

IPA[®] / DOUBLE IPA[®]

Integrated Patch Amplifier

ELECTROPHYSIOLOGY
PATCH-CLAMP SYSTEM
WITH
SutterPatch[®] SOFTWARE

Operation Manual



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The IPA and Double IPA systems have two international safety certifications:

- 1) The CE mark is for compliance to health, safety and environmental protection standards for products sold within the European Economic Area:



- 2) The RoHS (Restriction of Hazardous Substances) Directive 2002/95/EC restricts the use of hazardous substances for electronic equipment sold within the European Union:



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IPA®, Double IPA® and SutterPatch® are trademarks of Sutter Instrument Company. All other product and company names are trademarks or registered trademarks of their respective holders.

DISCLAIMER

The **IPA** system consists of one electronic amplifier with integrated digitizer and one headstage. The **Double IPA** system consists of one electronic amplifier with integrated digitizer and two headstages. All references to an IPA system also include a Double IPA system, unless otherwise noted. The purpose of the system is for the stimulation and measurement of cellular preparations. No other use is recommended.

This instrument is designed for use in a laboratory environment. It is not intended for, nor should it be used in human experimentation or applied to humans in any way. This is not a medical device.

Do not open or attempt to repair the instrument.

Do not allow an unauthorized and/or untrained operative to use this instrument.

Any misuse will be the sole responsibility of the user/owner, and Sutter Instrument Company assumes no implied or inferred liability for direct or consequential damages from this instrument if it is operated or used in any way other than for which it is designed.

SAFETY WARNINGS AND PRECAUTIONS

Electrical

- **Operate the IPA system using 100 – 240 VAC, 50 - 60 Hz line voltage. This instrument is designed for use in a laboratory environment that has low electromagnetic noise and mechanical vibration. Surge suppression is recommended at all times.**



Fuse Replacement: Replace only with the same type and rating:

Line Voltage: 100 – 240 VAC	
Fuse Rating	Manufacturer Examples
	RoHS Compliant (Lead Free)
T2.0, 250V	Bussmann: GMC-2-R, S506-2A Littelfuse: 239.002.P



IPA & DIPA Fuses

Type: 5 x 20 mm glass tube, Medium Time Delay (Slow Blow), RoHS compliant.

Rating: T2.0A 250V (Time Delay, 2 Amps, 250 Volts)



Examples: Bussmann: GMC-2-R, S506-2A
Littelfuse: 239.002.P

- **Avoiding Electrical Shock and Fire-related Injury**

-  Always use the grounded power cord provided to connect the system's power adapter to a grounded/earthed mains outlet. This is required to protect you from injury in the event that an electrical hazard occurs.
- Do not disassemble the system. Refer servicing to qualified personnel.
-  To prevent fire or shock hazard, do not expose the unit to rain or moisture.

Operational


Failure to comply with any of the following precautions may damage this instrument.

- This instrument is designed for operation in a laboratory environment (Pollution Degree I) that is free from mechanical vibrations, electrical noise and transients.
- Operate this instrument only according to the instructions included in this manual.
-  Do not operate this instrument near flammable materials. The use of any hazardous materials with this instrument is not recommended and, if undertaken, is done so at the users' own risk.
-  Do not operate if there is any obvious damage to any part of the instrument.

Other

- Retain the original packaging for future transport of the instrument.
- Sutter Instrument Company reserves the right to change specifications without prior notice.
- Use of this instrument is for research purposes only.

Handling Micropipettes

-  Failure to comply with any of the following precautions may result in injury to the users of this instrument as well as those working in the general area near the instrument.
- The micropipettes used with this instrument are very sharp and relatively fragile. Avoid contact with micropipette tips to prevent accidentally impaling yourself.

- Always dispose of micropipettes by placing them into a well-marked, spill-proof “sharps” container.

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1. INTRODUCTION

Welcome to a new era in patch-clamp technology! Our passion is creating the finest available electrophysiology research instrumentation. With over two centuries of combined experience from across the patch-clamp industry, our expert team has designed new microelectrode amplifier-recording systems that are powerful enough to satisfy experienced patch-clampers, yet easy-to-use for recent entrants.

Sutter Instrument Company is a leading manufacturer of innovative precision instrumentation in the neuroscience field. We have a worldwide reputation for the highest quality and performance of pipette pullers, micromanipulators, light sources and wavelength switchers. We are proud to apply this same commitment of excellence to the next generation of patch-clamp instrumentation.

1.1 Overview

Advanced Design

The IPA® Integrated Patch Amplifier is the world's first fully integrated microelectrode patch-clamp system, which facilitates and streamlines your experimental setup. All of the electronics (amplifier and digitizer) used in stimulating, compensating and recording from cells are integrated by design into a single printed circuit board (the Double IPA adds a second PCB).

The accompanying SutterPatch® software brings the controls and displays for full-featured data acquisition, data analysis, and graphics/layout together into a single, unified program, including a software control panel for direct access to all of the IPA amplifier functions.

The SutterPatch software was developed in the powerful Igor Pro system environment. Igor Pro, by WaveMetrics, Inc., is a data collection, management and analysis platform with a rich set of built-in functions and routines for scientific programs.

From concept to production, from hardware to software, these fully integrated systems' immediate benefits are leading-edge patch-clamp systems that are affordable, and easy-to-setup and use.

1.2 Software Highlights

- Full-featured electrophysiology package
- Single program for data acquisition, analysis and hardware control
- Complex experimental automation
- Publication-quality graphics

Convenient: All SutterPatch software is run by a single application. No need to launch multiple programs or to move data between programs.

- Comprehensive:** All data recordings, analyses, graphs, layouts, configurations and controls are saved in a single experiment file. This ensures that data are kept together with their complete contexts.
- Automation:** Automate your experiment using a rich set of data acquisition, data analysis, and amplifier controls. Create complex “Paradigms” that can respond to changing conditions via conditional steps and loops.
- Note:** Figures and examples in this manual may be from either an IPA or Double IPA system.

1.3 Experiment Structure

Experiment:

An Experiment is the highest-level structure in the SutterPatch world. An Experiment file can encompass all SutterPatch activity for the entire day, such as instructions (Paradigms), data acquisition parameters (Routines), recorded data (Series), execution settings, history, and comments. During reanalysis, data can be included from multiple experiments. Typically, one Experiment is created for each cell or preparation recorded from.

Paradigm:

A Paradigm is a sequence of control instructions used in an Experiment. Every Experiment contains at least one Paradigm, whether pre-planned by the user or automatically created by the system.

A loaded Paradigm “pool” file can contain multiple Paradigms for rapid access and execution. Such “planned” Paradigms can contain simple sequences, or sophisticated control structures, using a rich set of operations, such as conditional “If-then” decisions, nested loops, user-defined variables, hardware commands, and data acquisition Routines.

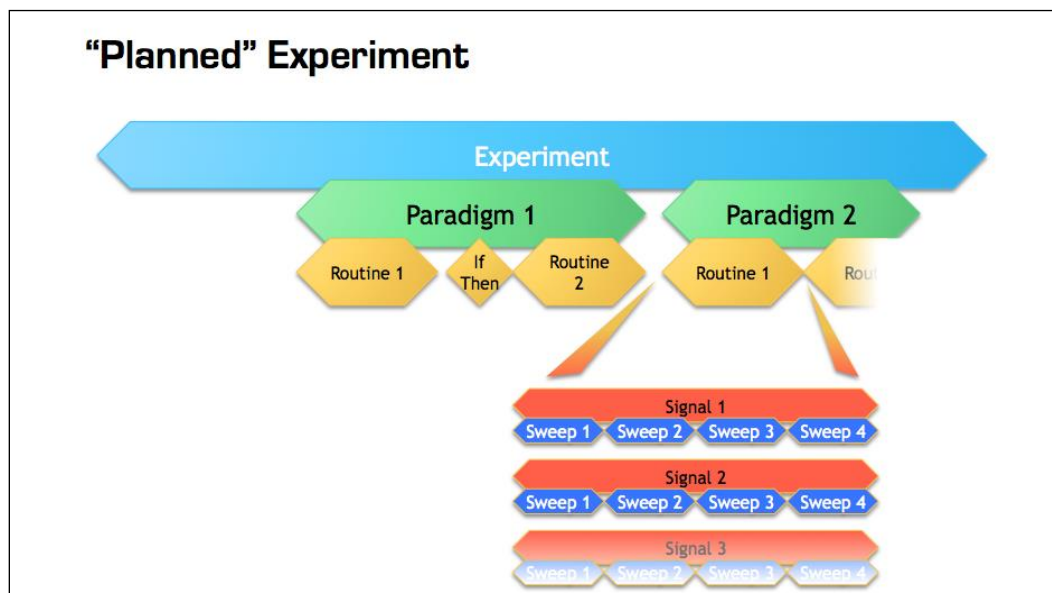


Figure 1-1. Data Structure - Planned Paradigms

An Experiment with two “planned” Paradigms running Routines.

However, if a Routine is manually run in the Scope window, an “auto-triggered” Paradigm is created as a container. This default Paradigm ensures that each Series is associated with a Paradigm in the context of an Experiment. If an auto-triggered Paradigm is already the active Paradigm, it is used for subsequent manually-run Routines.

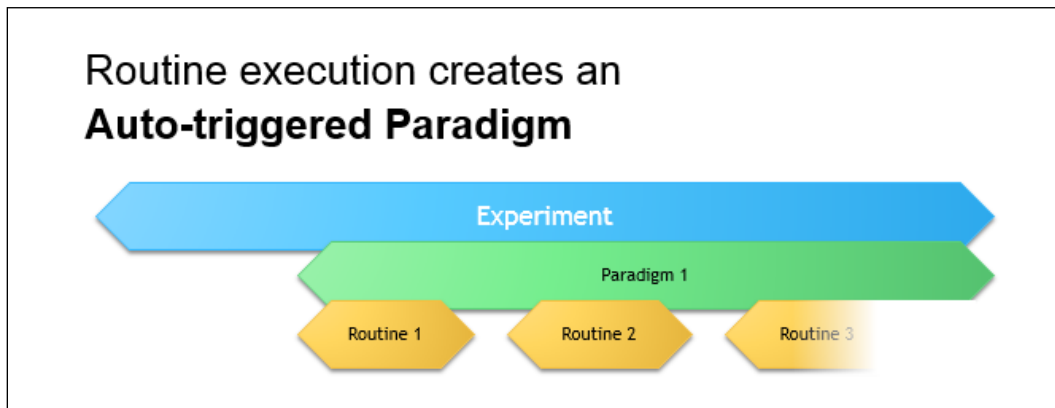


Figure 1-2. Data Structure - Auto-triggered Paradigms

An Experiment with manually run Routines using an “auto-triggered” Paradigm.

A Paradigm’s “data” includes all data points, variable values, and metadata tags from the course of a Paradigm. Altogether, this allows reconstruction of the exact course of an experiment. While a Paradigm could be compared to an itinerary, the Paradigm data correspond to the route a journey actually took. If conditional control is used in a Paradigm, e.g., for the number of loop cycles or a decision in an “If-then” step, these actions are recorded in the Paradigm data, even though they are not predetermined. (See the Data Browser.)

Routine:

A Routine is the set of data acquisition and online data analysis parameters that control input and output channel timing, triggering, command waveforms, display and real-time analysis.

A loaded Routine “pool” file can contain multiple Routines for rapid access and execution.

Series (Routine Data):

Recording Routine data creates a Series composed of all sweeps of data from all input signals. Multiple runs of a Routine create multiple Series of data. All Series are automatically stored in the current Experiment file.

Channel:

A Channel corresponds to a physical output (D/A) or a physical or virtual input (A/D) of the IPA system.

Analog input channels are used to record data, and are displayed in their own panes in the Scope window. There are two dedicated internal analog input channels ‘(Current’ and ‘Voltage’) for each attached headstage. General-purpose Auxiliary analog input channels (‘Aux-IN’) allow recording from external instruments. Virtual input channels allow creative processing of the physical input channels.

Analog output channels are used to send electrical stimuli, such as analog command waveforms to the preparation. There is a dedicated, internally configured, analog output channel ('StimOUT') for each attached headstage. General-purpose Auxiliary analog output channels ('AuxOUT') can send output signals to external instruments.

Digital Output bits are also referred to as digital output channels ('DigOUT').

All 'Aux' and 'DigOUT' channels are available via the included BNC "octopus" breakout cable or the optional Patch Panel rack mount panel.

Signal:

Named analog input and output channels are referred to as Signals. A Signal is either the scaled representation of a physical channel, or the virtual result of a computation.

Sweep:

A Sweep is the sum off all data points from all Signals, acquired for a single period from time zero, for a fixed duration. In SutterPatch Software, the Sweep Duration is determined by the duration of the command waveform.

Trace:

A Trace is a Sweep applied to a single Signal. Therefore, a Sweep can be described as the collection of Traces across all Signals.

Segment:

A Segment exists as a user-defined section of the command waveform. Each Segment has a waveform type, an amplitude, and a duration. A command waveform can be composed of up to 50 Segments.

Metadata:

Metadata are additional information associated with stored data. These can include information about the preparation (cell, tissue, animal), instrumentation (hardware, software), environmental parameters (temperature, atmospheric composition, etc.), stimuli (chemical compounds, light, acoustic, etc.) and other parameters. Metadata information is associated with the running of Paradigms and Routines, and their resulting data.

Metadata are dynamically recorded with a timestamp during an experiment. Information that can be determined by the system, such as the connected hardware, SutterPatch version, user Login Name, or the change of a digital output level, are automatically recorded without user intervention. In addition, the user can enter values for a large number of user-defined Metadata parameters, such as identifiers for the experimental animal or cell, the animal species, age and genotype, information about the recording solutions, and the electrodes or stimuli applied during the experiment. SutterPatch currently keeps track of ~ 600 Metadata parameters.

Terminology Comparison:

A table of equivalent terms to other electrophysiology software packages:

SutterPatch	PATCHMASTER	pCLAMP
--------------------	--------------------	---------------

Experiment	Compound Data	N/A
Paradigm	Protocol	Sequencing Keys
Routine	PGF Sequence	Protocol
Series	Series	Trial
Sweep	Sweep	Sweep
Signal	Signal	Signal
Trace	Trace	Trace
Segment	Segment	Epoch

Table 1-1. Software Terminology

2. INSTALLATION

2.1 Computer Requirements

Minimum Requirements

Operating System:	Windows:	7 or later (64-bit versions)
		Most language packs are compatible. (listed in OS: Control Panel > System)
	macOS:	10.11 (El Capitan) or later. (listed in OS: Apple > About this Mac)

CPU (Central Processing Unit): Dual-core

RAM (Random Access Memory): 3 GB

Hard Disk Free Space: 500 MB

Display Resolution: 1024 x 768 (XGA)

Computer Ports: (1) USB 2.0 High Speed port

Note: USB 2.0 computer ports are usually implemented with a 'High Speed' transfer rate, but a slower 'Full Speed' specification can sometimes be found on old computers or USB add-in cards.

To check for High Speed USB 2.0 ports on a PC computer running Windows, look in the Control Panel / Device Manager / Universal Serial Bus controller section for "Enhanced" host controllers.

As this does not provide any USB version information for the computer's USB ports, you might need to test the physical USB ports for operational performance.

Note: USB hubs are not supported.

Recommended Specifications

CPU (Central Processing Unit): Dual-core i5

RAM (Random Access Memory): 8 GB

SSD (Solid State Drive): 500 GB

Display Resolution: 1920 x 1080 (Full HD)

Note: High resolution (> 96-DPI) displays are not supported, such as Retina, 4K, 5K, Quad-HD, and Ultra-HD.

2.2 SutterPatch System Environment

The SutterPatch software runs in the Igor Pro 8 system environment. Igor Pro is widely used by scientists to acquire and analyze data, and to create publication-quality presentation graphics.

Igor Pro Features

- High-speed data display
- Large data set handling
- Waveform arithmetic
- Extensive set of built-in data analyses
- Image display and processing
- High-quality presentation graphics
- Graphical and command-line user interfaces
- Automation
- Extensibility via C and C++ modules
- Extensive online Help and PDF manual

SutterPatch 2.0 requires a 64-bit version of Igor Pro to run. The SutterPatch 2.0 full installer automatically installs Igor Pro 8 (64-bit English version) and SutterPatch 2.0, while the SutterPatch 2.0 Updater only updates SutterPatch.

Igor Pro 8 has a 30-day trial period where it is fully functional and fully supports SutterPatch. After 30-days, if the Igor Pro 8 license has not been activated, Igor Pro 8 runs in a demo mode with limited functionality that does not support the SutterPatch application.

Although Igor Pro 8 (64-bit) is strongly recommended for full support, SutterPatch 2.0 can still be run in Igor Pro 7 (64-bit), but only if the newly introduced 2.0 features (long names) are avoided.

For third-party applications that need a 32-bit version of Igor Pro 8:

- Windows: A 32-bit version of Igor Pro 8 is available in the installer.
 macOS: There is no 32-bit version of Igor Pro 8; run Igor Pro 7.

Note: Japanese versions of Igor Pro are not supported by SutterPatch.

2.3 Mounting Instructions

- Rack Mounting: The IPA amplifier is ready for mounting in a standard 19" wide equipment rack in a 1U (DIPA: 2U) space. A rack mount hardware kit consisting of hex screws, washers and cage nuts is included.
- Benchtop Usage: Attach the four included stick-on feet to the bottom of the IPA amplifier.

2.4 Electrical Connections

Typical AC Power: 60 Hz, 120 V
 50 Hz, 240V

The IPA amplifier can also run on power from 100 to 250 VAC - no switches need be set.

The AC power should be as clean as possible:

- At a minimum, a surge protector should be used to protect against high-voltage spikes; if lightning strikes are a concern, it should be rated > 1000 joules and > 40 kA.
- If you experience brownouts or voltage sags, a switching power supply (SPS) can be used to supply clean power to your instruments.
- To protect against power interruptions, use a universal power supply (UPS) for uninterrupted clean power.

2.5 Install Hardware

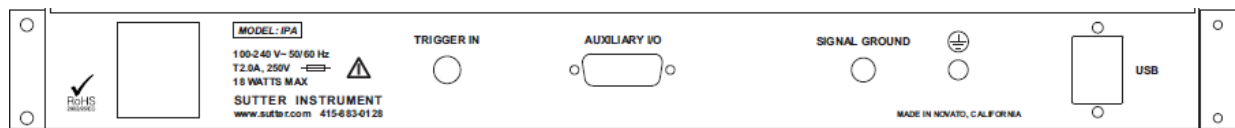


Figure 2-1. Rear of IPA Cabinet

1. Plug the female end of the power cord into the IPA rear-panel power receptacle.
2. Plug the male end of the included power cord into a grounded electrical mains outlet.



Figure 2-2. Front of IPA Cabinet

3. Set the IPA power button OFF (unlit position).



WARNING! Hot-swapping of headstages should be avoided – components can be damaged. Turn off the IPA system power before handling headstages.

4. Plug the IPA headstage into the HEADSTAGE port on the front of the IPA amplifier - the amplifier and headstage serial numbers should match.

For a Double IPA system, attach the lower serial-numbered headstage to HEADSTAGE 1, and the higher serial-numbered headstage to HEADSTAGE 2, as each headstage is individually tuned to its channel.

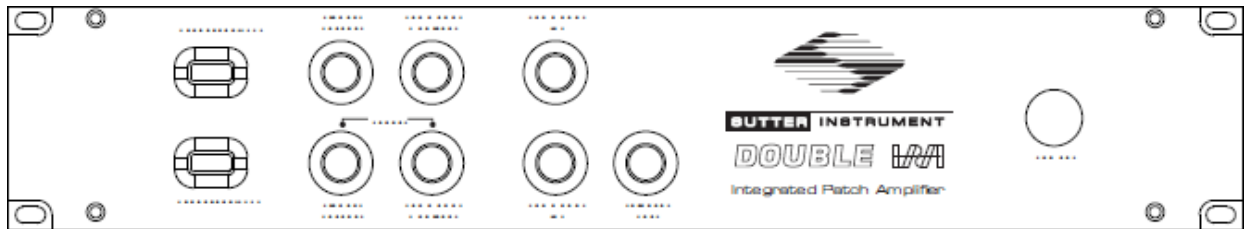


Figure 2-3. Front of Double IPA Cabinet

5. Plug the included I/O “octopus” breakout cable, or the optional Patch Panel cable, into the AUXILIARY I/O port on the back of the IPA amplifier.
6. Connect the supplied USB 2.0 cable to your computer’s USB 2.0 port, and to the IPA amplifier’s rear-panel USB port.
7. Connect the included electrode holder(s) to the headstage(s). See the Holder section for holder assembly instructions.

2.6 Install Software

Power on the computer.

A: It is strongly recommended to use the latest SutterPatch software version available.

Download the latest version of SutterPatch installer software from <https://www.sutter.com/AMPLIFIERS/SutterPatch.html> and choose the “Download” tab. Navigate your file browser to the download file and run it.

- B: If internet access is not available, attach the included USB flash drive to your computer USB port, and navigate to the flash drive.
1. Install the software for ‘All Users’ by double-clicking on:
 - Windows: SutterPatch_installer_with_Igor
 - Macintosh: sutterpatch_mac_full
 2. Follow the installer prompts:
 - If an existing version of Igor Pro 7 is found, it is recommended to replace it with Igor Pro 8. Make sure to keep a backup copy of all user files and parameters you may have placed into the program folder and its sub-folders.
 - However, older versions of Igor Pro can also be kept, as different versions of Igor Pro can coexist on the same computer.
 - If an existing version of Igor Pro 8 is found, SutterPatch sample files are overwritten.
 - The new install uses the Preferences from prior SutterPatch installations.
 3. Upon completion, the installer will report a successful installation. The following files and folders are installed:
 - IPA QuickStart Guide (PDF) (Includes Igor Pro 8 Serial Number and Key.)
 - Release Notes (PDF)
 - SutterPatch manual (PDF)
 - SutterPatch folder (Includes sample Routine Pool, Paradigm Pool, and Experiment data files).
 - SP_Code folder
 - SP_Driver folder (XOPs)
 4. Launch Igor Pro 8 and activate its license as instructed. You will need to enter the Igor Pro 8 Serial Number and Activation Key found in your IPA Quick Start Guide.
 5. “Eject” the flash drive - wait for the “Safe to Remove Hardware” prompt, and unplug it from the computer.

2.7 Test System

2.7.1 Install Model Cell

1. Attach the model cell to Headstage 1 and tighten the screw collar.
2. Plug the supplied 1 mm grounding wire into the gold sockets on the headstage and model cell.
3. If the headstage is not inside a Faraday cage, completely surround the model cell/headstage assembly with electromagnetic shielding, such as aluminum foil, and

connect the shielding material to the headstage ground wire or connector - a short wire with alligator clips on both ends makes a convenient shield-ground wire.

2.7.2 Startup

1. Power-on the IPA amplifier by pressing the silver POWER button on its front – it lights up as blue. (It can take a few seconds for the USB connection to be established.)
2. Launch the Igor Pro (SutterPatch) application by clicking on the 'Igor Pro 8' icon.

A SutterPatch splash screen displays while opening files.



Figure 2-4. Splash Screen

Then the Welcome to SutterPatch “start” window displays.



Figure 2-5. Welcome Screen

3. Click on the 'Start' button in the 'Welcome to SutterPatch' window, and the application begins compiling. This process may take several seconds.
4. Specify the experiment file name and storage location when prompted.
5. If the IPA amplifier is OFF or disconnected from the computer.
 - a. Reconnect it, and then click 'Retry', or...

