

You will see on exit that the Scope window is set up in preparation for the two input signals configured for this protocol, with units as currently telegraphed from Axoclamp.



Finished!

This completes our two protocols, "Voltage Clamp 1" and "Current Clamp 1", and is the completion of the guide for Axoclamp 900A setup.

Axoclamp Protocols 18

Alternative configuration using STEP-ACTIVATE command inputs

The Digidata 1320-series has two analog outputs, and therefore cannot output three analog waveforms– one for VC, and two for IC.

In this case, it is appropriate to use the STEP-ACTIVATE inputs on the rear of the Axoclamp 900A. There is one STEP-ACTIVATE input for each of the Axoclamp channels.

In current clamp, when a “digital” signal– a voltage of 5 V, sometimes called “high”– is applied to the STEP-ACTIVATE input, the Axoclamp 900A applies a command current step to the appropriate headstage.

The magnitude of the step is set in the Axoclamp 900A Commander. It is the same as the Tuning pulse for the channel.

The frequency setting is ignored. The current command is applied for as long as the input to STEP-ACTIVATE remains at 5 V.

Now we will make a protocol to use the STEP-ACTIVATE to apply a series of steps.

Axoclamp Protocols 19

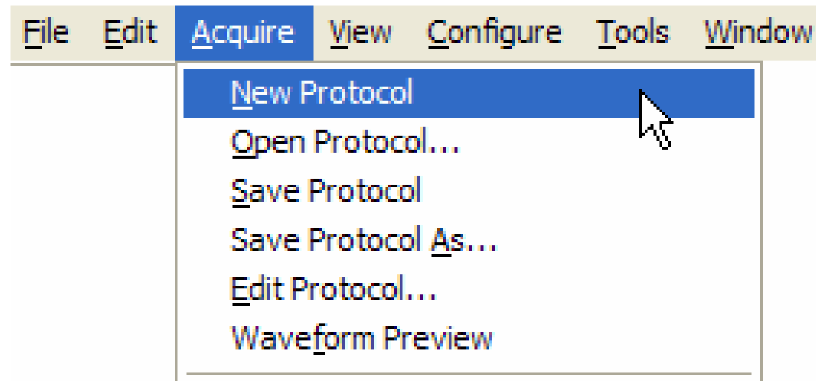
Alternative configuration using STEP-ACTIVATE command inputs

For this example we assume—

•The STEP-ACTIVATE inputs will be used to generate commands in current clamp

•If a voltage clamp mode is to be used, its command waveform is configured on Analog OUT #0

Open a new protocol



Note: If a previously saved protocol is not loaded in Clampex, it uses a place-holder protocol, labeled "(untitled)". If this is currently loaded you can open the editor to create a new protocol by selecting Edit Protocol, or by clicking the toolbutton:

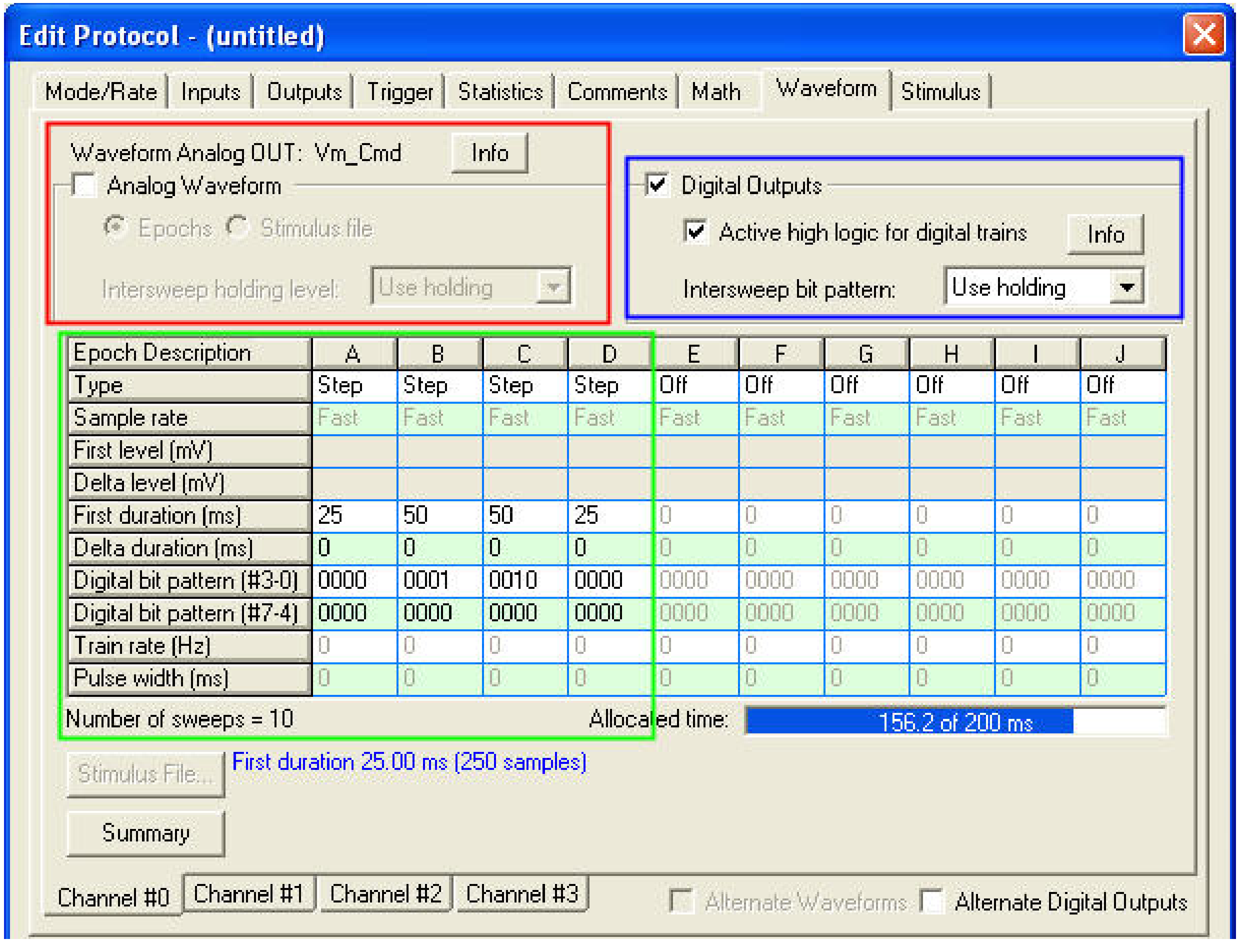
We will accept the defaults on the Mode/Rate panel. Proceed to the waveform tab

Axoclamp Protocols 20

Alternative configuration using STEP-ACTIVATE command inputs

These are the waveform settings for the use of STEP-ACTIVATE commands.

Each boxed section will be explained individually in the following slides.



Axoclamp Protocols 21

Alternative configuration using STEP-ACTIVATE command inputs

In this area you can see that the Analog OUT signal is Vm_Cmd. However, it is deactivated, because this protocol will use only the digital outputs to trigger STEP-ACTIVATE inputs.

De-activating the analog output waveform helps avoid confusion about the purpose of this protocol

This section activates the digital outputs, so that waveforms (or “bit patterns”) will be produced.

Note that the digital outputs can only be activated on one of the four analog waveform tabs in a particular protocol.

The “Active high logic for digital trains” refers to a feature we are not using at this time. Press Info for details.

Intersweep bit pattern indicates what happens on the digital outputs between episodic sweeps.

Axoclamp Protocols 22

Alternative configuration using STEP-ACTIVATE command inputs

As with analog waveforms, the digital output waveform is designed in the waveform table.

Duration entries function in the same manner as with analog output

Epoch Description	A	B	C	D
Type	Step	Step	Step	Step
Sample rate	Fast	Fast	Fast	Fast
First level (mV)				
Delta level (mV)				
First duration (ms)	25	50	50	25
Delta duration (ms)	0	0	0	0
Digital bit pattern (#3-0)	0000	0001	0010	0000
Digital bit pattern (#7-4)	0000	0000	0000	0000
Train rate (Hz)	0	0	0	0
Pulse width (ms)	0	0	0	0

Number of sweeps = 10 Allocat

Notice that the First level and Delta level are inactive– because we have turned off analog output on this channel

The Digital bit pattern entries define the digital waveform. They are described in detail on the following slide.

Axoclamp Protocols 23

Alternative configuration using STEP-ACTIVATE command inputs

Digital bit pattern entries define the digital waveform. Study the example shown below, and the resulting waveform.

•The digital outputs on a Digidata digitizer are labeled 0 to 7.

•The first digital bit pattern row– "Digital bit pattern (#3-0)"-- controls digital outputs 3, 2, 1 , and 0. The pattern is written in that order.