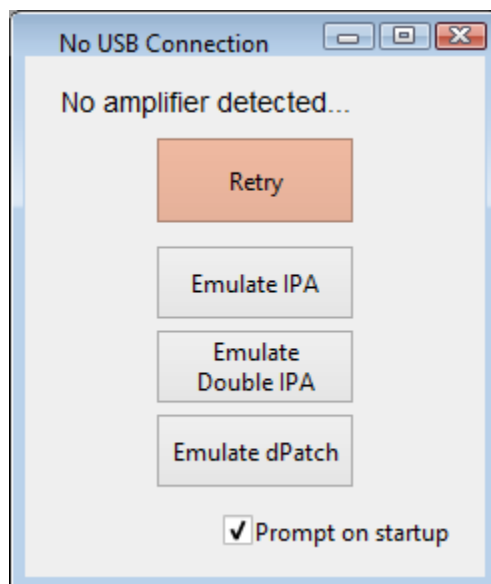


Figure 2-6. Emulation Modes

- b. Select a hardware emulation mode (IPA, Double IPA or dPatch amplifier), or
 - c. If 'Prompt on startup' is not enabled, and no headstages are attached, the software will be automatically configured to the last known state of the emulation mode.
6. The Dashboard panel is displayed.

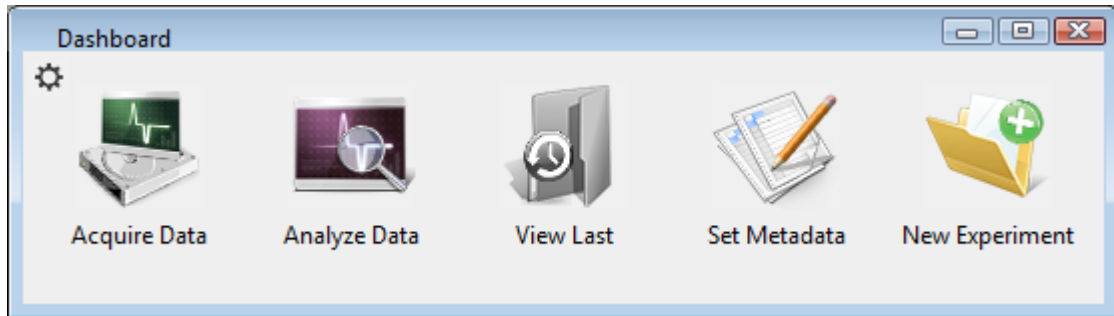


Figure 2-7. Dashboard.

7. Click on the 'Acquire Data' icon, and a second level of the Dashboard is displayed.

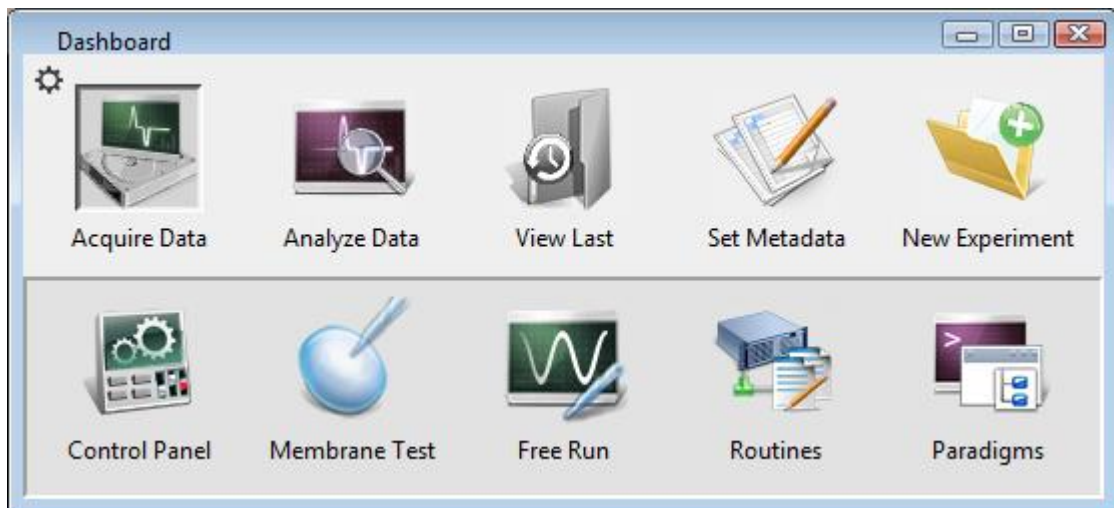


Figure 2-8. Acquisition Dashboard.

8. Click on the 'Control Panel' icon and the Amplifier Control Panel is displayed.

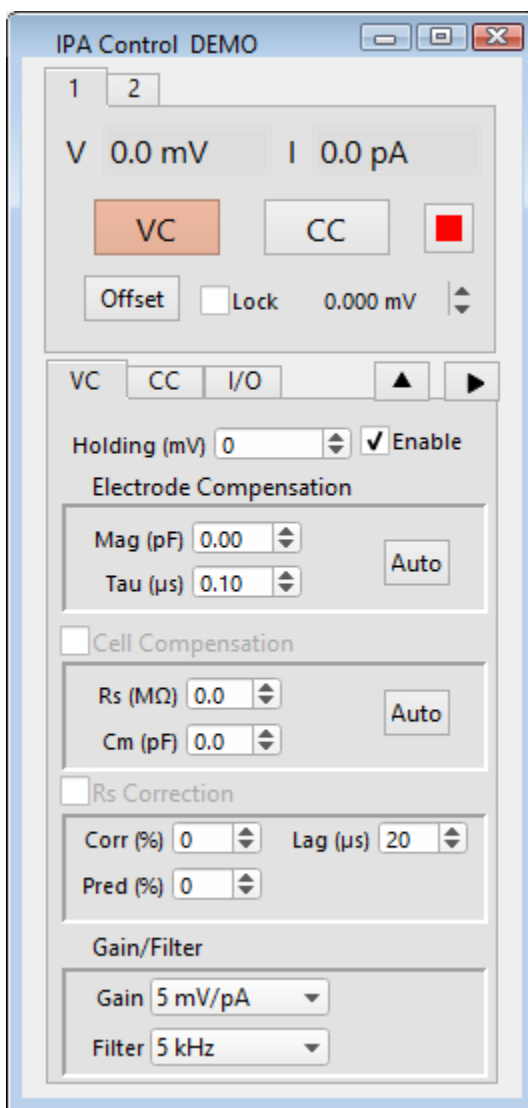


Figure 2-9. Amplifier Control Panel

- a. If “DEM...” is displayed in the Amplifier Control Panel title bar, you are running in a hardware emulation mode - ensure that the amplifier is “on” and its USB cable is connected, and then run a “New Experiment”.
- b. For the next step, make sure that the Amplifier Control Panel is in voltage-clamp mode – the VC button at the top of the Amplifier Control Panel should be highlighted in red.

2.7.3 Run a Membrane Test

The Membrane Test is useful for a quick check of the IPA system functionality. It tests the three basic steps necessary for recording in a whole-cell configuration.

1. Go to the Dashboard window and click on the 'Acquire Data' icon.

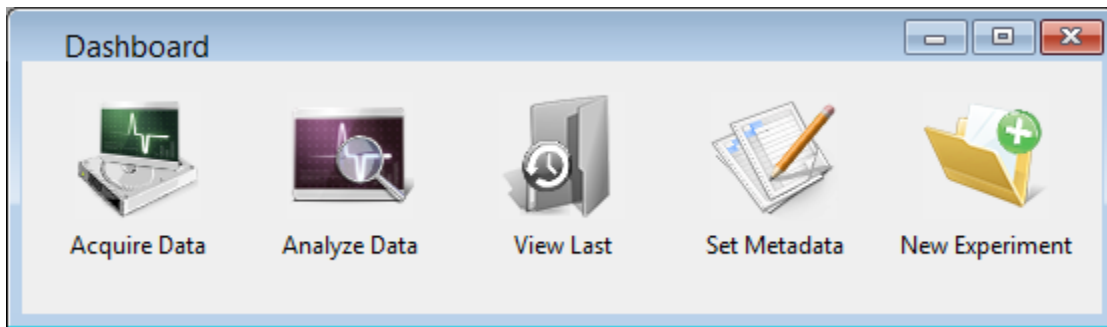


Figure 2-10. Dashboard

2. Click on the 'Membrane Test' icon.

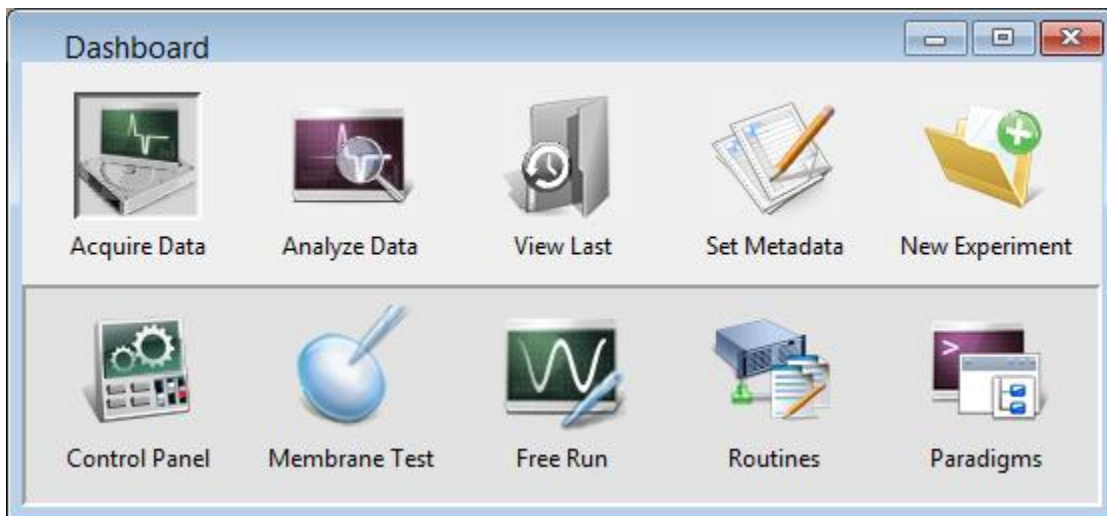


Figure 2-11. Dashboard – Acquisition

The following test values assume a 5 kHz filter...

3. Test the BATH mode:

This mode simulates placing an electrode into the bath solution and sending a voltage pulse through the solution.

- a. Set the Model Cell switch: **Bath**
- b. Click on the Membrane Test 'Bath' button.
- c. Verify readings: Pipette Resistance: $\sim 10 \text{ M}\Omega$

4. Test the SEAL mode:

This mode simulates an electrode making contact onto a cell and forming a high-resistance gigaohm seal with the membrane.

- a. Set the Model Cell switch: **Seal**
- b. Click on the Membrane Test 'Seal' button.
- c. Verify reading: Seal Resistance: > 10,000 M Ω

5. Test the CELL mode:

This mode simulates an electrode breaking into a cell and achieving a successful whole-cell patch.

- a. Set the Model Cell switch: **Cell**
- b. Click on the Membrane Test 'Cell' button.
- c. Verify readings:

Series Resistance:	~10 M Ω
Membrane Resistance:	~500 M Ω
Membrane Capacitance:	~28 pF

6. For a dual-headstage system:

- a. Move the model cell, ground wires and shielding to Headstage 2.
- b. Set the Scope window to 'Headstage 2'.
- c. Repeat steps 3 – 5.

3. HARDWARE OPERATION

3.1 IPA Front Panel

The front panel of the IPA system is used for the headstage and external I/O connections, and a power button.

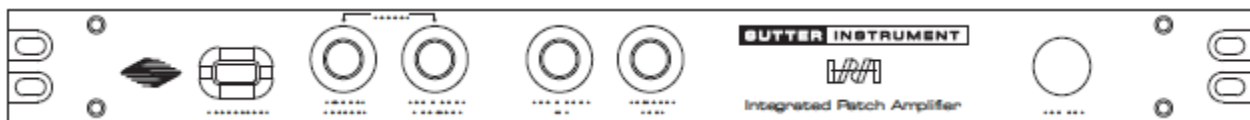


Figure 3-1. Front of IPA Cabinet

The front panel from left to right:

HEADSTAGE:	HDMI Port	For IPA headstage.
------------	-----------	--------------------

SCOPE-SIGNAL OUTPUT:	BNC	A scaled analog output signal of the headstage response signal.
• VC mode:	[#] mV/pA	Variable gain (Amplifier Control Panel)
	<u>Example:</u>	Membrane Test
	Model Cell:	BATH position (10 M Ω)
	Amplitude	= 10 mV
	Gain	= 5 mV/pA
	Current response	= 10 mV/10 M Ω = 1 nA (V/R = I)
	Scaled output voltage	= I * Gain 1 nA * 5 mV/pA = 5 V
• CC mode:	[#] mV/mV	Variable gain (Amplifier Control Panel)
	<u>Example:</u>	Current Clamp
	Gain	= 100 mV/mV
	Response signal	= 10 mV
	Scaled output voltage	= V * Gain = 10 mV * 100 mV/mV = 1 V
SCOPE-COMMAND MONITOR:	BNC	A scaled analog output of the headstage Stimulus signal (StimOUT).
• VC mode:	10 mV/mV	Constant gain
	<u>Example:</u>	Voltage Clamp
	Command voltage:	10 mV
	Scaled output voltage:	V * Gain = 10 mV * 10 mV/mV = 100 mV
• CC mode:	0.5 mV/pA	Constant gain
	<u>Example:</u>	Current Clamp
	Command current	= 200 pA

	Scaled output voltage	$= I * \text{Gain}$ $= 200 \text{ pA} * 0.5 \text{ mV/pA}$ $= 100 \text{ mV}$
COMMAND IN:	BNC	A scaled analog input that adds an external signal to the headstage Stimulus signal (StimOUT).
	• VC mode:	10 mV/mV Constant gain
	<u>Example:</u>	Voltage Clamp
	External Command	$= 5 \text{ mV}$
	Stimulus signal	$= 20 \text{ mV}$
	Total stimulation	$= (V * \text{Gain}) + \text{StimOUT}$ $= (5 \text{ mV} * 10 \text{ mV/mV}) + 20 \text{ mV}$ $= 70 \text{ mV}$
	• CC mode:	2 pA/mV Constant gain
	<u>Example:</u>	Current Clamp
	External Command	$= 1 \text{ mV}$
	Stimulus signal	$= 5 \text{ pA}$
	Total stimulation :	$(V * \text{Gain}) + \text{StimOUT}$ $= (1 \text{ mV} * 2 \text{ pA/mV}) + 5 \text{ pA}$ $= 7 \text{ pA}$
TRIGGER OUT:	BNC	Digital Trigger pulse output.
		Automatically sent at the start of continuous acquisition or each triggered sweep, including Membrane Test.
POWER:	Button	Turn power to unit On/Off. Lights up blue when 'On'

3.2 Double IPA Front Panel

The front panel of the Double IPA (vs. IPA) system adds support for a second headstage in an upper row of connectors:

HEADSTAGE 2

SCOPE-SIGNAL OUTPUT
SCOPE-COMMAND MONITOR
COMMAND IN

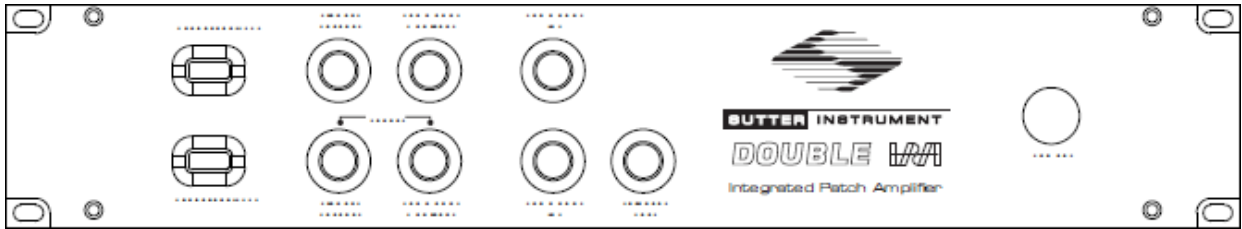


Figure 3-2. Front of Double IPA Cabinet

3.3 IPA Rear Panel

The rear panel of the IPA system is used for grounding, USB connection, and signal I/O. The rear panel of the Double IPA amplifier is essentially the same as for the IPA amplifier.

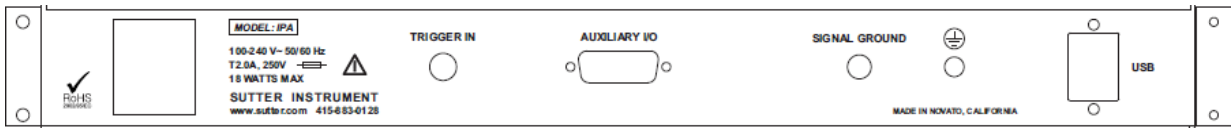


Figure 3-3. Rear of IPA Cabinet

[Unlabeled]:	Power-entry receptacle	For AC power cord
TRIGGER IN:	BNC	Digital input trigger.
AUXILIARY I/O:	DA-15 D-sub connector	External analog input and output channels, digital output channels, signal ground.
		See Appendix E for pin definitions.
SIGNAL GROUND:	4 mm Banana socket	Low-voltage grounding.
EARTH GROUND:	4 mm Banana socket	Instrument grounding.
[Unlabeled]:	USB Type B receptacle	USB 2.0 computer communication.

3.4 Grounding

An electrical connection is needed to an “earth” ground from your laboratory’s electrical system. If your building’s electrical grid does not provide an excellent earth ground, sometimes the plumbing system can be used. Or consider creating an earth ground for your own laboratory, using a heavy metal bar penetrating deep into the earth.

The equipment in a rig should all be grounded to a single point to avoid ground loops. Installing a bus bar to an earth ground can make this easier to accomplish.

“Signal” ground is a sensitive ground for very low voltages:

- BNC shields: Hard-wired to signal ground.
- Bath ground electrode: Connect to the headstage signal ground jack.
- Shielding: Connect to the IPA rear panel SIGNAL GROUND socket.

However, due to the complexity of grounding factors, despite any preconceived cabling notions, you may need to empirically determine the best grounding configuration of your system. For example, when multiple headstages are used, one or both headstages might need to be grounded.

3.5 Headstage

The IPA headstage supports both voltage- and (true) current-clamp in the same headstage.

Feedback resistor:	500 M Ω	
Whole-cell capacitance compensation:	0 – 100 pF	
Current-clamp rise time:	17.5 μ s	(100 M Ω load)

The headstage noise, as measured with an 8-pole Bessel filter:

<u>Bandwidth</u>	<u>Open-Circuit Noise (RMS)</u>
0.1 – 1 kHz	< 0.25 pA
0.1 – 5 kHz	< 0.75 pA
0.1 – 10 kHz	< 1.4 pA

Measuring “open-circuit”, i.e., with no attachments so the headstage input is exposed to the air, provides a fairly consistent baseline for such headstage noise measurements. Conversely, measuring noise with an electrode in the bath generates the worst noise conditions.

A 1 mm gold pin signal-ground socket is on the back of the headstage.

The IPA headstage cable length can be increased with a 6-foot HDMI (non-powered) extension cable.

Note: DIPA headstages are serialized, whereby the lower-numbered headstage is matched to the HEADSTAGE 1 port, and the higher-numbered headstage is matched to the HEADSTAGE 2 port.



WARNING! Hot-swapping of headstages should be avoided – components can be damaged. Turn off the IPA system power before handling headstages.

3.6 Holder

A “holder” attaches a microelectrode (pipette) to a headstage. It provides mechanical stability for the pipette, low-noise for the electrical circuit, and chemical inertness from its physical components. Our pipette holders accept electrode glass in the range of 1.0 – 1.7 mm OD (Outer Diameter) using sized-by-color silicone gaskets.

The standard pipette holder included with the IPA amplifier is composed of low-noise polycarbonate and Teflon. A suction tube projects at a right-angle from the middle of the barrel.

An ultra-low-noise quartz pipette holder is optionally available. While polycarbonate is a proven material for patch pipette holders, it undergoes significant thermal expansion. Uneven warming can lead to motion of the pipette tip, and is often incorrectly perceived as drift in the micromanipulator. Quartz has a significantly lower thermal expansion coefficient and virtually eliminates thermal drift.



WARNING! Quartz is fragile, and can crack or shatter on impact. Treat your quartz pipette holder with the same care as with an optical component.

3.6.1 Assembly

The holder is assembled from 8 parts incorporated into a main barrel:

End Cap – Gasket – Silver Wire – Barrel – Tubing – Gold Pin – Pin Cap - Lockdown Ring

Note: The silver wire must be chlorided before use. See “Chloriding Silver Wire”.

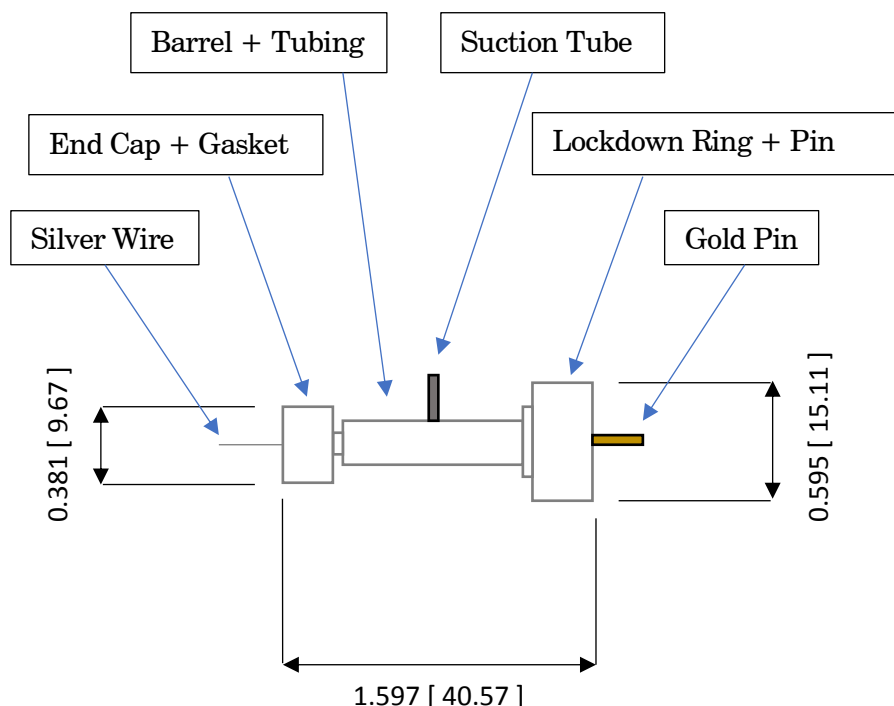


Figure 3-4. Electrode Holder

Figure dimensions are in “inches [mm]”.

1. Cut the silver wire to size: the depth it extends into the pipette plus half the length of the barrel.
2. Thread the silver wire through the barrel.
3. Cut a small piece of clear tubing sized to fill the tiny “end-cup” in the pin-side of the barrel – the end with the narrower shaft.
4. Thread the small piece of tubing over the silver wire into the end-cup.
5. Crimp the end of the silver wire just slightly over the end of the tubing.
6. Slide the lockdown ring over the tubing-side of the barrel, with the ring’s threads facing outwards.
7. Insert the gold pin into the recessed end of the pin cap - push it through the pin hole until it stops.
8. Screw the pin cap onto the barrel. Pressure from the compressed snippet of tubing ensures electrical contact between the silver wire and the gold pin.
9. Find a silicone gasket with an ID (inner diameter) just greater than your pipette OD (outer diameter):

<u>Gasket ID</u>	<u>Color</u>
1.1 mm	Clear
1.2 mm	Green
1.5 mm	Orange-Red
1.75 mm	Blue

10. Thread the gasket onto the silver wire on the end-cap side - the side with the narrower shaft.
11. Thread the end cap onto the silver wire and loosely tighten until it makes contact with the gasket.
12. Carefully thread a solution-filled micropipette onto the silver wire and into the gasket, and then push it into the barrel until it reaches the back end of the bore in the middle of the barrel.
13. Tighten the end cap onto the barrel.
14. Attach the holder to a headstage with the lockdown ring.

Assembly tips

1. The silver wire should be kept straight – do not bend or twist it.
2. Ensure good contact between the silver wire and the gold pin.

Check for proper tubing height and wire-crimping length – avoid excess or insufficient amounts.

For the most stable configuration, solder the silver wire to the end of the gold pin. Apply only a small bead of solder to the top of the pin in the very middle - avoid any excess solder that might interfere with the parts properly mating, as excess solder can result in air or solution leaks.

3. Fire-polish glass electrodes on both ends to prevent scratching the silver wire or the holder barrel.
4. The rubber gasket will wear out over time and need replacing.

3.6.2 Chloriding Silver Wire

The silver wire should be chlorided before first-time use, and then re-chlorinated monthly, or as needed.

Chemical Method

1. If needed, use a razor blade or fine sandpaper to rub off any insulation.
2. Optionally clean the silver (Ag) wire with ETOH (ethanol) to remove finger oils.
3. Immerse the silver wire in common household bleach (sodium hypochlorite) in glassware for 5 – 30 minutes until it turns purple-gray in color.
4. Remove the chlorided silver wire and rinse in distilled water.
5. Dry for storage.

Electrochemical Method

1. If needed, use a razor blade or fine sandpaper to rub off any insulation.
2. Optionally clean the silver (Ag) wire with ETOH (ethanol) to remove finger oils.
3. Connect a silver wire to each pole (positive & negative) of a household battery (1.5 V – 9 V).
4. Immerse the two silver wires in a solution of KCL (3 M) in glassware for 5 – 10 minutes. The wires should not touch each other. Bubbling around the silver wire indicates electroplating is occurring.

Alternatively, use HCL (1M) with a 2 hour immersion time.

5. The charging polarity for the wires should be reversed a few times during the process.
6. A fully chlorided silver wire should be purple-gray in color. Remove the chlorided silver wires and rinse in deionized water.
7. Dry for storage.

Re-Chloriding Silver Wire

1. Pass the used silver wire through a flame - the wire should become bright silver in color.

Alternatively, use a razor blade or fine sandpaper to scrape off any existing chloride.

2. Chloride the wire as described above.

3.6.3 Holder Maintenance

Holders must be properly maintained for good noise performance.

Storage:

1. Holders should be clean and dry.
2. Store in a container with dessicant.

Before 1st time use:

1. Disassemble the holder.
2. Rinse the polycarbonate parts in 70% ethanol.
3. Blot dry.
4. Store in a container with dessicant overnight.

After daily use:

1. Rinse holders with distilled water. For more thorough cleaning, wash with ethanol.
2. Blot dry.

Caution! Washing with soapy water can leave a film.

Continual cleaning with ethanol can degrade the polycarbonate parts.

Do not clean with methanol or strong organic solvents such as acetone.

Weekly Cleaning:

1. At least once per week, disassemble holder.
2. Clean the polycarbonate parts with 10 – 20 s sonication in distilled water.
3. Blot dry.
4. Store in a container with dessicant overnight.

3.7 Amplifier Control Panel (Software)

This software interface controls the IPA amplifier settings. It replaces all physical knobs, dials and meters, such as found on manually-controlled amplifiers.

Most of these settings can also be programmatically controlled in a Paradigm.

Most editable numeric fields can also be adjusted via a control panel with three slider bars (for 3 significant digits) by right-clicking on the field.

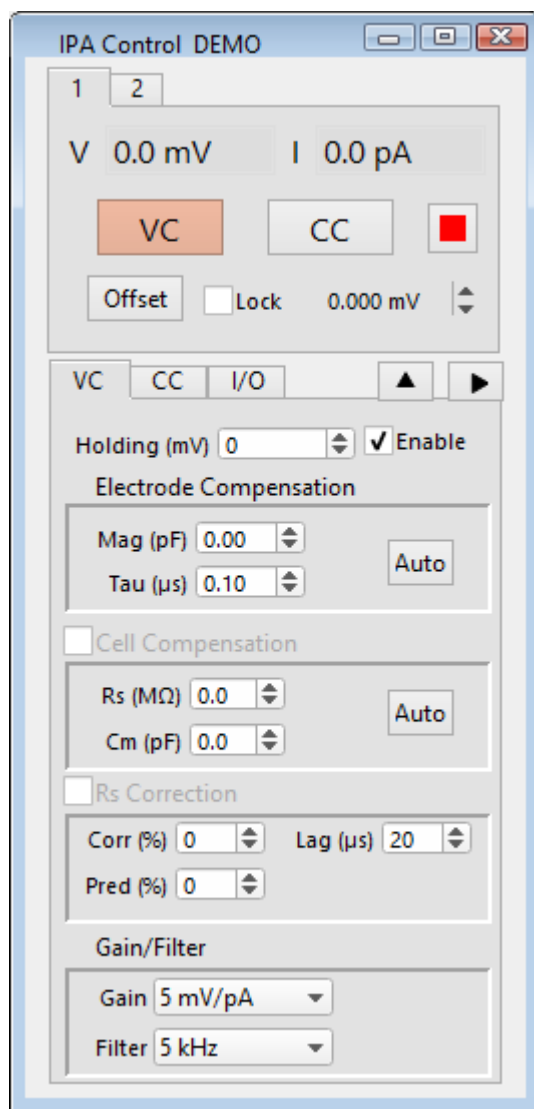


Figure 3-5. Amplifier Control Panel

General Controls

1 - 4 Headstage # tabs

When only a single IPA amplifier is being used, headstage selection tabs are not displayed.

When multiple headstages or amplifiers (IPA and/or Double IPA) are used, multiple headstage tabs will display. Each headstage maintains its own settings.

Clicking on a headstage tab will open its last-used “VC” or “CC” active mode settings. This will also blink the power light of the attached amplifier, which is useful in identifying which headstages are associated with which amplifiers.

Note: If a headstage is unattached while an IPA or Double IPA amplifier is in use, its input channel will be at “ground”. For older IPA amplifiers, the channel might appear as saturated.

V	'V' meter:	Displays the Voltage input channel level.
I	'I' meter:	Displays the Current input channel level.

VC	VC button:	Switch the IPA amplifier into Voltage Clamp mode.
----	------------	---

Uses a “gentle” switch, whereby the amount of voltage necessary to hold the current steady is initially injected into the cell, and then the headstage is switched to the new voltage level.

CC	CC button:	Switch the IPA amplifier into Current Clamp mode.
----	------------	---

Uses a “gentle” switch, whereby the amount of current necessary to hold the voltage steady is initially injected into the cell, and then the headstage is switched to the new current level.



Reset USB button: Click to re-establish the USB connection to the amplifier.

- All USB channels are reset.
- A green button indicates that a stable USB connection to the amplifier has been established.
- A red button indicates that there is no USB connection to the amplifier, or that SutterPatch is in Demo (hardware emulation) mode.
- When multiple amplifiers are connected, if any one instrument loses its connection, the button turns red.
- It can take several seconds for the USB connection to be re-established.
- If the amplifier is attached in demo mode, a USB reset will disable demo mode and run the hardware “live.”

Right-click menu Click almost anywhere in the Amplifier Control Panel to access:

- **Reset USB** Click to re-establish the USB connection to a Sutter amplifier.
 - All USB channels are reset.
 - It can take several seconds for the USB connection to be re-established.
 - If the amplifier is attached in demo mode, a USB reset will disable demo mode and run the hardware “live.”

- **Reset Amplifier Controls** Reset all hardware settings of attached Sutter amplifiers to their factory defaults; resets all attached headstages.

Offset The amount of offset applied is displayed in the numeric field below the Offset button. This value is independent of the holding potential.

[±250.000 mV]

The electrode offset should be initially adjusted in voltage-clamp mode, when the electrode is placed into the bath solution. This adjusts the electrode current signal to zero, providing a reference baseline for measurements. This offset counteracts any inherent hardware circuitry offsets, as well as electrode-in-the-solution offsets.

Click on the Offset button to apply an offset that zeroes the signal. The “auto-offset” value is an approximation that may need further adjustment.

Note: In Demo mode, this value is set to -0.2 mV.

Numeric values can be directly typed into the numeric field, or

- For fine adjustments, use the up and down spinners to increase or decrease the setting by ~0.015 mV.

Note: The Offset spinner step size (~0.015 mV) is based on the 16-bit resolution of a 1 V DAC. The actual spinner step size resolution is 0.0152588 mV.

- For moderate adjustments, increase the spinner increment by 10x (~0.15 mV) by holding down the Shift key and simultaneously clicking on the spinners.
- For fastest operation, select the offset field, hover the cursor over the numeric field or spinners, and hold down the Shift key while simultaneously scrolling up or down with the mouse wheel.

Lock Once an electrode offset has been applied, use the ‘Lock’ check box to prevent accidental changes to the Offset.

Voltage Clamp Controls

VC The ‘VC’ tab displays amplifier Voltage Clamp controls:

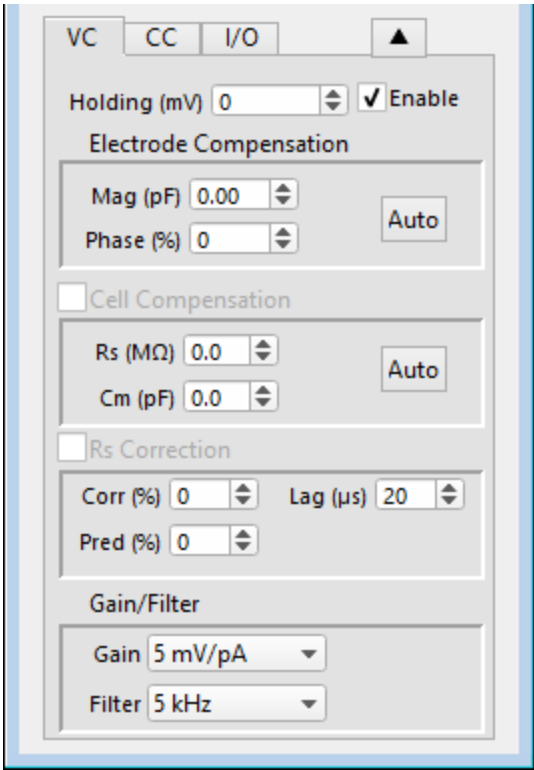


Figure 3-6. VC Control

Holding (mV): [±1000]

After achieving a seal, the holding voltage is typically set to the cell’s equilibrium or “resting” membrane potential (typically -60 to -80 mV for neurons).

Whenever you enable or leave the Amplifier Control Panel, a tag is written to the Log window with the Holding changes.

Electrode Compensation: Electrode capacitance compensation.

Mag (pF): [0.00 – 25.00] Magnitude

Tau (μs): [0.10 – 4.50]

 Opens a panel for simultaneous tuning of both parameters.

Auto Automatically sets approximate values.

Compensate for the microelectrode capacitive transients by clicking

the 'Auto' button, and then iteratively adjusting the 'Mag' and 'Phase' controls. For a square pulse command (like a Seal Test), the goal is to eliminate the sharp (fast) transients and reproduce a square waveform with minimal distortion.

Cell Compensation Whole-cell capacitance compensation.

Rs (M Ω): [0 – 100.0] Series Resistance

Cm (pF): [0 – 100.0] Membrane Capacitance



Opens a panel for simultaneous tuning of both parameters.

Auto Automatically sets approximate values (using small “gentle” steps to avoid hyperpolarization.)

After breaking into a cell, i.e., going “whole cell”, capacitive transients are now generated by the entire membrane of the cell, also producing a significant slow decay phase in the signal.

To remove the transients, click 'Auto', then iteratively adjust the 'Rs' and 'Cm' controls until the signal is adequately compensated (i.e., smooth transitions without transients.)

Note: When the Membrane Test is running, 'Cell Compensation' must be disabled, else capacitance spikes are suppressed, and Series Resistance and Membrane Capacitance values cannot be calculated.

The IPA system is optimized for real-world measurements from electrodes. But when used with the model cell, the compensation might need several more 'Auto' adjustments to compensate the model cell capacitance.

Rs Correction Series resistance compensation.

“Rs Correction” speeds up the rise time of the current response, reduces unwanted filtering effects, and corrects voltage drops.

Corr (%): [0 – 100] Correction

Pred (%): [0 – 99] Prediction

Lag (μ s): [20 – 200] (RC filter component)

$$\text{Lag} = 1 / (2 * \pi * \text{Bandwidth})$$

Control the speed of the correction vs. possible oscillations.

Rs Correction first requires the Electrode and Cell Compensations to

be applied.

Then set the Prediction (Pred) to “supercharge” the command potential. Small transients should become visible at the beginning and end of the current response.

Next, increase the Correction (Corr) current injected into the membrane. As the Corr setting is increased to sharpen the rise time, the current response transients increase in size. Try to avoid overshooting - if the correction is set too high, internal feedback can lead to oscillation of the circuit, i.e. “ringing”, and loss of a patch. Reduce oscillation of the circuit by adjusting the ‘Lag’ setting - larger values increase the stability of the circuit, but also increase the rise time.

Remove the Prediction/Correction transients in the signal by reducing the Cell Compensation ‘Rs’ setting until a minimum value is found.

Finally, adjust the Cell Compensation ‘Rs’ setting again until the best result is achieved, or try over again with lower Prediction/Correction settings.

Gain	Analog Input Gain														
	<table><tr><th><u>mV/pA</u></th><th><u>Signal Range</u></th></tr><tr><td>0.5</td><td>± 20 nA</td></tr><tr><td>1</td><td>± 10 nA</td></tr><tr><td>2.5</td><td>± 4 nA</td></tr><tr><td>5</td><td>± 2 nA</td></tr><tr><td>10</td><td>± 1 nA</td></tr><tr><td>25</td><td>± 400 pA</td></tr></table>	<u>mV/pA</u>	<u>Signal Range</u>	0.5	± 20 nA	1	± 10 nA	2.5	± 4 nA	5	± 2 nA	10	± 1 nA	25	± 400 pA
<u>mV/pA</u>	<u>Signal Range</u>														
0.5	± 20 nA														
1	± 10 nA														
2.5	± 4 nA														
5	± 2 nA														
10	± 1 nA														
25	± 400 pA														
Filter	Analog Input Filter (low-pass 4-pole Bessel)														
	<table><tr><th><u>kHz</u></th></tr><tr><td>0.5</td></tr><tr><td>1</td></tr><tr><td>2</td></tr><tr><td>5</td></tr><tr><td>10</td></tr><tr><td>20</td></tr></table>	<u>kHz</u>	0.5	1	2	5	10	20							
<u>kHz</u>															
0.5															
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10															
20															

Filter settings are applied to the active IPA headstage’s analog input signals when a Scope acquisition window is created.

Note: Demo mode bypasses the filter.

Current Clamp Controls

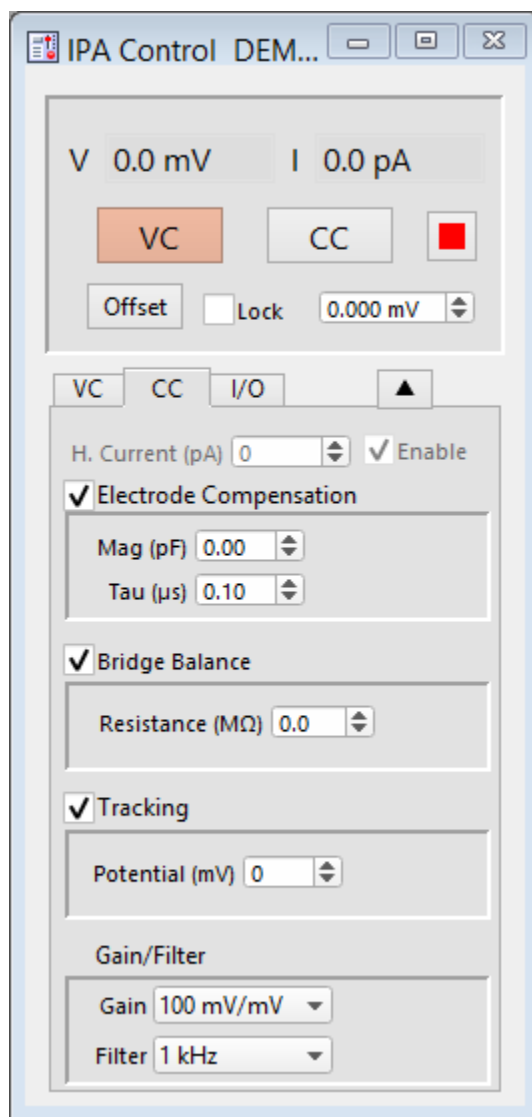


Figure 3-7. CC Control

CC The 'CC' tab displays the IPA Current Clamp controls:

H. Current (pA): [± 1000]

A Holding Current can be preset here. Use the Enable checkbox to activate it.

Electrode Compensation

Mag (pF): [0.00 – 25.00] Magnitude

Tau (μ s): [0.10 – 4.50]

After seal formation (i.e., in a cell-attached patch configuration), compensate for the microelectrode capacitive transients by iteratively adjusting these controls. For a square pulse (or Seal Test) command, the goal is to eliminate the sharp (fast) transients and produce a

square waveform with minimal distortion. This function is also known as capacitance neutralization.

Bridge Balance

Resistance (M Ω): [0 – 200.0]

Bridge balance compensation is used to correct distortion of the signal due to a voltage drop across the electrode during current flow.

Tracking

Potential (mV): [± 1000]

Slow tracking is used to keep the membrane voltage at a set target level without drifting over time. Enter the desired cell potential to be maintained.

The Tracking time constant (τ) is ~ 18 ms, equating to a rise time of 40 ms, or a bandwidth of 8.75 Hz.

Note: When Tracking is enabled, the Holding Current is automatically disabled, as the current output is dynamically adjusted by the system.

Gain

Input Gain

<u>mV/mV</u>	<u>Signal Range</u>
10	± 1 V
20	± 500 mV
50	± 200 mV
100	± 100 mV
200	± 50 mV
500	± 20 mV

Gain settings for the active IPA headstage are applied to its primary analog input signal ('Voltage' in CC Mode) when the Scope window is created.

Filter

Low-pass Filter

kHz
0.5
1
2
5
10
20

Filter settings for the active IPA headstage are applied to its primary

analog input signal ('Voltage' in CC Mode) when the Scope window is created.

Note: Demo mode bypasses the filter.

I/O Controls

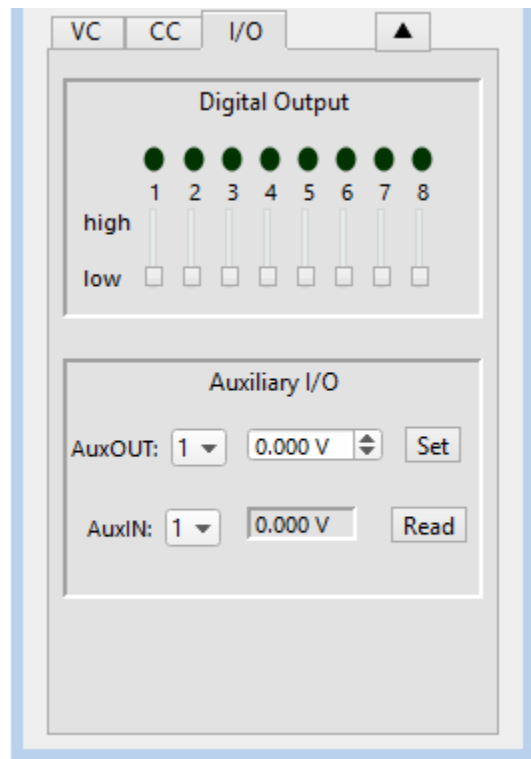


Figure 3-8. I/O Control

I/O

The 'I/O' tab contains the Digital Output and Auxiliary I/O controls. For a Double IPA system, the I/O tab is only visible when Headstage 1 is selected.

Digital Output

This section controls the holding bit pattern generated by the Digital Outputs of the IPA amplifier. Eight TTL-compatible digital channels are displayed. Switch between digital states by clicking on a channel slider 'high' or 'low' end, or by clicking and dragging the slider button. When a channel state changes, its dot also changes color:

- | | | | |
|-------|-----------|------|----------|
| • On | Red dot | high | (+3.3 V) |
| • Off | Black dot | low | (0 V) |

Note: When multiple Sutter amplifiers are connected, the Digital Outputs are only active for the main amplifier.

Auxiliary I/O

General purpose "auxiliary" analog input/output channels are

available.

AuxOUT 1 & 2: [± 10 V]

Select an auxiliary analog output channel from the drop-down list. Directly edit its voltage level, or use the spinners to change the value in 0.100 V increments.

When switching between auxiliary channels, hardware output channels maintain separate values, while demo output channels are reset to zero.

Tip: When the IPA system is used as a data acquisition system for external instruments, the auxiliary outputs can be used as holding levels.

Set Click the 'Set' button to apply the value to the selected output channel.

AuxIN 1 – 4: [± 10 V] (Read Only)
Select an auxiliary analog input channel from the drop-down list.

Read Click the 'Read' button to display a single-point reading of the selected input channel.

Tip: This is useful for monitoring slow-changing parameters, such as temperature.



The vertical Show/Hide button displays/hides the VC-CC-I/O panes.



The horizontal Show/Hide button displays/hides the Double IPA headstage monitor, which displays the Voltage and Current values for both headstages.

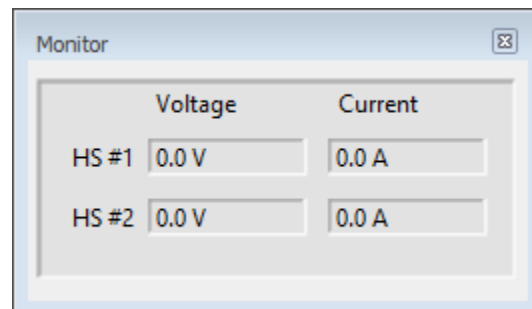


Figure 3-9. Headstage Monitor

3.8 Lock-In Adjustments

The dPatch “lock-in amplifier” system can be manually tuned via the menu item SutterPatch / Hardware Control / Lock-In Adjustments. Adjustments are made to the headstage(s), and should be done in voltage-clamp mode.

Enable Manual Adjustments	Adjustments can be made using direct field editing, spinners, or a field right-click slider panel.
Absolute Values	
Phase Delay Adjustment	Apply a phase delay to the calculations.
Reset	Reset to 0.00 s
Attenuation Adjustment	Apply a gain to the calculations.
Reset	Reset to 1.00
List Results	

The Lock-In computation is quite stable - its calibration values do not change day-to-day. However, Lock-In measurements can be affected by experimental conditions, including the amplifier itself. In particular, the electrode compensation has a large influence on the results, and needs to be properly set.

For higher frequencies (5 kHz and higher), the Lock-In sine frequency is increasingly sensitive to the Lock-In phase adjustment. For these cases, you might want to tune-up the Lock-In adjustments more frequently.

When making absolute capacitance measurements, you can improve the consistency of the measurements by adjusting the Lock-In phase adjustment to a given, known reference capacitance.

Note: Demo data is not designed to respond to Lock-In phase and attenuation adjustments.