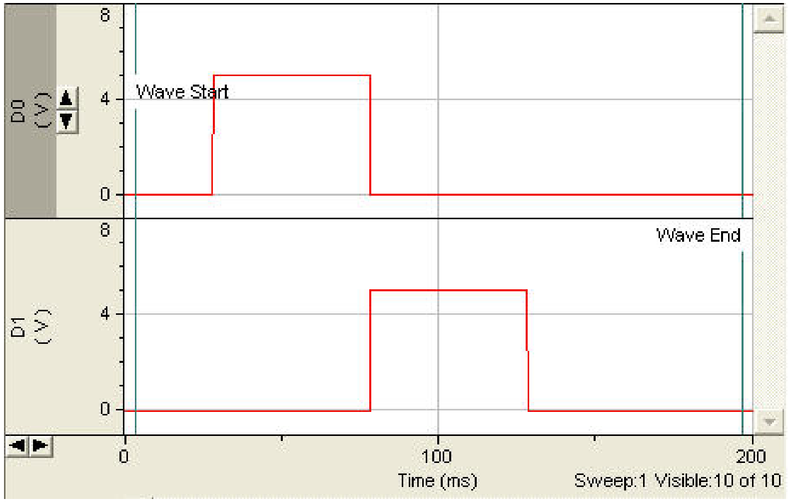
•The second digital bit pattern row– "Digital bit pattern (#7-4)"-- controls digital outputs 7, 6, 5 , and 4. The pattern is written in that order.

•A bit pattern consists of four digits, which can each be "0" or "1". "0" turns the corresponding digital output OFF (or "low"). "1" turns the corresponding digital output ON (or "high"= 5 V).

•The bit stays in the indicated state (1 or 0) for the length of the Epoch

Epoch Description	A	B	C	D
Type	Step	Step	Step	Step
Sample rate	Fast	Fast	Fast	Fast
First level (mV)				
Delta level (mV)				
First duration (ms)	25	50	50	25
Delta duration (ms)	0	0	0	0
Digital bit pattern (#3-0)	0000	0001	0010	0000
Digital bit pattern (#7-4)	0000	0000	0000	0000
Train rate (Hz)	0	0	0	0
Pulse width (ms)	0	0	0	0

Number of sweeps = 10 Allocat



Axoclamp Protocols 24

Alternative configuration using STEP-ACTIVATE command inputs

Epoch Description	A	B	C	D	E	F	G	H	I	J
Type	Step	Step	Step	Off	Off	Off	Off	Off	Off	Off
Sample rate	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast
First level (mV)										
Delta level (mV)										
First duration (ms)	25	100	25	0	0	0	0	0	0	0
Delta duration (ms)	0	0	0	0	0	0	0	0	0	0
Digital bit pattern (#3-0)	0000	001*	0000	0000	0000	0000	0000	0000	0000	0000
Digital bit pattern (#7-4)	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
Train rate (Hz)	0	100	0	0	0	0	0	0	0	0
Pulse width (ms)	0	5	0	0	0	0	0	0	0	0

Number of sweeps = 10 Allocated time: 156.2 of 200 ms

Stimulus File... First duration 100.00 ms (1000 samples)

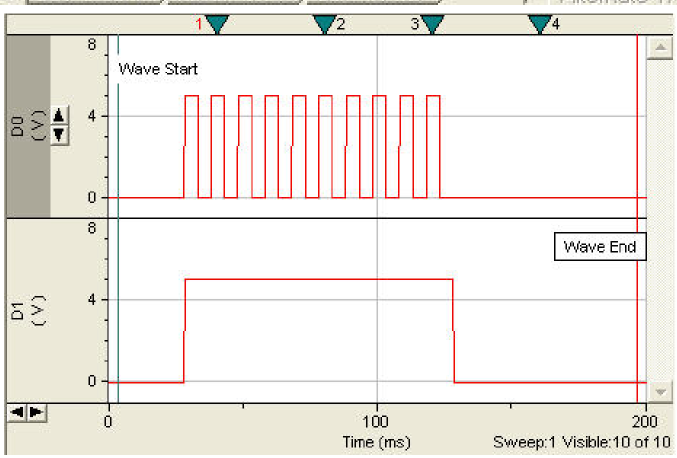
Pulse count 10
Train rate 100.00 Hz (100 samples)
Pulse width 5.00 ms => 50 % (50 samples)

Summary

Channel #0 Channel #1 Channel #2 Channel #3 Alternate Waveforms Alternate Digital Output

Digital bit patterns can also be configured as pulse trains.