3.9 Using Peripheral Equipment

The IPA system can control peripheral equipment, such as:

- solution changers
- light sources
- wavelength switchers
- pulse generators

Auxiliary analog output signals can be used to control other instruments within a range of ± 10 V. Digital outputs use TTL-compatible voltage signals. Analog and digital holding levels are set in the Amplifier Control Panel

The digital command output can be formatted as either an 8-bit “word” or a single “bit”, as selected in the Routine Editor / Output Channels & Waveform section. Actual command output patterns are configured in the Routine Editor / Output Channels & Waveform / Waveform Editor.

Note: The Analog and Digital controls in the Amplifier Control Panel provide a way to quickly and easily test the behavior and operation of peripherals, without the need to create or modify Routines.

3.10 Using Multiple Sutter Amplifiers

Any combination of two IPA or Double IPA amplifiers can be connected and run simultaneously by the SutterPatch program. When more than one IPA headstage is attached, each headstage will have its own numbered tab [1 – 4] in the Amplifier Control Panel. Up to 16 combined analog input channels are available, however Digital Outputs are only available from the “main” amplifier

One amplifier is designated the “main” amplifier, and provides a clock signal to control the timing of the “secondary” amplifier. To setup, install the amplifiers, and then connect the main amplifier front panel TRIGGER OUT BNC to the secondary amplifier rear panel TRIGGER IN BNC.

When a computer is powered on, its USB ports and attached hardware are detected and “enumerated”, i.e., receive a sequence number. The Amplifier Control Panel displays each attached Sutter headstage in a numbered tab based on its enumerated sequence. You will need to determine the actual headstage sequence during installation, but these numbers should not change after installation unless the attached equipment is changed or the USB ports are re-configured.

Note: Emulation (demo) mode does not support multiple-amplifier configurations.

3.11 Using Non-Sutter Amplifiers

An IPA or Double IPA system can also be operated as a stand-alone data acquisition system interfacing to non-Sutter amplifiers. You continue to control the Sutter digitizer via the Amplifier Control Panel and SutterPatch software.

The IPA digitizer interfaces to external amplifiers via the rear panel Auxiliary I/O Cable or optional Patch Panel BNCs:

AuxOUT1 & 2

These two auxiliary analog output channels can be used to send stimulus waveforms to external instruments, such as non-Sutter microelectrode amplifiers.

AuxIN1 – 4

These four auxiliary analog input channels can be used to digitize signals from external instruments, such as

non-Sutter microelectrode amplifiers.

DigOUT1 – 8

Digital output patterns can be sent via eight digital output channels to a variety of peripheral equipment.

Auxiliary analog and digital holding levels are set in the Amplifier Control Panel I/O tab.

Note: Sutter amplifier output levels into Sutter systems attenuate by < 0.2%.

HEKA amplifier output levels into Sutter systems attenuate by 0.%.

Axon amplifier output levels into Sutter systems attenuate by 5%.

3.12 Using Non-Sutter Data Acquisition Systems

The IPA or Double IPA system can also be operated as a stand-alone amplifier using non-Sutter data acquisition systems, while the Sutter amplifier continues to be controlled via the Amplifier Control Panel.

IPA System Front Panel Connections

BNC

Channel

COMMAND IN

Stimulus to the preparation.

Combines the analog input signal from an external source with the stimulus (command waveform) sent to the preparation.

The Command In external signal is summed with the IPA internal StimOUT output signal, and is then sent to the IPA headstage.

SCOPE - SIGNAL OUTPUT

Data from the preparation.

This BNC supplies the response from the IPA preparation.

The current or voltage response from the headstage is directly available from this analog BNC output, and can be connected to an external data acquisition system for digitization and recording.

SCOPE - COMMAND MONITOR

Data from the Stimulus signal.

This BNC allows you to monitor the stimulus channel.

The analog stimulus delivered to the IPA headstage (voltage or current) is directly

available on this BNC, and can be connected to an external data acquisition system for digitization and recording.

4. SOFTWARE OPERATION

4.1 Acquisition

SutterPatch acquisition operations.

4.1.1 Camera Module

The SP_Camera window displays:

Camera	Select a camera name from those attached to the computer.
Start	View live video.
Capture	Take a single picture. If live video is running, this will take a picture while live video continues to run. The image time-stamp is reported in the Log window.

[video screen]

Last Capture A thumbnail of the last picture taken in the Experiment is displayed.

All pictures are stored in the current Experiment. To review pictures, go to:

Data Navigator	Select a Paradigm or Routine. Any associated images are listed in the Preview pane. Click on an image name to display the image.
Data Browser	Navigate to the Data / Images folder. Right-click on an image name, and select 'New Image'.
Tip:	For dark-room experiments, the window background color can be adjusted by the operating system.
Windows:	In the Control Panel / Appearance / Personalization window, scroll down and select the High Contrast Black theme, or use the Magnifier tool with option 'Turn on color inversion' enabled.
macOS:	Press 'Control-Option-Command-8' to set the System Preferences / Accessibility / Display / Invert Display colors option, or open its menu with 'Command-Option-5'.

Full-camera drivers have been successfully tested for the following camera models:

Sentech drivers:

STC-MC33USBVGA
 STC-MCS231U3V
 STC-MB83USBVGA
 STC-MBCM401U3V
 STC-MBCM401U3V-NIR
 STC-HD203DV

Photometrics PVCAM drivers :

Photometrics:	Prime 95B Prime 95B 25mm
Qimaging:	Electro

4.1.2 Free Run

The Free Run (Scope) window simulates a one-channel oscilloscope, and is a quick method of viewing repetitive data. It operates similarly to the Scope acquisition window, with unsupported controls removed or disabled.

After selecting this operation, an Input Channel dialog needs to be configured:

Select channel: Select an input channel from a list of all input channels.

The default channel is 'Current1' in VC mode, and
 'Voltage1' in CC mode.

Select sampling frequency: 1, 2, 5, 10, 20, 50 kHz

Input sweep length (s): The duration of the acquired/displayed sweep.

4.1.3 Membrane Test

The Membrane Test is primarily used to monitor seal formation and cell health in a voltage-clamp whole-cell patch-clamp configuration. However, current-clamp mode operation is also supported.

Scope Window

The default Scope window top pane displays the Current signal from the active Sutter headstage, and the pane beneath it displays the corresponding Voltage command signal.

This Scope: Membrane Test window operates similarly to the Scope: Acquisition window, however Persistence display and sweep counts are not supported, and during an Experiment the Autoscale button state persists between windows.

Whenever the Membrane Test is opened in an IPA Experiment, it opens in a “running” state. Closing the Membrane Test Scope or Analysis windows automatically stops Membrane Test acquisition.

Membrane Test Analysis Window

Configure all Membrane Test settings in the Membrane Test Analysis window.

The screenshot shows the 'Analysis' window with a 'Show' dropdown set to 'All Settings'. Below this are three main sections: BATH, SEAL, and CELL. The BATH section includes fields for Rpipette (10.0 MΩ), Rmembrane (OFF), and Cmembrane (OFF), with a 'Write to Log' button. The Test Pulse Parameters section includes a Pulse Type dropdown (Single Pulse), Amplitude (-0.010 V), Duration (20 ms), and Repetition Interval (0.2 s). The Zap Parameters section includes Amplitude (0.5 ms), Duration (1.0 V), and a 'Do it' button. The Signal Parameters section includes a Channel dropdown (Headstage 1), A/C Line Reduction (Off), and Sampling rate (50 kHz).

Figure 4-1. Membrane Test Monitor

Show Monitor Only
 All Settings

The Membrane Test Analysis window contains the three basic steps to form a whole-cell seal:

- 1) Bath With a new pipette in the bath solution, a low-resistance square pulse is visible. The pipette resistance should be very low if the tip is not clogged. With dissociated cells, typical pipette resistances are 1 – 5 MΩ. In slice patch experiments, pipette resistances well above 10 MΩ are not uncommon.

	Triangle	A train of 5 symmetrical bipolar triangular pulses. [dPatch only]
	Sine	A train of 5 sine wave pulses. [dPatch only]
	RMS Noise	No pulse – the holding level is output.
Amplitude	[± 0.100 V]	Amplitude is relative to the 'Holding' level in the Amplifier Control Panel.
Duration	[10, 20, 50, 100, 200 ms]	
Repetition Interval	[0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10 s]	Sweep start-to-start interval.

Zap Parameters

After a gigaohm patch has been achieved, use Zap in the Seal mode as an alternative to suction in creating a whole-cell patch.

Amplitude	Set the amplitude of the square wave zap pulse [0.1 - 1.0 V]
Duration	[0.1 – 2.0 ms]
Do It	Click the 'Do It' button to send a single square wave voltage pulse from the headstage to the preparation to disrupt the cell membrane.

Signal Parameters

Channel	Headstage 1 Headstage 2
A/C Line Reduction	Off 50 Hz 60 Hz
Sampling rate	10 kHz 20 kHz 50 kHz

4.1.4 Paradigm Editor

The Paradigm Editor is an advanced feature that opens up a world of complex experimental control via Paradigms and Paradigm Pools. A rich set of operators and actions are available to control and/or automate data acquisition and analysis.

The Paradigm Editor allows you to create “Planned Paradigms”, which offer almost unlimited flexibility in creating and/or automating your patch-clamp experiments, such as running Routines and directly controlling amplifier settings.

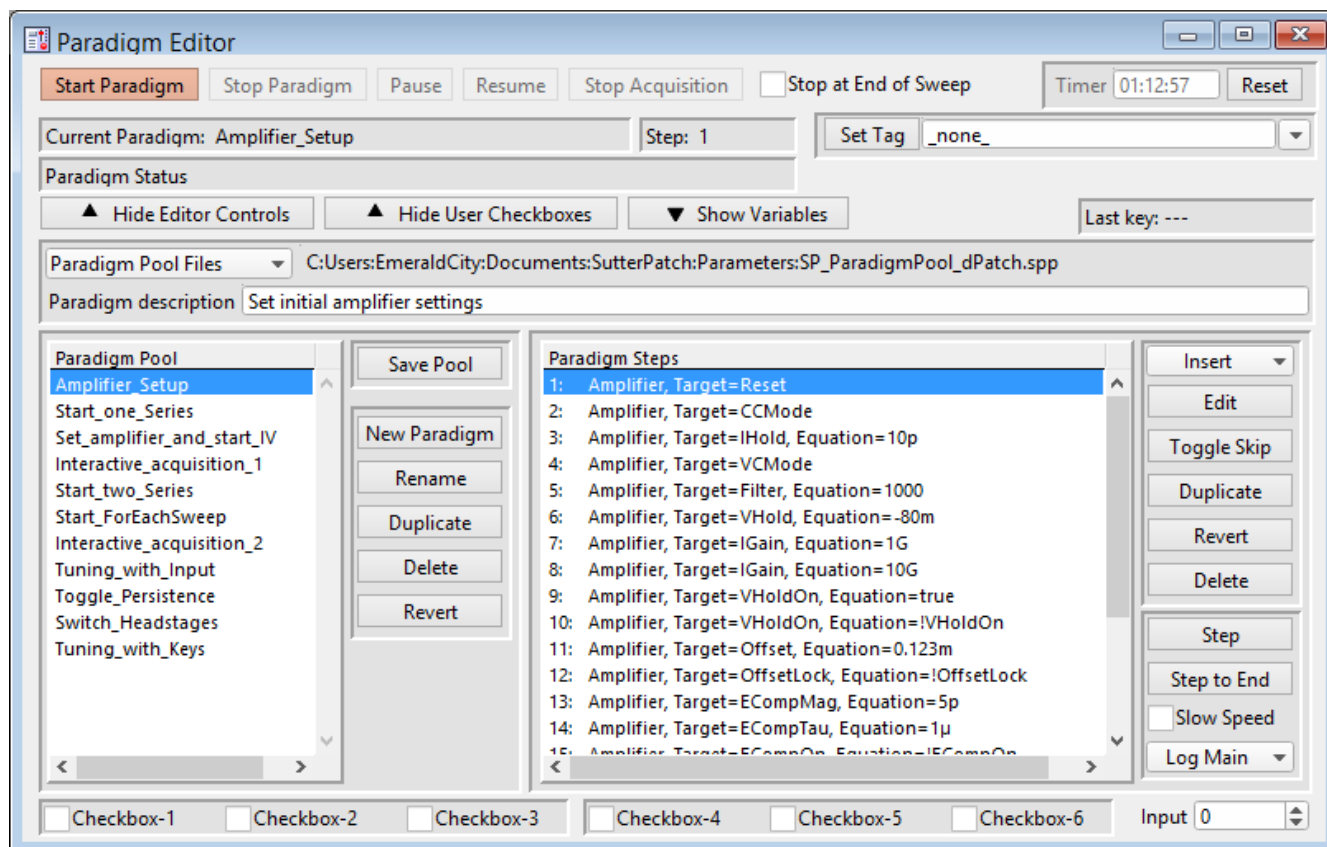


Figure 4-2. Paradigm Editor

Loaded Paradigms display on the left, while loaded Paradigm Steps display on the right. A bottom section can display interactive checkbox controls and/or variables.

Controls

Start Paradigm

Run the selected Paradigm highlighted in the Paradigm Pool list. A “Planned Paradigm” is created, which terminates after the end of the last paradigm step.

Acquisition in the Scope window (for Membrane Test or Free Run) is stopped.

This button turns into a ‘Stop Paradigm’ button when the Paradigm is running.

Stop Paradigm

Stop a running Paradigm.

Pause	Manually pause a running Paradigm.
Resume	Manually resume running the Paradigm.
Stop Acquisition	End a Routine's acquisition.
Stop at End of Sweep	Wait until the sweep-in-progress has completed before stopping an acquisition.
Timer	A running clock displays the time in "hh:mm:ss" since the last timer reset, or since a new experiment established a USB connection or emulation mode.
Reset:	Reset the Timer to 00:00:00.
Current Paradigm:	The name of the currently loaded Paradigm.
Step:	The highlighted Paradigm Step.
Paradigm Status:	Status information about Paradigm execution.
Set Tag	<p>A comment tag can be manually written to the Paradigm metadata with this button. Enter the comment text in the field, or select text from a drop-down list of the most recently used entries. Click this button to write the tag text. The last selection is used for all subsequent entries within the same Paradigm.</p> <p>When this button is clicked during acquisition, a black vertical "tag" cursor is displayed in the Scope window data at that time point, and time-stamped metadata entries are created.</p> <p>Note: In emulation mode, tag timing is not accurate, and no tags are set in the first sweep.</p> <p>Tip: When using the Set Tag button, you can minimize the rest of the Paradigm window by hiding its Editor Controls and Variables.</p>
Show/Hide Editor Controls	The Paradigm Editor controls (and checkboxes) for the Paradigm Pool and Paradigm Steps can be displayed or hidden.
Show/Hide User Checkboxes	Checkbox controls are displayed at the bottom of the Paradigm Editor controls, for use in conditional Paradigm step execution. This display is dependent upon Show Editor Controls.
Show/Hide Variables:	A Variables table can be displayed at the bottom of the Paradigm Editor. These paradigm variables can be utilized in any equation.

Variable names can be edited to any label, but they are only informational, and are not supported in equations.

Last key: The last key (or key combination) pressed on the keyboard is displayed here, such as used in Shortcuts or the ‘If’ and ‘ElseIf’ Paradigm steps. (See sample Paradigm ‘Tuning with Keys’.)

Note: Function and Control (Ctrl/Cmd) Shortcut key combinations are not displayed.

Paradigm Pool Files These operations affect the entire “Paradigm Pool”.

New Paradigm Pool	Create a new blank Paradigm Pool and optionally copy Paradigms into it from the existing Paradigm Pool. The suggested name is auto-incremented from the previously loaded Paradigm Pool name.
Load Paradigm Pool	Load the Paradigms of a previously saved Paradigm Pool file into the Paradigm Pool.
Revert to Last Saved	Undo any unsaved changes to the Paradigm Pool.
Save Paradigm Pool	Save the Paradigm Pool using its existing file name and path.
Save Paradigm Pool As	Save the Paradigm Pool to a new file, and switch to the new file. The default file name is the same as the original file name.
Save Paradigm Pool Copy	Save the Paradigm Pool to a new file, but do not switch to the new file. The default file name has ‘Copy of’ prepended to it.
Merge Paradigm Pools	Insert the Paradigms from a previously saved Paradigm Pool file into the loaded Paradigm Pool.

[The file path and file name of the loaded Paradigm Pool file are displayed.]

Paradigm description: A user description of the active paradigm.

Paradigm Pool A column of paradigm names from the loaded Paradigm Pool.

- Click on a paradigm name to highlight it as the active paradigm and display its steps.
- Double-click on a paradigm name to start execution of the paradigm and display its steps.
- Click-and-drag a paradigm name to change its position

in the column.

Save Pool	Save the Paradigm Pool using its existing file name.
New Paradigm	Create a new blank Paradigm in the Paradigm Pool.
Rename	Rename the selected Paradigm. <ul style="list-style-type: none"> Valid characters are A-Z, a-z, 0-9, and “_”. Special characters are not allowed. Spaces are replaced by an underscore. The name cannot start with a number – such entries will have an ‘X’ prepended to the name.
Duplicate	Add a copy of the selected Paradigm to the Paradigm Pool. The Paradigm name number is appended or incremented.
Delete	Remove the selected paradigm from the Paradigm Pool.
Revert	Select a paradigm and click the ‘Revert’ button. All editable steps are reset to their originally loaded values, as long as the Paradigm Pool has not been saved.
Paradigm Steps	A column of instructions from the active paradigm is displayed. These instructions are sequentially run by the paradigm. <ul style="list-style-type: none"> Click on a paradigm step to highlight it as the active step. Double-click on a paradigm step to view or edit its settings. Click-and-drag a paradigm step to change its position in the column.

Note: Step values are usually in SI standard units, i.e. "Volts" and "Amperes".

Step Buttons

Insert	Insert a new command Step into the Paradigm Steps column:
	Amplifier
	Each Sweep
	Routine

Analysis

Camera

Clear Key

Execute

Export

Front Window

Hide Window

Reset Timer

Scope Operation

Set Axis

Set Checkbox

Set Metadata

Set Solution

Set Tag

Set Variable

Sound

View Last

Write Log

Alert

Beep

Comment

Wait

Pause

	Break
	Chain
	For Loop
	Jump
	Label

	If
	ElseIf
	Else
	(See details in Insertable Steps list below.)
Edit	<p>If a highlighted Step is configurable, clicking the Edit button (or double-clicking the step) will open it in the Paradigm Steps Editor for configuration.</p> <p>Also, if a highlighted Step's text is partially hidden, use the Edit button to view the entire entry.</p>
Toggle Skip	<p>Mark a step so it is not executed.</p> <p>A semicolon is prepended to the Step number to "comment out" the instructions, and a Skip status is appended to the Step text.</p>
Example:	<p>A 'Beep' command in Paradigm step #2:</p> <pre style="margin-left: 40px;">; 2 Beep, Skip=true</pre> <p>The leading semicolon ";" prevents this step from being executed by the instruction queue, and the 'Skip' status is displayed.</p>
Duplicate	Insert a copy of the selected step.
Revert	<p>Select a Step to be reverted, and click the Revert button. Editable fields are reset to their originally loaded values, as long as another Paradigm has not been loaded.</p>
Delete	Delete the selected step. For multi-line steps, optionally delete the entire group.

Step	Execute the selected step, then move to the next step. Executing a single step does not terminate a running Paradigm, even if it is the last step in the Paradigm. Note: A 'For' loop is processed as a single step.
Step to End	Execute the selected step and all following steps as fast as the system allows.
Slow Speed	Execute 'Step to End' at ~1 second per step.
Log Main Steps	Action-oriented steps are recorded in the Paradigm metadata (visible in the Metadata 'By Event' view): Amplifier Break Camera Chain Execute For Each Sweep Reset Timer Routine Set Checkbox Set Solution Set Variable Wait
Log All Steps	Log the main steps and additional steps into the Paradigm metadata (visible in the Metadata 'By Event' view.)

Insertable Steps:

Amplifier

Control the IPA amplifier hardware.

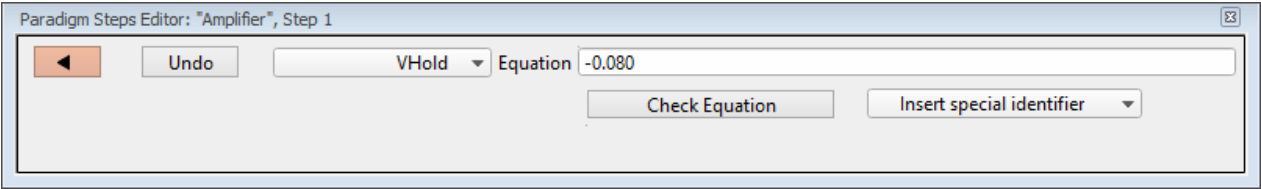


Figure 4-3. Step: Amplifier

Default Setting: *Amplifier, Target=VHold, Equation=-0.080*

Undo Removes any unsaved edits to this step.

[drop-down list] Amplifier settings:

SelectProbe (select active probe)

[1 - 4]

Most Paradigm Step commands apply to the “active” probe, the Sutter headstage presently controlled by the Amplifier Control Panel. Select the target headstage.

For a single headstage system, the active probe is always headstage number "1".

CCMode (amplifier current clamp)

Place the amplifier into Current-Clamp mode.

VCMODE (amplifier voltage clamp)

Place the amplifier into Voltage-Clamp mode.

Hold (IHold in CC-mode, VHold in VC-mode)

[±0.000,000,020 A (±20 nA), or ±1.000 V (±1000 mV)]

Set the active headstage holding level.

IHold (amplifier holding current, A)

[± 0.000,000,020 A (±20 nA)]

Set the active headstage holding level in

Current-Clamp mode.

IHoldOn (amplifier holding current On)

Enable the active headstage holding level in Current-Clamp mode.

VHold (amplifier holding voltage, V)

[± 1.000 V (± 1000 mV)]

VHoldOn (amplifier holding voltage On)

Enable the active headstage holding level in Voltage-Clamp mode

IGain (amplifier current gain, V/A)

Set the gain for the active 'Current' input channel using standard unit numbers (V/A) or scientific notation (1 mV/pA = "1e9"). The value is converted to a preset Gain level:

- 0.5 mV/pA
- 1 mV/pA
- 2.5 mV/pA
- 5 mV/pA
- 10 mV/pA
- 25 mV/pA

To help reduce signal saturation from too high a gain, a 90% threshold promotes the equation value to the next higher Gain setting.

VGain (amplifier voltage gain, V/V)

Set the gain for the active 'Voltage' input channel using standard unit numbers (V) or scientific notation (1 mV = "1e3"). The value is converted to a preset Gain setting:

- 10 mV/mV
- 20 mV/mV
- 50 mV/mV

- 100 mV/mV
- 200 mV/mV
- 500 mV/mV

To help reduce signal saturation from too high a gain, a 90% threshold promotes the equation value to the next higher Gain setting.

Filter (amplifier input filter, Hz)

Apply a preset filter level to the input channels:

- 500 (500 Hz)
- 1000 (1 kHz)
- 2000 (2 kHz)
- 5000 (5 kHz)
- 10000 (10 kHz)
- 20000 (20 kHz)

To help prevent over filtering, a 10% threshold promotes the equation value to the next higher filter level.

Offset (amplifier pipette offset, V)

[± 0.5 (± 500 mV)]

OffsetLock (amplifier pipette offset lock On)

[1 = On, 0 = Off]

VTrack (amplifier tracking potential, V)

[± 1 (± 1000 mV)]

Set a value and enable slow CC Tracking

VTrackOn (amplifier tracking potential On)

[1 = On, 0 = Off]

Enable slow CC Tracking

ECompMag (amplifier electrode compensation magnitude, F)

ECompPhase	(amplifier electrode compensation phase, fraction)
ECompOn	(amplifier electrode compensation phase On) [1 = On, 0 = Off]
CmComp	(amplifier cell compensation Cm, F) Set a cell capacitance value and enable cell capacitance compensation
RsComp	(amplifier cell compensation Rs, Ohm) Set a series resistance value and enable cell capacitance compensation
RsCompOn	(amplifier cell compensation On) [1 = On, 0 = Off]
RsCorr	(amplifier Rs correction, fraction) [0.00 – 1.00] Converted to a percentile
RsLag	(amplifier Rs correction lag, s)
RsCorrOn	(amplifier Rs correction On) [1 = On, 0 = Off]
Bridge	(amplifier bridge balance, Ohm)
Reset	(reset amplifier controls)
AutoEComp	(amplifier auto electrode compensation)
AutoCellComp	(amplifier auto cell compensation)
AuxOUT1	(Auxiliary Output-1, V)
AuxOUT2	(Auxiliary Output-2, V)
DigOUTWord	(Digital Output Word)
DigOUT1	(Digital Output-1)
DigOUT2	(Digital Output-2)

DigOUT3 (Digital Output-3)

DigOUT4 (Digital Output-4)

DigOUT5 (Digital Output-5)

DigOUT6 (Digital Output-6)

DigOUT7 (Digital Output-7)

DigOUT8 (Digital Output-8)

LockInPhaseAdj (set LockIn phase delay adjustment)

LockInAttenAdj (set LockIn attenuation adjustment)

[Equation field] A free-form text field. This field is evaluated and its value passed to the “target” function.

[Errors are reported under this field.]

Note: Values in the Equation field are rounded to whole numbers.

While Amplifier steps are configured in standard units (Amperes, Volts), the Amplifier Control Panel displays values in scaled units.

Check Equation Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports “Syntax is ok.”

Insert special identifier Acquisition, amplifier and reference settings are available for use in equations. (See_list_below.)

Each Sweep

Control the Paradigm operations on a “per sweep” basis of a Routine. Commands to be executed are inserted between the “EachSweep, Target” line and the “ForEachEnd” line.

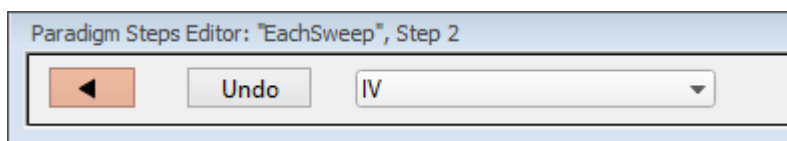


Figure 4-4. Step: Each Sweep

Default Setting: *ForEachSweep*
 EachSweep, Target=untitled
 ForEachEnd

Undo Removes any unsaved edits to this step.

[drop-down list] Select a Routine name from the loaded Routine Pool.

For example, when tracking whole-cell R_s values in real-time measurement graphs, update the Cell Compensation sweep-by-sweep, by inserting an Amplifier step (with its AutoCellComp command selected) within an EachSweep loop

Note: When using 'Each Sweep' to record data, the minimum sweep start-to-start time is +200 ms. For faster execution times, use the 'Routine' step.

Routine

Run acquisition.

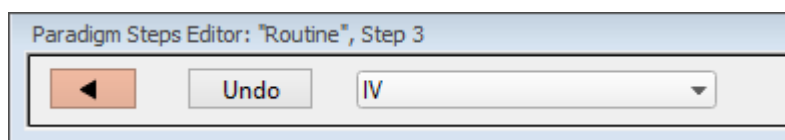


Figure 4-5. Step: Routine

Default Setting: *Routine, Target=untitled*

Undo Removes any unsaved edits to this step.

[drop-down list] Select an acquisition type, or a Routine to record a data Series.

Membrane Test Once

Indefinitely

Free Run

Duration [100 ms – 999.9 s]

Indefinitely

Add Channel

Clear

List of input channels

[<selected channels>]

List of Routine names from Routine Pool

[<selected Routines>]

Note: The time from starting this command to recording data is +300 ms.

“Single-stepping” this command (when no Paradigm is running) will create an auto-triggered Paradigm.

Analysis

Save an analysis to the Analysis Editor, or combine it with prior analyses.

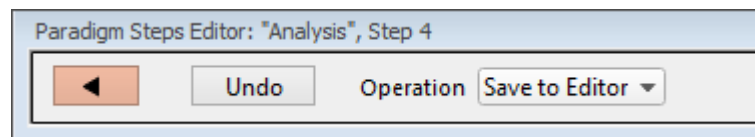


Figure 4-6. Step: Analysis

Default Setting: *Analysis, Operation=Save to Editor*

Undo Removes any unsaved edits to this step.

Operation

- Save to Editor Save the latest analysis
- Append to Last Append to the prior analysis
- Average with Last Average with the prior analysis
- Show Table Display analysis as numeric table
- Show Graph (1 – 8) Display analysis as visual graph

Camera

Take a single picture and/or run a live video preview. A Camera window is opened behind the Paradigm Editor and Scope windows.

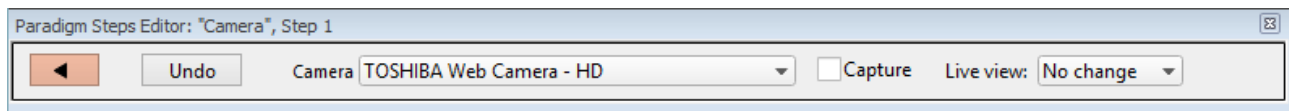


Figure 4-7. Step: Camera

Default Setting:	<i>Camera, Camera=_Camera_Name, Capture=true</i>
Undo	Removes any unsaved edits to this step.
Camera	Select a camera on the computer system.
Capture	Take a picture when executed.
Live view:	Configure the state of the live view: <ul style="list-style-type: none"> • No Change Keep last settings • Stop Stop live view • Start Start live view

Clear Key

Clears the 'Last key' field in the Paradigm Editor, which holds the last-pressed keyboard key since the start of the Paradigm.

Default Setting: *ClearKey*

Execute

Extend the functionality of SutterPatch by running an Igor command.

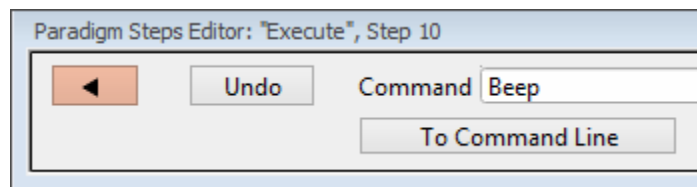


Figure 4-8. Step: Execute

Default Setting: *Execute, Command=Beep*

Undo Removes any unsaved edits to this step.

Command Run any Igor command accepted by the Command window, including user-created Functions.

Note: Igor syntax usually requires that open/close parentheses “()” be appended to the end of a command, however exceptions include the “beep” and “print” commands, for which no parentheses are used.

Special references can also be used within commands:

- p[#] n'th Paradigm variable.
- s[series-count, sweep-count, trace-count, routine name]

Reference an input trace (data wave) via counts of Series #, Sweep #, Trace # (Scope position), and the Routine name.

The “current” item is the “active” trace in the Scope window, and has a count value of zero.

If a “count” number is non-zero, it is used as an offset from the current count value of zero. Any fractions in count numbers are truncated to integers.

If the routine name is left blank, the current routine

name is used.

Ex: s[0,0,0,]

The current series,
current sweep,
current trace, of the
current routine.

- t[#] Reference the input trace (data wave) in Scope position “n” for the last sweep of the current Series.

Copy to Command Line Append the Command text to the Command window's Command line.

Expand to Command Line Append the Command entry to the Command window's command line after processing it to be compatible with Command window execution - any variables are replaced by their values.

Example 1: Reset the Timer.

Set the Execute 'Command' to:

Paradigm_ResetTimer()

Note the open and close parentheses at the end.

Example 2: Create a FFT graph of your data.

The Paradigm Steps:

1. ForEachSweep
2. EachSweep, Target=*YourRoutineName*
3. Execute,
Command=FFT/OUT=3/DEST=Voltage1_
FFT t[2]
4. If, Left=sweep, Operation="=", Right=1
5. Execute, Command=Display Voltage1_FFT
6. EndIf
7. Execute, Command=SetAxis Bottom 0,60

8. ResetTimer

9. ForEachEnd

In Step 2: Replace “*YourRoutineName*” with your own Routine name, or use the sample “IV” Routine.

In Step 3: The Igor ‘FFT’ command is run, and “t[2]” retrieves the Scope’s second input trace.

Export

Export data graphs into a Layout window.

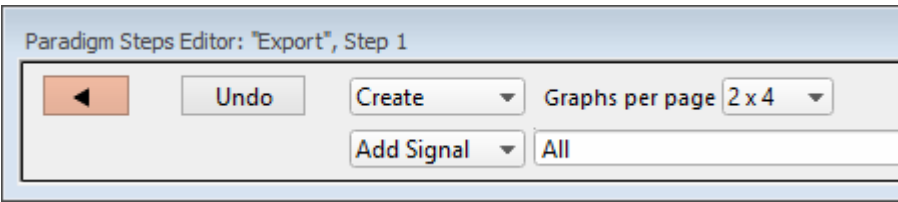


Figure 4-9. Step: Export

Default Setting: *Export, Signal=Layout*

Undo Removes any unsaved edits to this step.

[drop-down list]

Append Append graphs to an open Layout window, or into a new window.

Create Create a new Layout window.

Graphs per page Set the graph layout configuration for new Layout windows:

1 Graph fills entire page

2 Graphs stacked

3 Graphs stacked

2 x 2 matrix

2 x 4 matrix

[drop-down list]	Select signals to be exported from a list of default names.
Clear	Clear the signal field, set it to 'off'.
All	Selects all entries.
All Signals	Selects all input signals.
< List of input signals >	
All Analyses	Selects all Analysis graphs
< List of Analysis graphs >	
[List of selected signals]	User-edited names can be directly entered into the signal field.

Note: The sequence of signals is not used for positioning in the Layout window – signal positioning is based on their Scope window sequence.

Front Window

Set the specified window as the front window.

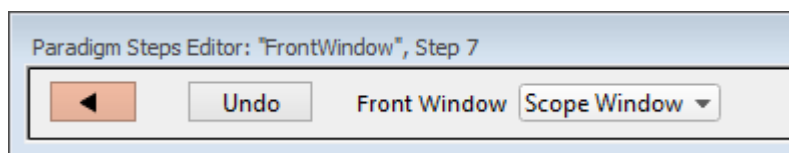


Figure 4-10. Step: Front Window

Default Setting:	Front Window, Target=Scope Window
Undo	Removes any unsaved edits to this step.
Front Window	Analysis Editor Camera Window Control Panel Dashboard Data Navigator Equation Editor Log Window

- Paradigm Editor
- Routine Editor
- Scope Window
- Shortcut Editor
- Solution Editor
- Template Editor

Hide Window

Hide the specified window.

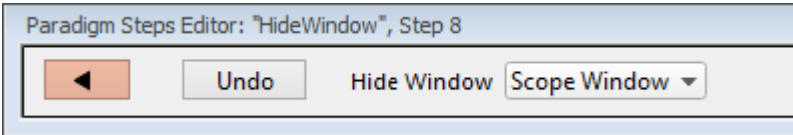


Figure 4-11. Step: Hide Window

Default Setting:	HideWindow,Target=Scope Window
Undo	Removes any unsaved edits to this step.
Hide Window	Analysis Editor Camera Window Control Panel Dashboard Data Navigator Equation Editor Log Window Paradigm Editor Routine Editor Scope Window Shortcut Editor Solution Editor Template Editor

Reset Timer

Reset the Paradigm Editor Timer to 00:00:00.

Default Setting: *ResetTimer*

Scope Operation

Control which Scope window signals are displayed, and how the sweep display operates.

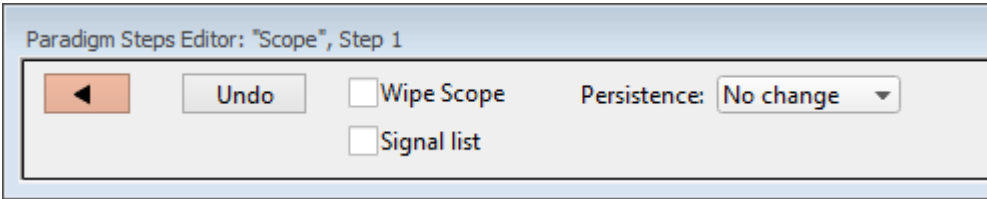


Figure 4-12. Step: Scope Operation

- Default Setting: Scope, Wipe=false
- Undo Removes any unsaved edits to this step.
- Wipe Scope Clears the Scope window of sweeps
- Persistence: No change
 On
 Off
- Signal list: Enable to display a list of input signals
- Add Signal

Clear
All Signals

Clears the signal list
Selects all signals
- [List of available input signals]
- [List of selected input signals]
- You can directly edit the list.
User-defined signal labels can
also be used.

Set Axis

Modify the Axis scaling of a signal.

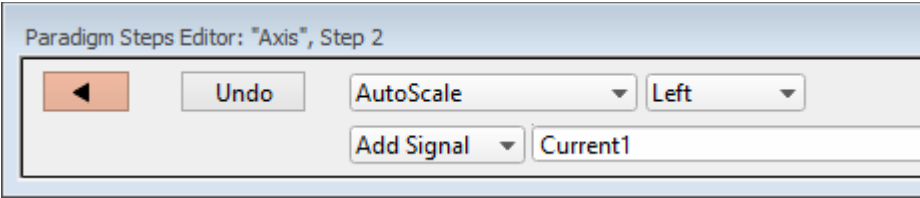


Figure 4-13. Step: Set Axis

Default Setting: *Axis, Axis=Autoscale, Kind=Left, Target=Current1*

Undo Removes any unsaved edits to this step.

[Drop-down list]

- | | |
|---------------------|--|
| Autoscale | Match the axis range to the data range |
| Autoscale from Zero | Display from zero to the largest value |
| Full scale | Display the full range of the axis |
| Use last | Keep using the last-used settings |

[Drop-down list]

- | | | |
|--------|---------------|-----------------|
| Left | Select Y-Axis | Add a signal |
| Bottom | Select X-Axis | For all signals |

Add Signal

- | | |
|-------------|------------------------|
| Clear | Clears the signal list |
| All Signals | Selects all signals |

[List of all input signals]

[List of selected signals] Select an input signal to modify from a list and/or directly enter user-defined signal labels.

Set Checkbox

Set Checkbox uses simple “on/off” toggles. Checkbox status can be read by ‘If’ and ‘ElseIf’ steps to make “yes/no” decisions and control the execution path of the Paradigm. If the equation evaluates to a non-zero value, the checkbox is enabled, i.e., ”on”.

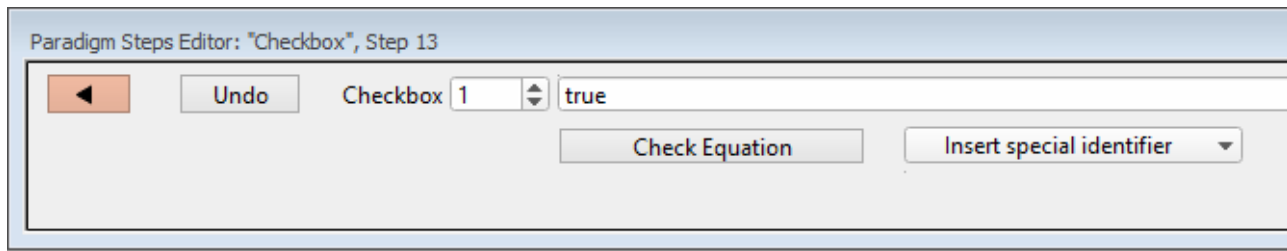


Figure 4-14. Step: Set Checkbox

Default Setting:	<i>Checkbox, Count=1, Equation=true</i>
Undo	Removes any unsaved edits to this step.
Checkbox	<p>Checkboxes [1 – 3] are local: they are cleared whenever a Paradigm is started.</p> <p>Checkboxes [4 – 6] are global: they are not automatically cleared, so their status persists across all Paradigms in the Experiment.</p>
[Equation field]	<p>A free-form text field, evaluated to a value, and applied to the Checkbox.</p> <p>[Errors are reported under this field.]</p>
Check Equation	<p>Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports "Syntax is ok."</p> <ul style="list-style-type: none"> • The constant "True" evaluates to '1.000'. • The constant "False" evaluates to '0.0000'.
Insert special identifier	Acquisition, amplifier and reference settings are available for use in equations. (See list below.)

Set Metadata

Define Metadata parameter values to apply. The “Set Metadata Paradigm Step Value” dialog opens.

Go to Preferences > Metadata to set the detail level.

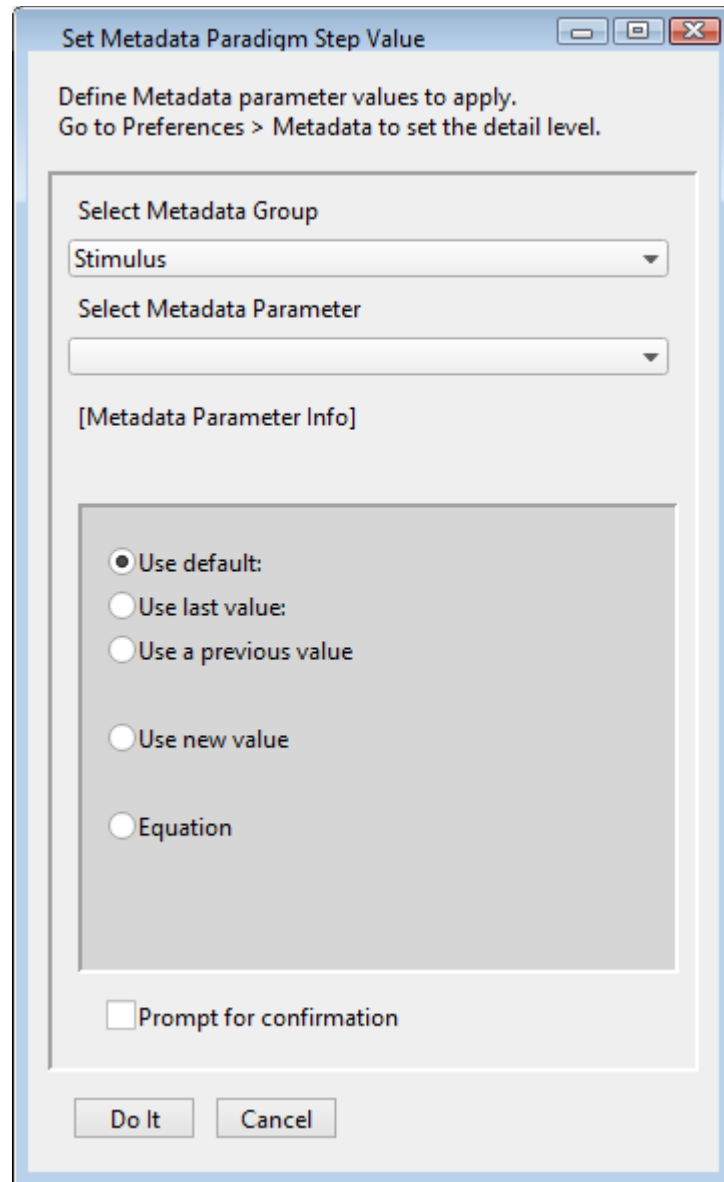


Figure 4-15. Step: Set Metadata

Select Metadata Group

- Operator (Full detail level)
- Preparation – Animal (Basic detail level)

- Preparation – Tissue (Basic detail level)
- Preparation – Cell (Basic detail level)
- Experiment (Basic detail level)
- Electrode (Extended detail level)
- Recording Solutions (Extended detail level)
- Paradigm (Full detail level)
- Cell Health / Quality Control (Full detail level)
- Series (= Routine Data) (Full detail level)
- Stimulus (Basic detail level)

Select Metadata Parameter

[Metadata Parameter Info]

- Use default:
- Use last value:
- Use a previous value
- Use new value
- Equation

[<Define Equation>]

Check

Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports "Syntax is ok."

Special identifier

Special functions for use in equations. (See list below.)

Prompt for confirmation

Display a metadata prompt before acquisition.

Set Solution

A “solution” command is used to turn solution valves ‘on’ or ‘off’ in perfusion systems. A predefined digital pattern or analog level can be automatically output with this step. Solution settings are configured and numbered in the Solution Editor.

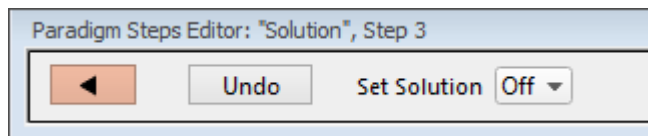


Figure 4-16. Step: Set Solution

Default Setting: *Solution, Target=Off*

Undo Removes any unsaved edits to this step.

Set Solution Select a solution number to activate its valve. The number of available solutions depends on the Solution Editor configuration.

[1 – 24]

Set Tag

A comment tag can be automatically written to the Paradigm metadata with this step. Enter the comment text in the ‘Tag text’ field. The last selection is used for all subsequent recordings within the same Paradigm.

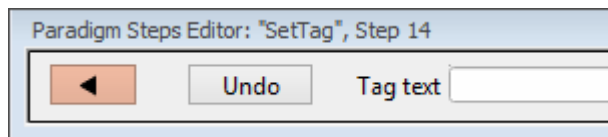


Figure 4-17. Step: Set Tag

Default Setting: *SetTag, Text=*

Undo Removes any unsaved edits to this step.

Tag text Enter the comment text.

Note: “Step” comment text is maintained separately from the manually triggered ‘Set Tag’ control comments.

Set Variable

Variables allow flexible control of any operation using equations.

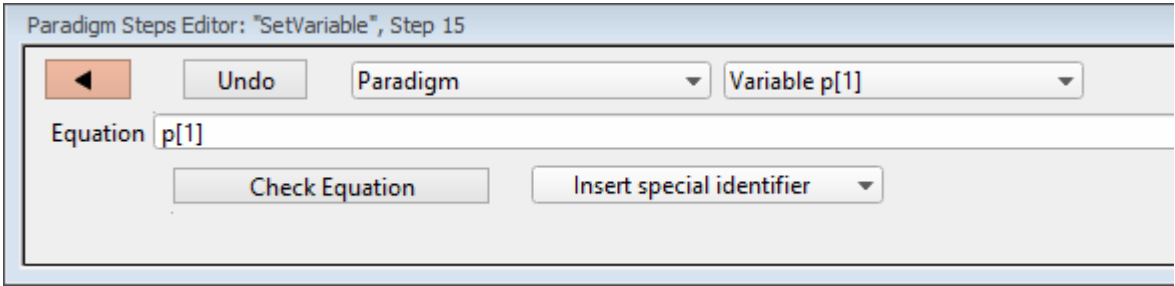


Figure 4-18. Step: Set Variable

Default Setting: *SetVariable, Target=Paradigm, Count=1, Equation=p[1]*

Undo Removes any unsaved edits to this step.

[List of targets]

- Paradigm Set the value of a Paradigm Variable_p[#]

[1 – 16, All Variables]

When 'All Variables' is selected, if varying values are desired, enter their values into the Equation field as a comma-separated list; simple equations (those without internal commas) can also be used in place of a value.) If there are more variables than list values, the “extra” variables are unchanged. If a list value is blank, the corresponding variable is unchanged.

- Paradigm_Input Set the value of the Paradigm Editor 'Input' control.
- < Routine Names > Select a Routine and set the value of its Variable_r[#]

[1 – 16, All Variables]

When 'All Variables' is selected, if varying values are desired, enter

their values into the Equation field as a comma-separated list; simple equations (those without internal commas) can also be used instead of a value.) . If there are more variables than list values, the “extra” variables are unchanged. If a list value is blank, the corresponding variable is unchanged.