- To activate a train on a particular digital output, set its corresponding bit to “*”. Here bit #0 (digital OUT #0) is set to “*”.
- Enter the train rate and the width of each pulse. Clampex calculates the number of pulses resulting.



The Waveform Preview shows the result. Here a train is output from digital OUT #0, which digital OUT #1 is HIGH for the duration of the epoch.

Applied to STEP-ACTIVATE, this would cause the Axoclamp to apply a train of pulses to the headstage.

MOLECULAR DEVICES

Axoclamp Protocols 25

Alternative configuration using STEP-ACTIVATE command inputs

We have now designed a protocol to use the STEP-ACTIVATE inputs to apply pulses to each headstage in current clamp.

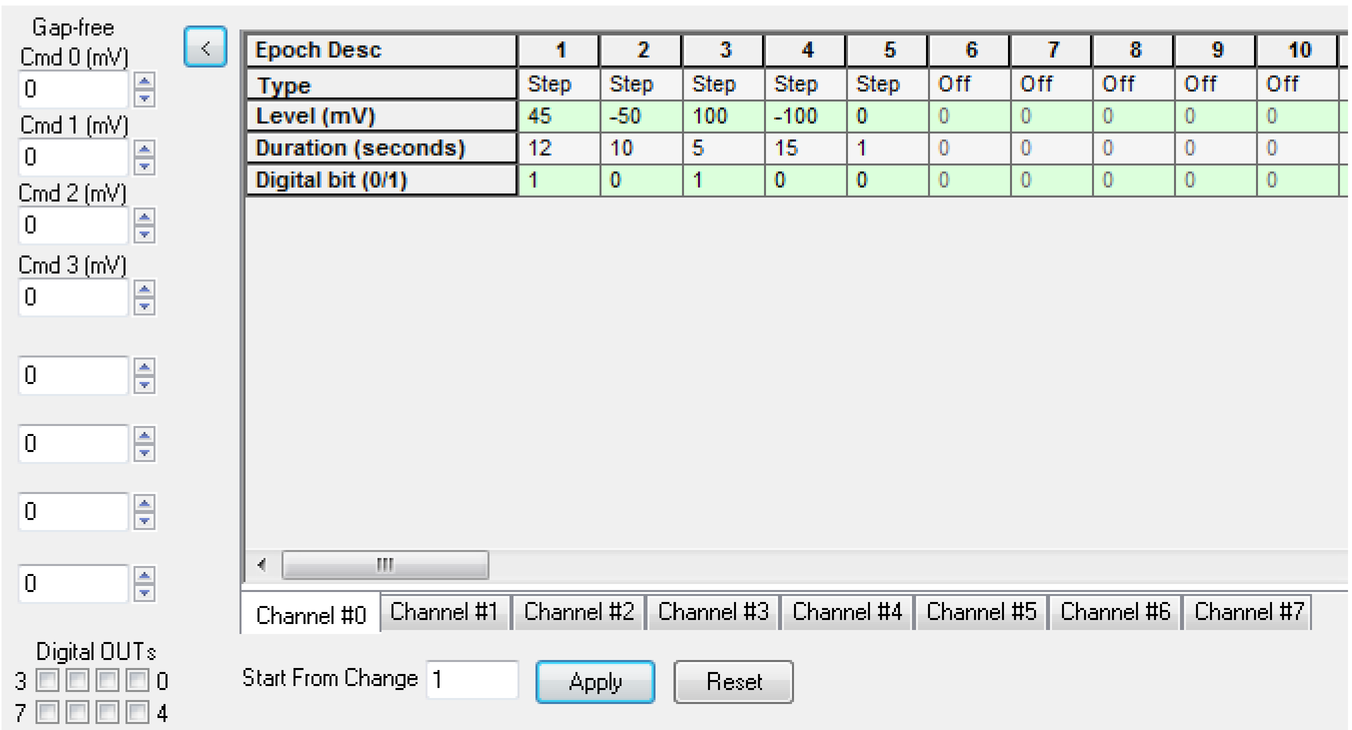
Remember, the amplitude of the applied step is determined by the Tuning amplitude on the Axoclamp 900A Commander. Therefore it cannot be changed from Clampex.

Since the Axoclamp 900A cannot be in both a voltage clamp mode and current clamp at the same time, the analog waveform for voltage clamp commands is disabled.

Note: STEP-ACTIVATE **1** may also be used to apply command steps while in voltage clamp. The basic approach is the same, except that the amplitude of the applied pulse is set with the Seal Test feature on the Axoclamp 900A Commander.