Equation	Evaluates to a value to set variables or the Paradigm Editor ‘Input’ control. You can likewise set the value of a variable by inserting special identifiers; for example, ‘Input’ reads the ‘Input’ control. (See sample Paradigm ‘Tuning_with_Input’.)
Check Equation	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports "Syntax is ok."
Insert special identifier	Acquisition, amplifier and reference settings are available for use in equations. (See_list_below.)

Sound

Output a note from the computer speaker.

The frequency can be defined by a fixed value or an equation.

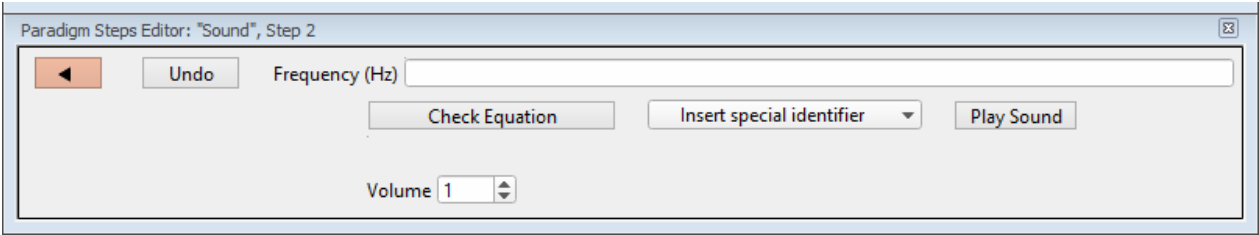


Figure 4-19. Step: Sound

Default Setting:	<i>Sound, Equation=, Volume=1</i>
Undo	Removes any unsaved edits to this step.

Frequency (Hz)	[250 – 8000]
	Specify as an equation or fixed value.
	The sound output has a linear frequency response range within its limits.
	< 250 Hz: two clicks
	> 8 kHz: 8 kHz tone
Check Equation	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports "Syntax is ok."
Insert special identifier	Acquisition, amplifier and reference settings are available for use in equations. (See_list_below)
Play Sound	Test the sound output.
Volume	[0.1 – 1.0]
	Use the spinners for 10% increments, or directly edit the field.

Output is via the standard sound output that Igor uses:

- Windows: Built-in speakers, or a computer sound card with external speakers
- Note: Lower frequency tones are attenuated in volume on lower-quality speakers
- macOS: Built-in speakers

This paradigm step can also be utilized as an Igor programming command. For instance, using an equation, one could listen to the membrane resistance of the cell under investigation

Example: Output a note.

Enter this equation in the Command window command line:

SutterPatch#Paradigm_PlaySound(400, 1)

[View Last](#)

Display the data from the last recording in a Scope (reanalysis) window.

Write Log

Enter text to be written to the Log window.

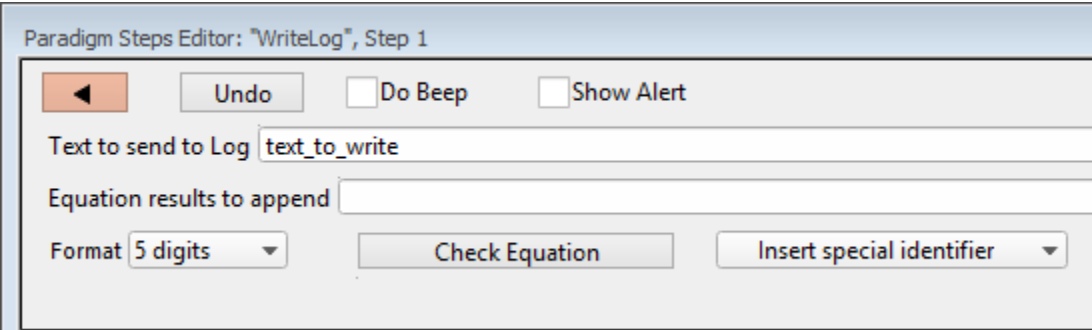


Figure 4-20. Step: Write Log

Default Setting:	<i>WriteLog, Alert=true, Text=text_to_write, Equation=, DoBeep</i>
Undo	Removes any unsaved edits to this step.
Do Beep	Generate a beep before writing.
Show Alert	Display and/or edit the Alert text, then write it to the Log.
Text to send to Log	[]
Equation result to append	[]
	Multiple equations in a comma-separated list can be evaluated.
Format	Time Date 1 – 12 digits
Check Equation	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports "Syntax is ok."
Insert special identifier	Acquisition, amplifier and reference settings are available for use in equations. (See list below.)

Run-time dialog: Write to Log Input

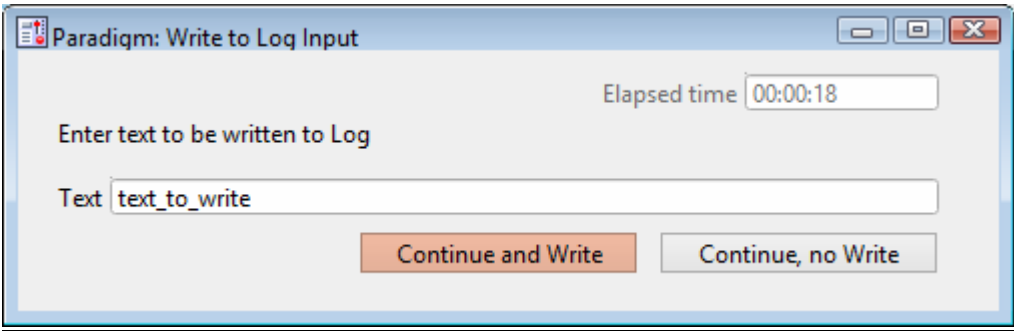


Figure 4-21. Write to Log Input

Elapsed time	A time counter for the Alert.
Text	Edit the text message.
Continue and Write	Write to the Log.
Continue, no Write	Do not write to the Log.

Alert

Display an “Alert” dialog box that pauses Paradigm execution until manually dismissed.



Figure 4-22. Step: Alert

Default Setting:	<i>Alert, Text=alert_text, DoBeep=true</i>
Undo	Removes any unsaved edits to this step.
Do Beep	Sound a “beep” from the computer
Text to show in Alert	Enter a message to the user.

Beep

Generate a “beep” sound from the computer speaker.

Default Setting: *Beep*

Comment

A text message can be displayed in a floating window.

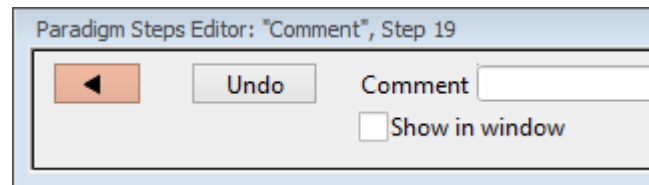


Figure 4-23. Step: Comment

Default Setting: *Comment, Text=*

Undo Removes any unsaved edits to this step.

Comment Enter the comment text.

Show in window A ‘Paradigm Comment’ window is displayed with the comment text, and closes when the paradigm ends.

Wait

Temporarily pause execution of the Paradigm for a defined duration.

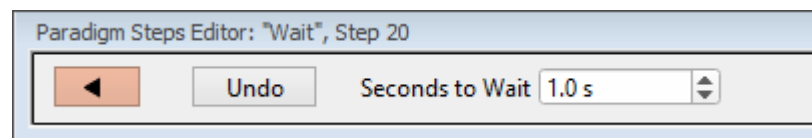


Figure 4-24. Step: Wait

Default Setting: *Wait, Time=1*

Undo Removes any unsaved edits to this step.

Seconds to Wait Click the spinners for 0.1 s increments, or type in a value. The precision of the wait time is 5 ms.

Pause

Pause execution of the Paradigm until the Resume button is manually clicked.

Break

Use a Break step to stop the execution of a Paradigm, or to interrupt For Loop and For Each Sweep loops.

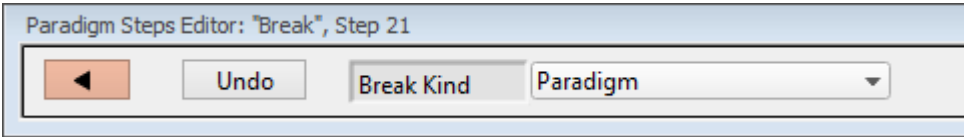


Figure 4-25. Step: Break

- Default Setting: *Break, Kind=Paradigm*
- Undo Removes any unsaved edits to this step.
- Break Kind: Paradigm
 ForLoop

Chain

Use to link step execution to another Paradigm.

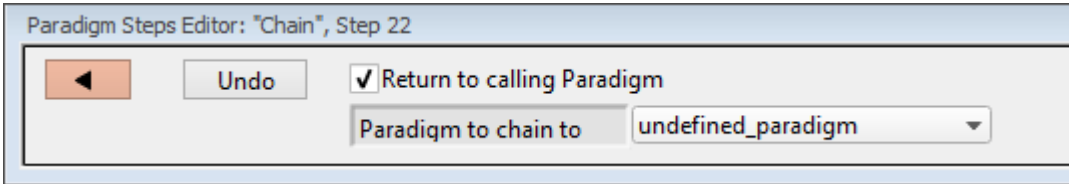


Figure 4-26. Step: Chain

- Default Setting: *Chain, Target=undefined_Paradigm, Return=true*
- Undo Removes any unsaved edits to this step.
- Return to calling Paradigm: Once execution of the target Paradigm has completed, return execution to this Paradigm.
- Paradigm to chain to: Paradigm execution will shift to the selected Paradigm.

For multiple Chains (or recursive calls), you can link a maximum of eight Paradigms.

For Loop

Use a standard programming “For loop” to repeat a set of steps.

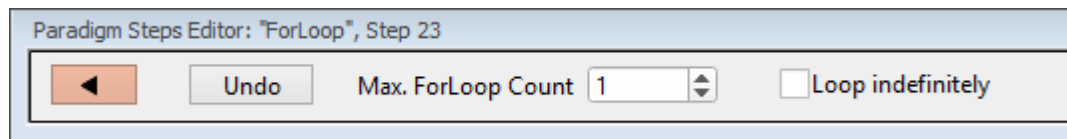


Figure 4-27. Step: For Loop

Default Setting: *ForLoop, Max=1*
 ForEnd

Undo Removes any unsaved edits to this step.

Max. ForLoop Count Number of loop cycles to run

Loop Indefinitely Sets ‘Max. ForLoop Count’ to ‘inf’

Note: A ‘For’ loop is processed as one step

Jump

Shift the Paradigm sequence to an arbitrary step. When executed, a jump occurs to the step after the target Label.

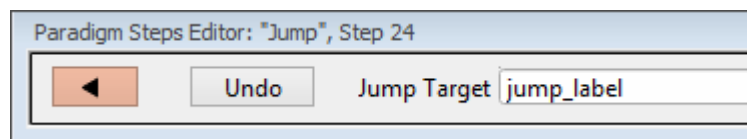


Figure 4-28. Step: Jump

Default Setting: *Jump, Target=jump_label*

Undo Removes any unsaved edits to this step.

Jump Target Enter the Label of the step to jump to.

Label

Create a Label for a Jump step.

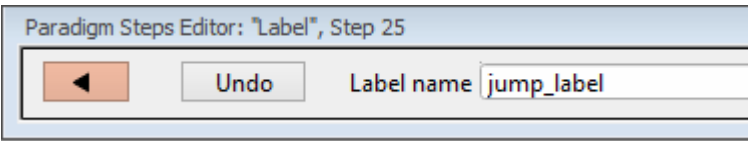


Figure 4-29. Step: Label

Default Setting:	<i>Label, Target=jump_label</i>
Undo	Removes any unsaved edits to this step.
Label name	Assign a name to the Label.

If

This step allows conditional Paradigm flow control between multiple choices.

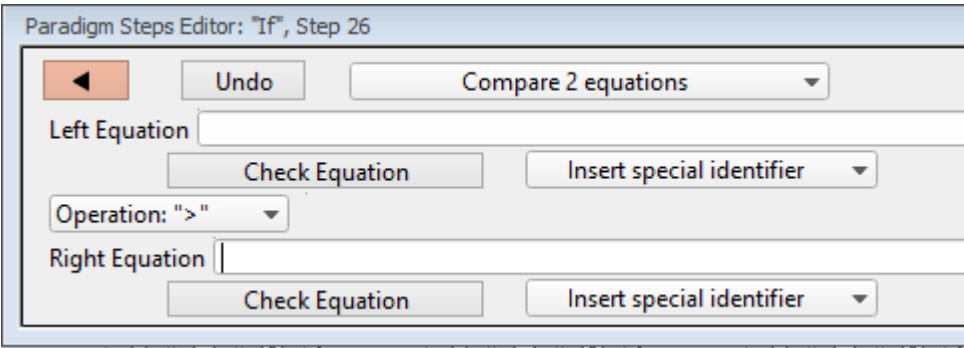


Figure 4-30. Step: If

Default Setting:	<i>If, Left=, Operation='>', Right= EndIf</i>
Undo	Removes any unsaved edits to this step.
[drop-down list]	Operation selection.
<ul style="list-style-type: none">• Compare 2 equations	
Left Equation	Evaluated to a value.
Check Equation	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports "Syntax

	is ok.”
Insert special identifier	Acquisition, amplifier and reference settings are available for use in equations. (See_list_below.)
Operation:	Comparison operators.
>	Greater than
>=	Greater than or equal to
=	Equal to
!=	Not equal to
<=	Less than or equal to
<	Less than
Right Equation	Evaluated to a value.
Check Equation	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports "Syntax is ok.”
Insert special identifier	Acquisition, amplifier and reference settings are available for use in equations. (See_list_below.)
• Check for key pressed	<p>“Last key” typed on the keyboard during the current Paradigm.</p> <p>The “Last key” field is cleared at the start of a Paradigm. .</p>
Key to check for	Enter a text key, or insert a “special” key.
Insert special key	Use a “non-text” key.
<ul style="list-style-type: none"> ○ Space ○ Return ○ Esc 	
• Check checkbox status	<p>Select a checkbox to monitor for “on/off” status.</p> <p>Checkboxes are displayed at the</p>

bottom of the Paradigm Editor window.

Checkbox

- [1 – 3] Paradigm-level “local” checkboxes, cleared at start of Paradigm.
- [4 – 6] Experiment-level “global” checkboxes, persist across Paradigms.

- Answer of yes-no-alert

- Do Beep Your computer beeps once when the alert displays.
 - Alert Text [] Enter your alert question text.

Run-time dialog

- Elapsed Time A time counter for the alert.
 - Yes ‘Yes’ button (value = 1)
 - No ‘No’ button (value = 0)
 - Stop Paradigm Manually abort the Paradigm.

ElseIf

Allow conditional Paradigm flow control between multiple choices.

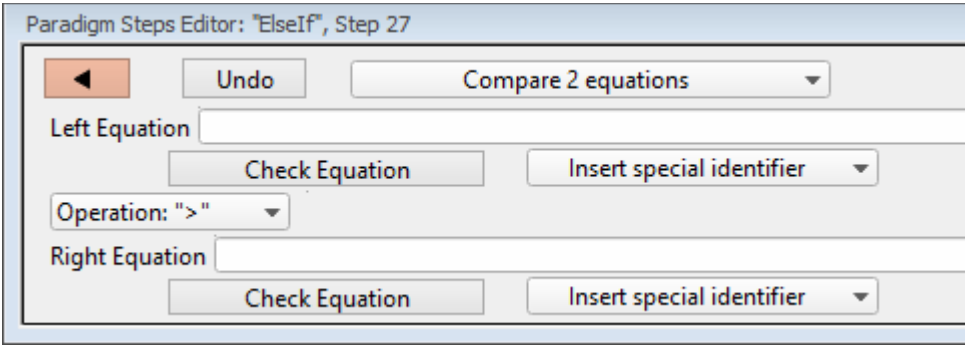


Figure 4-31. Step: Else If

- Default Setting: *ElseIf, Left=, Operation=>, Right=*
- Undo Removes any unsaved edits to this step.
- [drop-down list] Operation selection.

- Compare 2 equations

Left Equation	Evaluated to a value.
Check Equation	Check the equation syntax (for sweep #1). The equation is evaluated, and if valid, it reports "Syntax is ok."
Insert special identifier	Acquisition, amplifier and reference settings are available for use in equations. (See list below.)
Operation	Comparison operators.
>	Greater than
>=	Greater than or equal to
=	Equal to
!=	Not equal to
<=	Less than or equal to
<	Less than
Right Equation	Evaluated to a value.
Check Equation	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports "Syntax is ok."
Insert special identifier	Acquisition, amplifier and reference settings are available for use in equations. (See list below.)

- Check for key pressed

"Last key" typed on the keyboard during the current Paradigm.

The "Last key" field is cleared at the start of a Paradigm.

Key to check for	Enter a text key, or insert a "special" key.
Insert special key	Use a "non-text" key.
o Space	

- Return
 - Esc
- Check checkbox status Select a checkbox to monitor for “on/off” status.

Checkboxes are displayed at the bottom of the Paradigm Editor window.

Checkbox	
[1 – 3]	Paradigm-level “local” checkboxes.
[4 – 6]	Experiment-level “global” checkboxes.

- Answer of “Yes/No” alert

Do Beep Your computer beeps once when the alert displays.

Alert Text [] Enter your alert question text.

Run-time dialog

Elapsed Time A timer of how long the Alert has been displayed.

Yes ‘Yes’ button (value = 1)

No ‘No’ button (value = 0)

Stop Paradigm Manually abort the Paradigm.

Else

This step allows Paradigm flow control to continue to the next step if the previous condition fails.

Default Setting: *Else*

Checkboxes Checkboxes are useful for quick conditional control of Paradigm steps. They are visible at the bottom of the Paradigm Editor window.

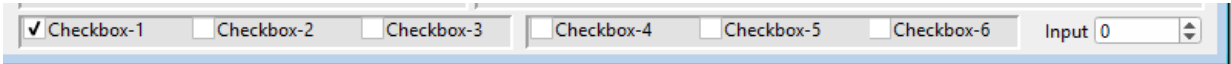


Figure 4-32. Checkboxes

- [1 – 3]

These “local” checkboxes are cleared when a Paradigm starts. They provide Paradigm-specific controls that are only valid for the current Paradigm session.
- [4 – 6]

These “global” checkboxes are cleared when an Experiment starts. They can be used across all Paradigm Pools for the entire Experiment.

Input

Routine and paradigm variables can be set to this value. Manually enter a value, or set via the paradigm step ‘Set Variable’.

- [-1.00 – 1.00]

This value can be rescaled when used in an equation.

Paradigm Variables Pane

You can Show/Hide a Paradigm Variables table at the bottom of the Paradigm Editor.

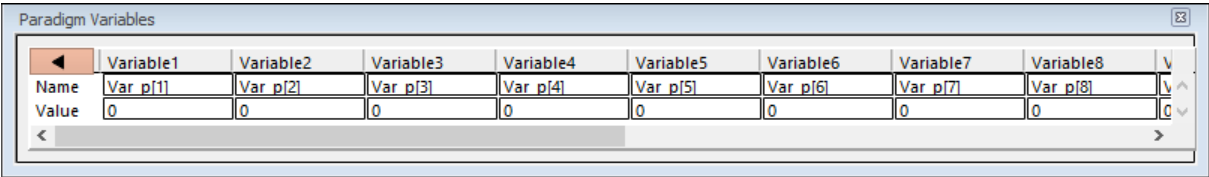


Figure 4-33. Paradigm Variables

These variable can be used in any equation, or in the paradigm step Execute, and persist across experiments. The table can be directly edited during non-acquisition, or set via the paradigm step Set Variable.

- ‘Close’ button

Closes the Variables table.
- Variable[1 – 16]

16 columns of Paradigm Variables.
- Name: Var_p[1 – 16]

Paradigm Variable names can be edited to any text.
- Note:

These names are for display only, and cannot be used in equations.
- Value: []

Numeric values can be manually entered, or programmatically set via

the paradigm step 'Set Variable'.

List of special identifiers

	Acquisition, amplifier and reference settings are available for use in equations.
Loop	(active paradigm ForLoop count)
Sweep	(active Routine sweep count)
	Sweep count of the active sweep in the Scope window.
LastSweep	(total number of sweeps in active Routine)
	During acquisition, this is set according to the routine parameters. Once acquisition terminates, this is replaced by the count of the last acquired sweep, i.e., the last sweep in the stored series.
	Processing can occur before or after the last sweep of a series.
	Example: Use in a 'ForEachSweep' loop Routine, to compare an 'If' step equation to the sweep number.
	<pre> ForEachSweep EachSweep, Target=IV If, Left=sweep, Operation='=', Right=LastSweep- 1 Alert, Text=LAST SWEEP, DoBeep=true EndIf ForEachEnd </pre>
AqStopped	(last acquisition was stopped)
	1= the last acquisition was stopped 0 = the last acquisition completed
ParadigmTime	(time at start of paradigm, s)
RoutineTime	(time at start of routine, s)
Stimulant	(last applied stimulant concentration)
	From the Solution Editor 'Concentration' setting, for a solution configured as a 'Chemical Stimulant'.
Time	(present date-time, s)
Timer	(timer time, s)

m[1..16]	(n'th analysis measurement value)
gx[1..8]	(n'th analysis graph x value) The X-value of the last data point in the latest version of graph[#]
gy[1..8]	(n'th analysis graph y value) The Y-value of the last data point in the latest version of graph[#]
r[1..16]	(n'th routine stimulus variable)
p[1..16]	(n'th paradigm variable)
Hold[1..8]	(holding of n'th output channel)
Input	(Input variable on paradigm window)

AuxIN[1..4]	(reading of auxiliary input, V) A single-point reading
Imon	(amplifier current reading, A)
Vmon	(amplifier voltage reading, V)
Mean[name or count,start,width]	(mean of given input signal) 'name' = signal name 'count' = window-signal position 'start' = time of start, s (of measurement range) 'width' = duration, s (of measurement range)

ActiveProbe	(active probe) [1 – 4] The “active” probe number is the Sutter headstage presently controlled by the Amplifier Control Panel. For a single headstage system, the active probe is always headstage number "1".
NumProbes	(number of probes) The number of IPA headstages attached to the system.

CCMode	(amplifier current clamp)

VCMODE	(amplifier voltage clamp)
Hold	(IHold in CC-mode, VHold in VC-mode) [in Routines] [$\pm 0.000,020$ A ($\pm 20,000$ pA), or ± 1.000 V (± 1000 mV)]
IHold	(amplifier holding current, A) [$\pm 0.000,020$ A (± 20 nA)]
IHoldOn	(amplifier holding current On)
VHold	(amplifier holding voltage, V) [± 1.000 V (± 1000 mV)]
VHoldOn	(amplifier holding voltage On)

IGain	(amplifier current gain, V/A)

The gain of the active voltage-clamp 'Current' input channel is implemented in discrete levels:

0.5	mV/pA
1	mV/pA
2.5	mV/pA
5	mV/pA
10	mV/pA
25	mV/pA

Use a preset discrete level in an equation, or else a 90% threshold is used to promote values between the preset levels (to help avoid saturation of the input signal.)

VGain	(amplifier voltage gain, V/V)
-------	-------------------------------

V/V evaluates to mV/mV.

The gain of the active current-clamp 'Voltage' input channel is implemented in discrete levels:

10	mV/mV
20	mV/mV
50	mV/mV
100	mV/mV

200 mV/mV

500 mV/mV

Use a preset level in an equation, or else a 90% threshold is used to promote values between the preset levels (to help avoid saturation of the input signal.)

Filter (amplifier input filter, Hz)

Apply a filter to the input channels.

Use a preset level in an equation, or a 10% threshold is used to promote values between the preset levels (to help avoid over-filtering).

500 (500 Hz)

1000 (1 kHz)

2000 (2 kHz)

5000 (5 kHz)

10000 (10 kHz)

20000 (20 kHz)

Offset (amplifier pipette offset, V)

OffsetLock (amplifier pipette offset lock On)

VTrack (amplifier tracking potential, V)

VTrackOn (amplifier tracking potential On)

ECompMag (amplifier electrode compensation magnitude, F)

ECompPhase (amplifier electrode compensation phase, fraction)

ECompOn (amplifier electrode compensation On in CC-mode)

CmComp (amplifier cell compensation Cm, F)

RsComp (amplifier cell compensation Rs, Ohm)

RsCompOn (amplifier cell compensation Rs On)

RsCorr (amplifier Rs correction, fraction)

RsLag (amplifier Rs correction lag, s)

RsCorrOn	(amplifier Rs correction On)
Bridge	(amplifier bridge balance, Ohm)

Relectr	(electrode/seal/access resistance, Ohm)
	Value from last Membrane Test
Rmemb	(membrane resistance (Cell mode), Ohm)
	Value from last Membrane Test
Cmemb	(membrane capacitance (Cell mode), F)
	Value from last Membrane Test
RMSNoise[1..2]	(membrane test RMS noise, A)
	Value from last Membrane Test
LockinAttenAdj	(Lockin phase delay adjustment, s)
LockinAttenAdj	(Lockin attenuation adjustment)

4.1.5 Routine Editor

Routines contain the settings that are in effect during data acquisition. The Routine Editor allows you to define acquisition parameters, set input and output channels, and to create stimulus waveforms and online analyses. The Routine Editor is the central place to create and manage saved Routine Pools and data acquisition settings.

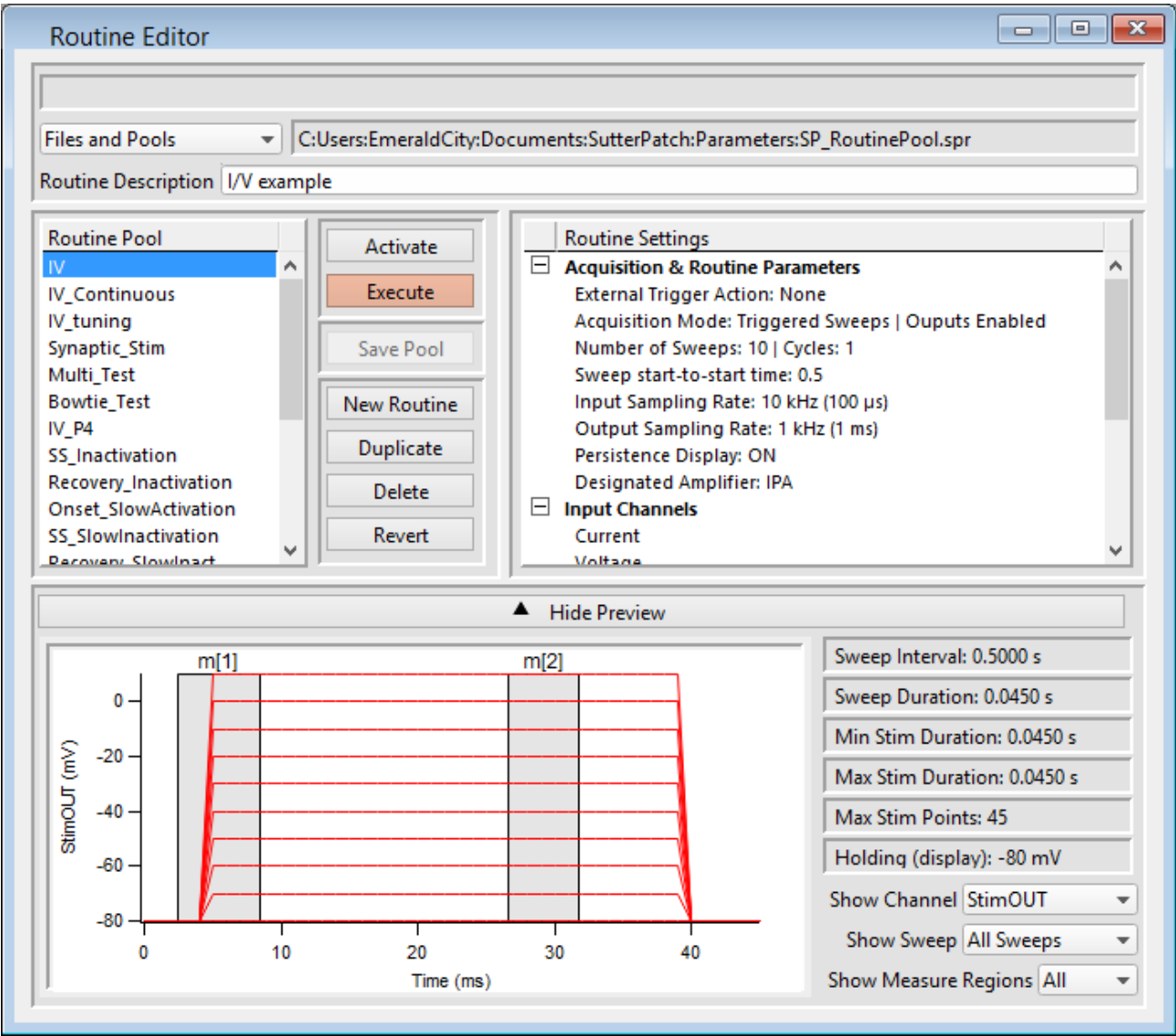


Figure 4-34. Routine Editor

The Routine Editor is structured to hold one or more Routines within its Routine Pool. The Routine Pool thus provides easy access to the set of Routines used in an experiment.

Tip: SutterPatch comes with a Sample Routine Pool that contains a collection of frequently used experimental scenarios. Rather than creating a new Routine, it might be easier to Duplicate a sample Routine and modify it until it meets your particular needs.

<u>Status Field</u>	Notifications on edits and Routine names are displayed here.	
<u>Files and Pools</u>	[drop-down list ...]	
	Most recently used list of the last 5 Routine Pool files	
	New Routine Pool	Create a new Routine Pool either with a blank

		default Routine, or populated with Routines from the currently loaded Routine Pool.
	Load Routine Pool	Load the Routines of a previously saved Routine Pool file into the Routine Pool
	Revert to Last Saved	Undo any unsaved changes to the Routine Pool
	Save Routine Pool	Save the Routine Pool using its existing file name and path.
	Save Routine Pool as	Save the Routine Pool to a new file, and switch to the new file. The default file name has an increment number appended to the original file name.
	Save Routine Pool Copy	Save the Routine Pool to a new file, but do not switch to the new file. The default file name has 'Copy of' prepended to the original file name.
	Merge Routine Pools	Insert the Routines from a previously saved Routine Pool file into the loaded Routine Pool.
	Merge PatchMaster PGF File	Insert the "Routines" from a PatchMaster PGF file into the loaded Routine Pool.
	Convert Routine Pool	Convert the loaded Routines to be compatible with the attached amplifier or emulation mode. The original file is overwritten.
	Send Last Used List to Command	Copy the pathname of the last used Routine and paste it into the Command window history.

Table 4-1. Routine Files and Pools

New Routine Pool sample dialog

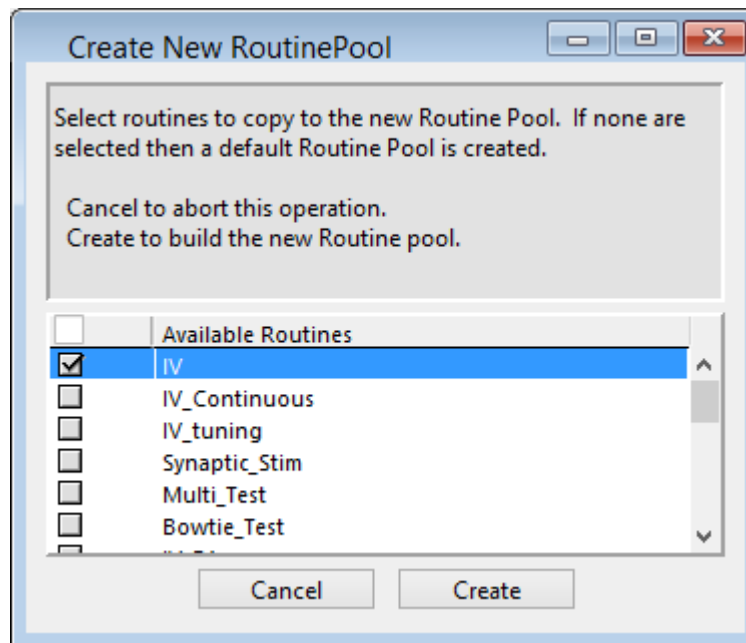


Figure 4-35. New Routine Pool

[File Path field]

The file path of the current Routine Pool displays on the right of the 'Files and Pools' list. If the Routine Pool has not been previously saved, this field is blank.

Routine Description

A Routine Description text comment can be edited and saved with the Routine.

Routine Pool

The Routine Pool section lists the names of all currently loaded Routines. Selecting a Routine name loads it into the Routine Settings section for editing and activation. As the Routine Pool contents are held in memory, the switching times between Routines are very fast.

Double click a Routine name to edit it.

- Allowable characters are A-Z, a-z, 0-9, and “_”.
- Special characters and spaces are not allowed.
- The maximum length of a Routine name is 22 characters.

Note: When a Routine is selected, if it was created for a different system (i.e., IPA vs. Double IPA), and the Preferences / Hardware / Routine Conversion is set to 'Prompt user', then a Routine Conversion dialog displays.

Activate	<p>Open or refresh the Scope window with the latest Routine settings, but do not start acquisition.</p> <p>This button is renamed to “In Progress” during a recording.</p>
Execute	<p>Open or refresh the Scope window and immediately start recording. The latest Routine settings are applied to the Scope window.</p> <p>This button is renamed to “Convert” if a routine designed for a different amplifier type is selected.</p>
Save Pool	Save the Routine Pool using its existing file name.
New Routine	Add a blank Routine to the Routine Pool, and open it for editing. The default Routine name is “untitled” with an increment number appended.
Duplicate	Add a copy of the selected Routine to the Routine Pool. The Routine name number is appended or incremented.
Delete	Remove the selected Routine from the Routine Pool.
Revert	Discard any unsaved changes to the selected Routine.

Table 4-2. Routine Editor Buttons

Waveform Preview

The stimulus waveform is graphically displayed at the bottom of the Routine Editor.

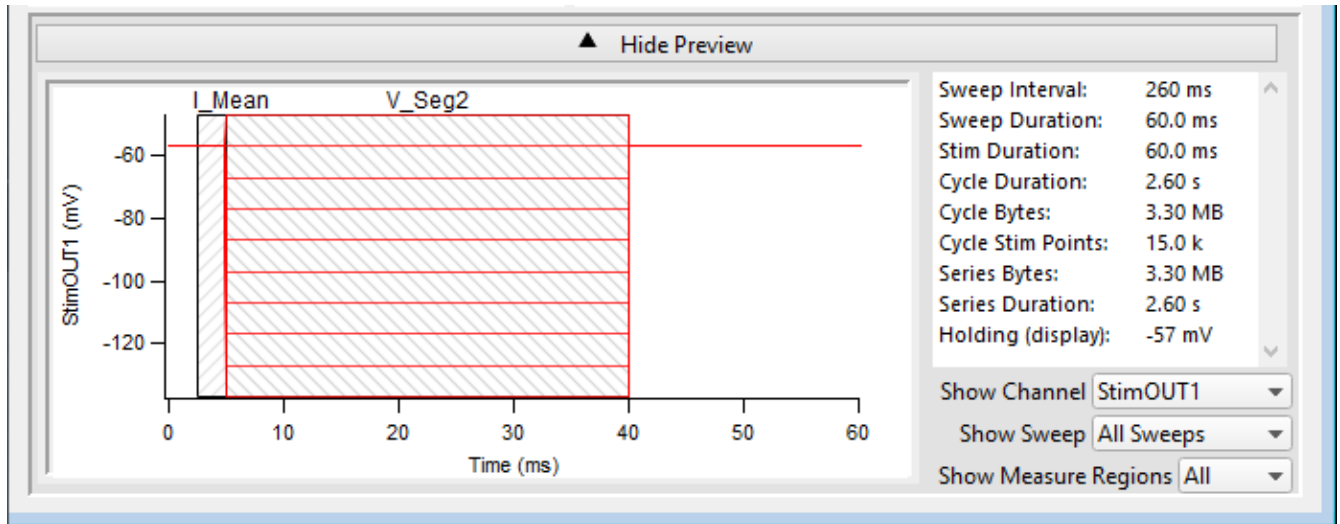


Figure 4-36. Waveform Preview Pane

The waveform preview and its settings are updated live to reflect changes in the Waveform Editor and Amplifier Control Panel.

‘Sweep Time’ measurement cursors can be manually dragged to update their Start/End settings in the Routine, but only if the Routine Settings section Real Time Measurements & Graphs is the active section; measurement cursors defined as relative to ‘Segment Time’ cannot be dragged.

The preview for the digital output ‘DigOUTWord’ sets its Y-axis to ‘Digital State (Word)’, and displays the decimal value of the selected bits.

Note: A “Cityscape” display mode is used, i.e. plotting with straight horizontal and vertical lines connecting the preview sample points (vs. smooth interpolated transitions).

X- and Y-axis Control

- Hover the mouse cursor over an axis line until the cursor turns into a double-headed arrow, then scroll up or down to contract/expand the axis.
- In the preview pane, click and drag the mouse cursor to surround a region of interest with a bounding box (the “marquee”). Right-click in the box and select one of the expand/shrink options.

Some key settings and display controls are listed on the right of the Preview pane.

Units are in ‘s’, or if < 1 s, then in ‘ms’.

Sweep Interval:

The interval of time between the starts of consecutive triggered sweeps (Sweep Start-to-Start Time) in the active Routine.

When set to ‘Shortest’, this equals the longest Sweep Duration + 200 ms.

Sweep Duration:	<p>The amount of time in a sweep during which signal recording occurs with the active Routine.</p> <ul style="list-style-type: none"> • Outputs enabled <p>The sweep duration is based upon the longest stimulus waveform duration set in Output Channels & Waveform / Waveform Editor.</p> • Outputs disabled <p>The sweep duration is based upon the longest duration set in Input Channels / Edit Signals / Waveform Editor.</p>
Stim Duration:	<p>The maximum amount of time during which output stimulation occurs in a sweep.</p> <p>Set in Output Channels & Waveform / Waveform Editor / Duration.</p>
Cycle Duration:	<p>The amount of time for a cycle.</p> <p>Set in Acquisition & Routine Parameters.</p>
Cycle Bytes:	The number of bytes of data in a cycle.
Cycle Stim Points:	The number of points in which output stimulation occurs in a cycle.
Series Bytes:	The number of bytes of data in the Series.
Series Duration:	The amount of time for the Series.
Preview Holding:	The holding level in the Amplifier Control Panel.
Show Channel:	A list of output channel to preview.
Show Sweep:	<p>Control the sweeps display in the preview pane (autoscaled).</p> <ul style="list-style-type: none"> • Time Course All sweeps in continuous linear time. • All Channels The first sweep of all channels. • All Sweeps All sweeps overlaid from time zero. • Sweep # Select a single sweep.
Show Measure Regions:	<p>A list of measurement regions to preview.</p> <ul style="list-style-type: none"> • None No regions displayed.

- All All regions displayed.
- m[#] Select a single region to display.

Routine Settings

The Routine Settings are split into 4 main sections. Click on a section header or item to open its sub-window.

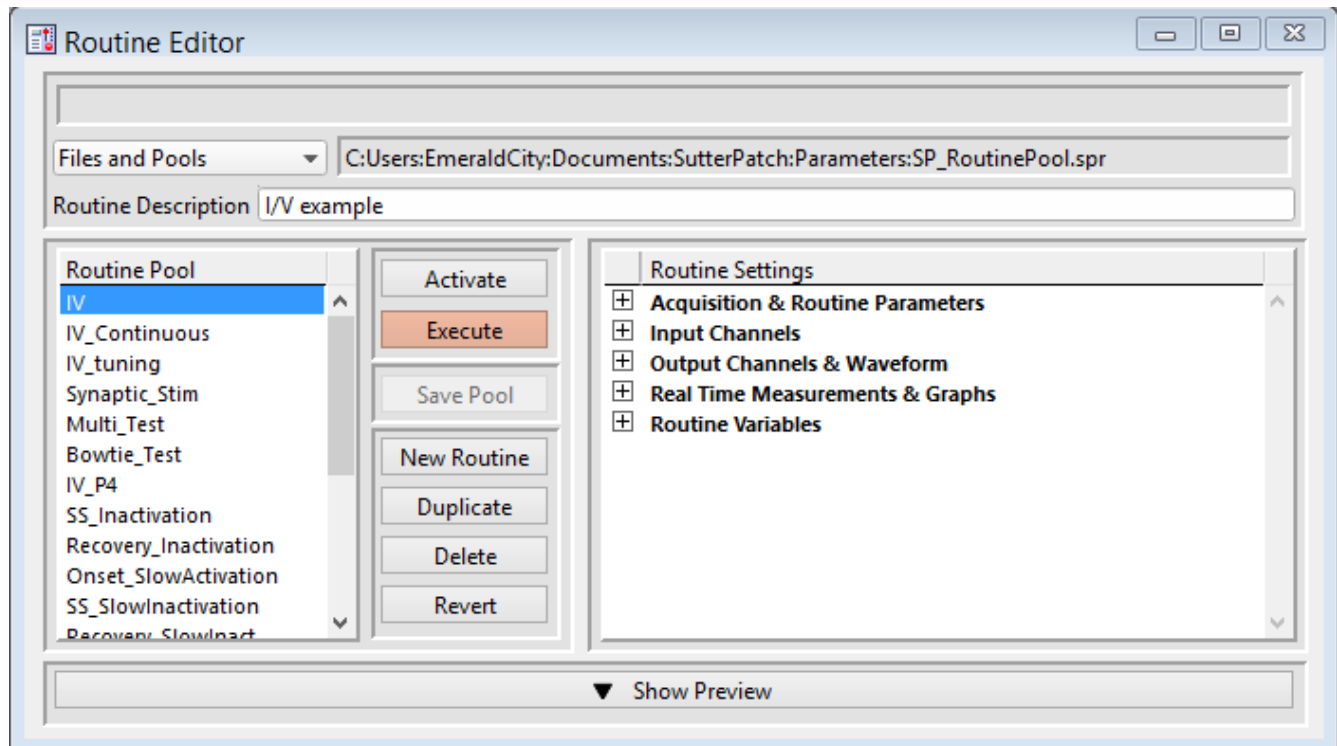


Figure 4-37. Routine Settings

Routine Editor: Acquisition & Routine Parameters

Acquisition timing parameters are controlled in this section, such as sweep duration and sampling rates. The settings in this section are shared by all input and output channels.

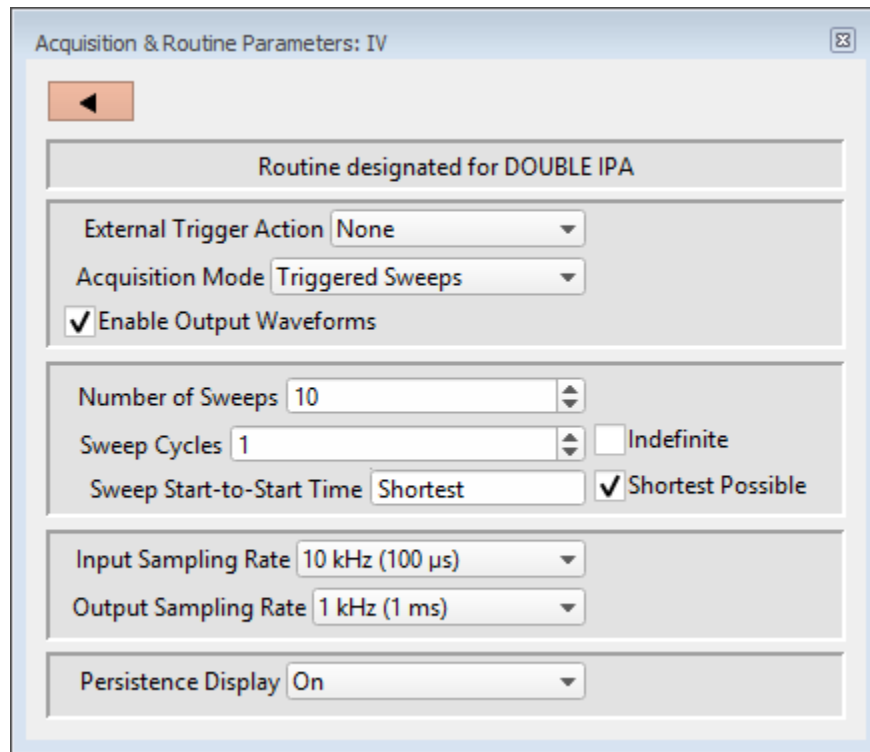


Figure 4-38. Acquisition & Routine Parameters

External Trigger Action

Control how and when recordings occur.

- **None:** Start a recording timed by the SutterPatch program. Hardware trigger inputs are ignored.
- **Start Sweep:** Use a hardware trigger to start recording the first sweep of a Series; each subsequent sweep waits for another external trigger pulse to start recording, until the Routine is completed.

To account for triggering delays, there is a short gap (~200 ms) between sweeps.

Use the digital input 'Trigger In' BNC on the back panel of the IPA amplifier.

However, if the Paradigm step 'Each Sweep' is run with the Routine, then the trigger is generated by the Paradigm.

Acquisition Mode

- **Triggered Sweeps:** Each sweep is started by a software trigger from a Routine or Paradigm, or by an external hardware trigger.

To account for triggering delays, there is a short gap (~200 ms) between sweeps. There can also be a few microseconds of jitter in the Sweep Start-to-Start time

- **Continuous Sweeps:** Uninterrupted recordings without time gaps between sweeps are supported in this mode, by setting the Sweep Start-to-Start Time equal to the Sweep Duration. Data recordings are displayed as sweeps (not a continuous or “rolling” display).

This option does not support:

- Pausing of sweeps during recording.
- Paradigm step ‘For Each Sweep’.
- Very short sweeps.

Also, there is the potential for data overrun from data processing overhead.

Enable Output Waveforms

Output channel waveforms are enabled by default, but can be optionally disabled. When outputs are disabled, sweep and segment durations (for analysis measurements) can be controlled via the Input Channels / Edit Signal / Waveform Editor.

Note: When output waveforms are disabled, the waveform information is discarded. To recover disabled waveforms, you will need to ‘revert’ the Routine, i.e. discard any changes made to the Routine.

Number of Sweeps [1 – 65000]

The number of sweeps to record.

Sweep Cycles [1 – 65000]

The number of times to automatically repeat the entire set of sweeps to be recorded within a single Series.

Indefinite The cycles are repeated until acquisition is stopped.

Note: A command waveform (if enabled) is only generated for the first cycle.

Sweep Start-to-Start Time [‘s’, or if < 1 s, then in ‘ms’]

The time from the start of recording a sweep to the start of the next sweep.

Shortest Possible The waveform duration + 100 ms for overhead processing.

Note: In demo mode, the sweep start-to-start times can vary during acquisition on slower computers.

Input Sampling Rate: Applies to all input channels.