Axoclamp Protocols 26

When you use Gap-free mode in the Real Time Controls panel, open the pre-programming dialog by clicking the **<** button. You can pre-program voltage level and holding duration values for each channel, as well as turning the digital bit on or off. You can pre-program up to 50 epochs. You can also manually change values during a recording.



Finish

This completes the protocol tutorial and completes this guide.

Keep Your Software Up to Date

At Molecular Devices, we aim to make improvements to released software frequently, and to fix reported problems quickly.

pCLAMP 11 and Axoclamp Commander 900A are both regularly updated. The programs have internal settings to remind you to check our website for updates. Alternatively, you may force the program to update.

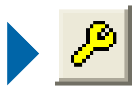
•To update **pCLAMP 11**, open Clampex, and go to the Help menu and select “About Clampex”. Note the complete version number, and close the window.

•Return to the Help menu and select “Molecular Devices on the Web”. This should launch a browser and open the Molecular Devices homepage. Navigate to *Service > Knowledge Base > Conventional Electrophysiology / Downloads > pCLAMP 11*, if the version number on the web page is later than yours, download and install the update.

•To update **MultiClamp 700B Commander** or **Axoclamp 900A Commander**, go to the *Options > About* dialog

•Remember the full version number displayed

•Go to *www.moldev.com /Service/ Knowledge Base/Conventional Electrophysiology/Downloads/MultiClamp* to check if a newer version is available.



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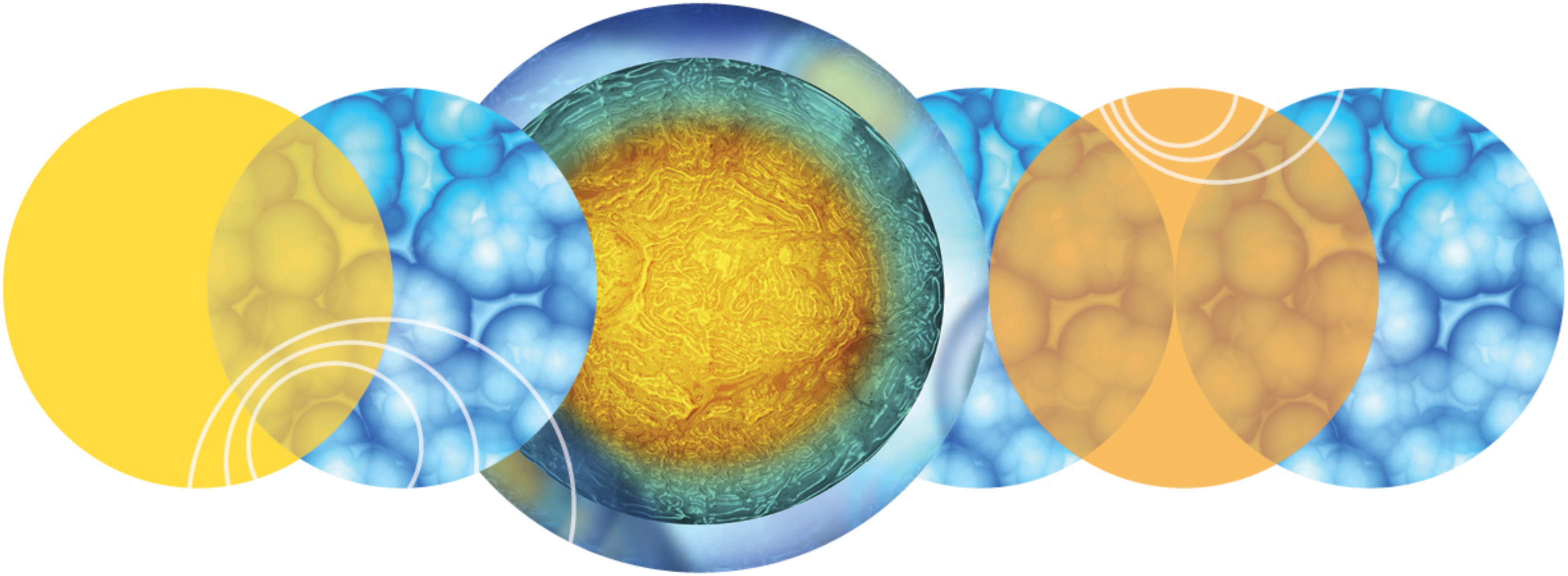
Technical Support – including a searchable knowledge base and a link for finding regional technical support:
http://www.moleculardevices.com/support.html

Web site:
http://www.moleculardevices.com/



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