<u>Sampling Rate</u>	<u>Sampling Interval</u>
100 Hz	(10 ms)
200 Hz	(5 ms)
400 Hz	(2.5 ms)
800 Hz	(2 ms)
1 kHz	(1 ms)
2 kHz	(500 μ s)
4 kHz	(250 μ s)
5 kHz	(200 μ s)
8 kHz	(125 μ s)
10 kHz	(100 μ s)
20 kHz	(50 μ s)
25 kHz	(40 μ s)
40 kHz	(25 μ s)
50 kHz	(20 μ s)
100 kHz	(10 μ s)
200 kHz	(5 μ s)

Tip: A 10 kHz input channel sampling rate is standardly used, with 20 kHz occasionally used for faster events, and 5 kHz for slower events.

Note: Slow acquisition can take a long time to respond, due to 16k buffers to fill.

Output Sampling Rate: Applies to all output channels.

<u>Sampling Rate</u>	<u>Sampling Interval</u>
100 Hz	(10 ms)

200 Hz	(5 ms)
400 Hz	(2.5 ms)
800 Hz	(2 ms)
1 kHz	(1 ms)
2 kHz	(500 μ s)
4 kHz	(250 μ s)
5 kHz	(200 μ s)
8 kHz	(125 μ s)
10 kHz	(100 μ s)
20 kHz	(50 μ s)
25 kHz	(40 μ s)
40 kHz	(25 μ s)
50 kHz	(20 μ s)
100 kHz	(10 μ s)
200 kHz	(5 μ s)

Note: The sample Routines use a 1 kHz default output channel sampling rate, as command waveforms usually do not require high resolution time changes - increase this setting as needed.

Persistence Display

For a recording, display each new sweep without erasing any previous sweeps.

- Off Set the Scope window into non-persistence display mode.
- On Set the Scope window into persistence display mode.
- Keep current setting Do not change the Scope window's prior setting.

Routine Editor: Input Channels

Configure the input channels.

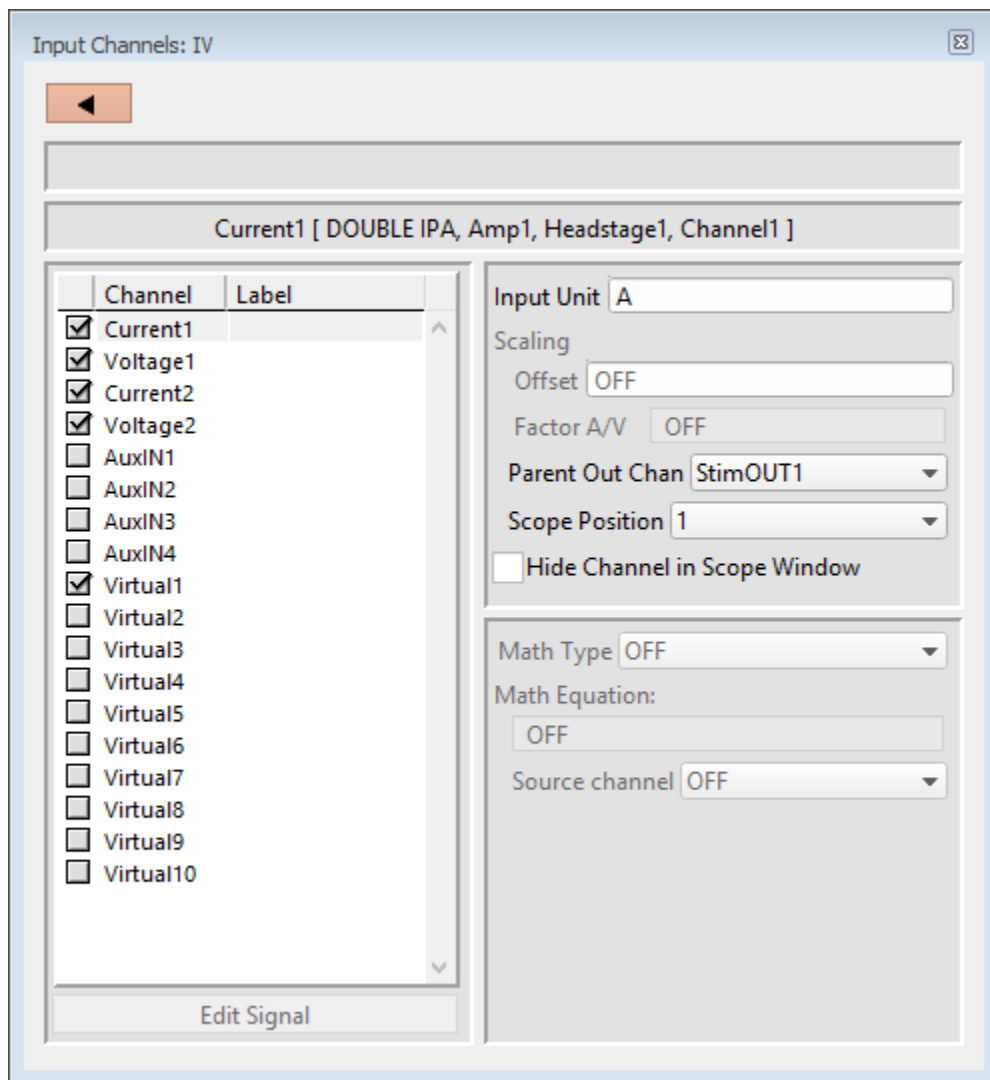


Figure 4-39. Input Channels

[Status field] Notifications on edits are displayed.

[HW Status field] Hardware information on the selected channel is displayed.

Channel

Enable up to 16 Input Channels for recording data:

Current# Analog input current channels hardwired from the IPA headstage.

Voltage# Analog input voltage channels hardwired from the IPA headstage.

AuxIN[1 – 4] Four auxiliary analog input channels allow you to directly digitize and record input signals from connected non-Sutter

external equipment.

Note: In Emulation mode, the AuxIN channels display a ± 20 mV sine wave.

Virtual[1 – 10] Ten virtual channels are available.

Virtual channel data are mathematically transformed data from another input channel, or are entirely computed from an equation.

Label

A user-editable signal name for a channel.

These labels are used in:

- Routine Settings overview for Input and Output Channels
- Parent Out Chan'
- Virtual channel Math Equations and Source Channels
- Scope window signal panes
- Data Navigator Preview pane
- Metadata Input Signal Name

To rename an Input Channel, first enable it, then double-click it and enter the new name. If the label is used by another channel, an underscore and increment number are appended to the new label.

Note: If a virtual input channel is set to 'Math Type: Leak', a default 'LeakSubtracted' label is automatically created.

Edit Signal

When Output Waveforms are disabled in the Acquisition Parameters section, the Input Channel timing can be set in a modified Waveform Editor. The segment timing can be used by cursors to make measurements.

Input Unit

The base unit of measurement.

- | | |
|----------------------|---|
| • 'Current' Channels | Fixed at 'A' for current. |
| • 'Voltage' Channels | Fixed at 'V' for voltage. |
| • 'AuxIN' Channels | Default is 'V', but is editable. |
| • 'Virtual' Channels | Same as its Source channel units; is only |

editable for virtual Math Type 'Equation'.

Note: The resolution of the signal units are automatically set.

Scaling [for AuxIN and Virtual Input channels]

Offset Apply an amplitude offset to an input signal (after any scaling).

Tip: To use 'mV' units, enter: #m' or '#e-3'
To use 'pA' units, enter: #p' or '#e-12'

Factor Change the scaling factor of input channels using a fixed value or equation.

Note: The IPA digitizer uses a high-resolution 16-bit ADC with 64-bit data, so data resolution is not an issue when scaling input signals.

Parent Out Chan

The "Parent Output Channel" shows which output channel is associated with an input channel. This also controls the Segment timing for measurement 'Cursors Relative to Segments'.

For Auxiliary Input channels, you can change the 'Parent Out Chan'.

Virtual channels cannot directly select Parent Output channels. Instead, they utilize their Source channel Parent channels.

Scope Position The Input Channel panes can be repositioned in the Scope window.

Hide Channel in Scope Window

The selected input channel is hidden in the Scope window.

Virtual Input Channels

If a virtual input channel is enabled, its configuration fields are ungrayed:

Math Type Apply a data transformation to a virtual input channel.

BaselineSubtract Subtract a value from an input trace.

Source channel Select an input channel to process.

Baseline From Select the value to subtract.

- Value Subtract a fixed value from the input trace.

Value Spinner adjusts in 1 pA or 1 mV increments.

- Trace Subtract from the input trace.
 - Sweep Time Subtract the average of the Start/End Time data from the input trace.
- | | |
|------------|---|
| Start Time | Set the starting time of the data to be averaged. |
| End Time | Set the ending time of the data to be averaged. |
- Segment #s Subtract the average of a Segment from the input trace.
- | | |
|-------------|--|
| Start Ratio | Set the starting time of the data to be averaged, as a ratio relative to the Segment duration. |
| Start Time | [reported value] |
| End Ratio | Set the ending time of the data to be averaged, as a ratio relative to the Segment duration. |
| End Time | [reported value] |

BesselFilter A frequency-domain filter.

- | | |
|----------------|------------------------------------|
| Source channel | Select an input channel to filter. |
| Filter Band | Select a frequency range. |
- LowPass Allow signal frequencies less than the cutoff frequency.
 - HighPass Allow signal frequencies greater than the cutoff frequency.
- | | |
|-----------------------|--|
| Filter Order | [1, 2, 4, 8] |
| | Number of “poles” in the filter. |
| Cutoff Frequency (Hz) | [100 to < ½ the sampling rate] |
| | Restrict frequencies from this boundary point onwards. |

Differentiate Data transformation using differentiation.

- | | |
|----------------|---|
| Source channel | Select an input channel to differentiate. |
|----------------|---|

Equation Specify an equation to create virtual data.

(See the Equation Editor for more details.)

Source channel Transform data from the selected input channel.

Equation [] Click field to access the ‘Specify math equation’ editor.

Note: The full equation is always visible as a tool tip, by hovering the mouse cursor over the ‘Math Equation’ field.

Specify math equation for virtual signal

[<equation>] A free-form text field.

[Errors are reported under this field.]

Check Equation Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports that the syntax is “ok”.

Insert special identifier Special references can also be used within commands.

- p[#] n’t h paradigm variable
- s[series-count, sweep-count, trace-count, routine name]

Reference an arbitrary input trace (data wave) via counts of Series #, Sweep #, Trace # (Scope position), and the Routine name.

The “current” item is the “active” trace in the Scope window, and has a count value of zero.

If a “count” number is non-zero, it is used as an offset from the current count value of zero. Any fractions in count numbers are truncated to integers.

If the routine name is left blank, the current routine name is used.

Ex: s[0,0,0,]

		The current series, current sweep, current trace, of the current routine.
	• t[#]	n'th input trace
		The trace wave of the input channel in Scope Position "n" is accessed using this substitute for the data folder path.
		You can duplicate a trace by using a virtual signal of Math type "equation" with the equation "t[1]" for the first Scope signal.
	Undo	All changes in the equation editing session are discarded.
Integrate	Data transformation using integration.	
	Source channel	Select an input channel to integrate.
Leak	Remove leakage current from the data.	
	Source channel	Select an input channel to transform.
	Show leak	Displays the accumulated leak currents after the subtracted data in a sweep.
	Leak zero segment	Identify a segment with no active cellular response to the command signal.
		When set to zero, the field is set to 'OFF'. To re-display the numeric spinners, enter a non-zero number into the field.
	Note:	The mean of the second half of the specified segment is used to compute an averaged leak current, which is then used to correct the P/N leak average. This option reduces the influence of a constant leak-current, which is otherwise added to the leak current of the main pulse.
LineFreq	Remove AC line frequency noise (hum) from the data.	
	Source channel	Select an input channel to apply noise reduction to.
	Line Frequency	50 Hz

60 Hz

Alternating current (AC) power contains 50 or 60 Hz oscillations that can cause sinusoidal line-frequency noise in recorded signals. This FFT-based filter reduces such noise by > 90% over 6 harmonics. The adjusted signal is displayed in real time.

LockIn Measure cell characteristics (such as membrane capacitance) with high signal-to-noise sensitivity using a dual-phase software lock-in amplifier.

This feature is only enabled when the Routine includes an output channel with a waveform set to 'Sine / Sine Wave Cycles / For LockIn'.

Note: Calculations are made in voltage-clamp mode, using 'conductance' (1 / resistance) instead of 'resistance'.

Current Channel Select an input channel with a current signal.

Trace kind	CM	Computed membrane capacitance.
	GM	Computed membrane conductance.
	GS	Computed series conductance.
	DC	DC component of measured signal.
	RealY	Real number part of the lock-in response signal.
	ImagY	Imaginary number part of the lock-in response signal.

The selected 'Trace kind' is automatically set as the Virtual Channel label.

Cycles to Average	[1 – 1000]	
Cycles to Skip	[1 – 1000]	
V-reversal	[±1000 mV]	When using a calculated stimulus trace.

Math used for the LockIn computation:

$$\text{Factor} = (2.0 / \text{SinePointsPerCycle}) / \text{sine_amplitude}^2$$

$$A = \text{Factor} * \sum (\text{current} * \text{stim_real}) \\ \sum \text{ over one SinePointsPerCycle}$$

$$B = \text{Factor} * \sum (\text{current} * \text{stim_imag}) \\ \sum \text{ over one SinePointsPerCycle}$$

$$\text{DC} = 1/\text{SinePointsPerCycle} * \sum (\text{current}) \\ \sum \text{ over one SinePointsPerCycle}$$

$$\text{Phase} = \frac{\text{VC-mode}}{\text{atan}(B/A)} = \frac{\text{CC-mode}}{\text{atan}(B/A)}$$

$$\text{RealY} = A = A / (A^2 + B^2)$$

$$\text{ImagY} = B = B / (A^2 + B^2)$$

$$\text{Omega} = (2 * \pi) / \text{SineCycleDuration}$$

$$\text{Gt} = \text{Idc} / (\text{Vdc} - \text{Et})$$

$$\text{Gs} = (A^2 + B^2 - A * \text{Gt}) / (A - \text{Gt})$$

$$\text{Gm} = \text{Gt} * \text{Gs} / (\text{Gs} - \text{Gt})$$

$$\text{Cm} = (A^2 + B^2 - A * \text{Gt})^2 / ((A - \text{Gt})^2 + B^2) / (\text{Omega} * B)$$

Smooth Smooth the data with a “moving average” noise-reduction filter.

Source channel Select an input channel to smooth.

Smoothing type:

- Gaussian A standard filter with excellent 10 – 90% rise-time response.
Smooth operations [1 – 32767]
of smoothing operations to perform.
- Boxcar A fast time-domain filter with excellent 0 – 100% rise-time response.
Smooth repetitions [1 – 32767]

		# of smoothing repetitions to perform.
	Boxcar window points	[1 – 99] # of points in boxcar window (odd values only).
Stimulus	Replicate the Command Waveform.	
	Source channel	Select an input channel – the waveform from its ‘Parent Out Chan’ is used.
SweepAverage	Average the input traces.	
	Source channel	Select an input channel to process.
	Average Type	Cumulative Run Average Number of Sweeps
SweepSubtract	Subtract a sweep from the input trace.	
	Source channel	Select an input channel to process.
	Source channel	Select an input channel to process.
	Reference Sweep	The sweep that is subtracted from all other sweeps. If the sweep does not yet exist, no subtraction occurs.

Routine Editor: Output Channels & Waveform

Configure the output channels and command waveform.

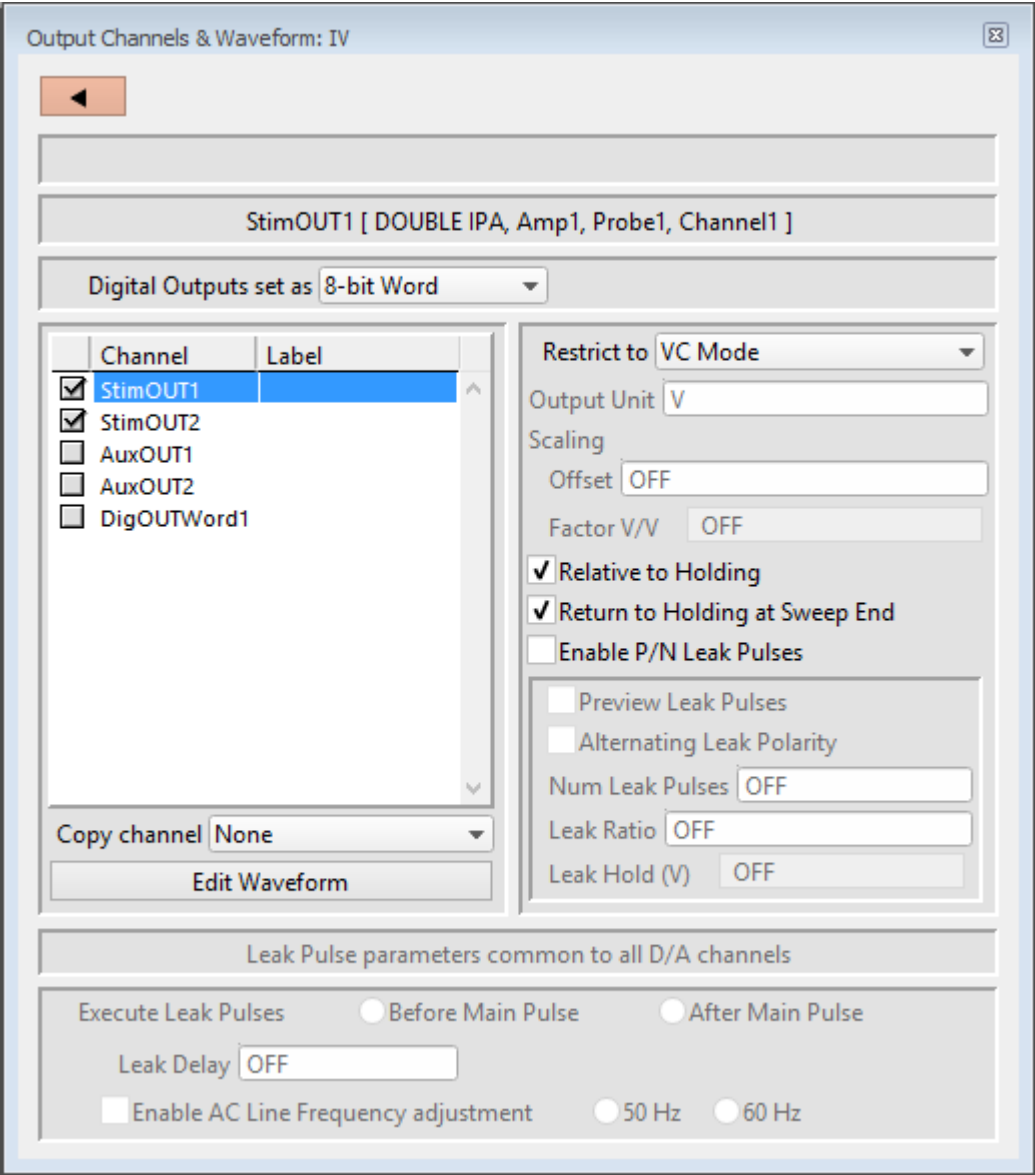


Figure 4-40. Output Channels & Waveform

[HW Status field] Hardware information on the selected channel is displayed.

Digital Outputs set as

Eight digital output channels (bits) can be set individually or as a group.

- Individual bits Each DigOUT(1 – 8) bit is individually set in its own Waveform Editor table.

The waveform preview uses the bit’s binary word value for its Y-axis value, i.e. if bit 3 is ‘HIGH’, it has a ”word” value of 4.
- 8-bit Word The 8-bit digital output pattern is controlled by a single decimal number (0 - 250), which is also the

waveform preview amplitude value.

The waveform preview uses the binary bit pattern word value for its Y-axis value, i.e., if bits '1' and '3' are 'HIGH', the 8-bit word has a value of 5.

Edit Waveform

Click the Edit Waveform button to access the Waveform Editor table and create a stimulus waveform. Or alternatively, double-click on an Output Channel name. (See the Waveform Editor section below.)

Channel

Click on the Output Channel checkboxes to enable analog and digital output channels in the Routine. Click on a channel name to highlight and select it – the channel output parameters are displayed for configuration.

The default StimOUT channels are hardwired to the IPA headstage.

The two rear panel auxiliary analog output channels (AuxOUT1 & 2) can be used to send stimulus waveforms to external instruments.

The digital outputs are available as either a single 8-bit “word”, or as 8 individual 1-bit channels, as set in the Acquisition & Parameters section.

To rename any of the Output Channels, double-click on the respective name and enter a new one.

Tip: If a signal is connected to the front panel ‘COMMAND IN’ BNC, that signal is summed with the StimOUT waveform that is sent to the headstage.

Note: For StimOUT channels, the actual DAC output signal is passed through a 20 kHz low-pass filter before entering the headstage.

Label

A user-defined signal name for the channel.

These are used in:

- ‘Copy Channel’
- Waveform Preview pane ‘Show Channel’
- Metadata: Output Signal Name

To rename an Output Channel, first enable it, then double-click it and enter the new name. If the same label is reused for another channel, an underscore and increment number will be appended to the new label.

Copy channel

Copies one channel waveform to another channel, but only works between output channels of the same type (i.e., “Stim”, “Aux”, or Digital). If a channel is enabled, then highlighting another or blank channel of the same type ungrays its ‘Copy channel’ field, and changes it from ‘OFF’ to ‘None’, with a drop-down list of available channels to copy from.

Restrict to

The routine’s IPA headstage StimOUT channels have to match the VC/CC mode of the IPA amplifier, or else the Routine cannot be activated or executed.

The default setting for new routines is ‘VC Mode’. This prevents CC mode pA (10^{-12} A) current outputs from being accidentally overscaled by VC mode routines using mV (10^{-3} V) voltage outputs.

- VC Mode The Amplifier Control Panel must be in VC mode to run this routine.
- CC Mode The Amplifier Control Panel must be in CC mode to run this routine.

Note: The IPA amplifier can be switched into any mode (VC or CC) while a recording is in progress, however it is your own responsibility to interpret data from mixed recording modes.

Output Unit

Enter the base unit of measurement. The signal unit resolution is automatically adjusted.

- StimOUT Channels

Fixed at ‘V’ for voltage clamp and “other” experiment types; fixed at ‘A’ for current clamp experiments.

- AuxOUT Channels

Set at ‘V’, but can be edited to any setting.

Scaling

Offset Apply an offset to the Auxiliary output channel (after any scaling.)

Factor Apply a scaling factor as a fixed value or an equation.

(See the Equation Editor for more details.)

Relative to Holding

If 'Relative to Holding' is enabled, the output signal is the command waveform summed with the 'Holding' level in the Amplifier Control Panel. If the Holding level is set to '0', this setting has no effect.

Note: This setting is updated live by holding level changes in the Amplifier Control Panel.

Return to Holding at Sweep End

During a Series, set the output signal to the holding level while the system is not acquiring data, i.e., the time between sweeps after a sweep acquisition ends, but before the the next sweep starts.

This ensures that your cells are kept in a resting state as much as possible, and that each output sweep starts from the same holding level.

When 'Return to Holding at Sweep End' is not enabled, the output signal uses the last value of the command waveform during the time between sweeps, and returns to the original holding potential (as shown on the Amplifier Control Panel) when the routine stops.

Note: You might want to avoid returning to an unwanted holding level at the end of a series, as such a situation could send a short but disruptive glitch to your preparation. To avoid this, create a Paradigm and set a new target holding level in an "Each Sweep" loop just before the last sweep is started.

This setting is updated live by holding level changes in the Amplifier Control Panel if 'Relative to Holding' is enabled, otherwise it is only updated at the time of routine activation.

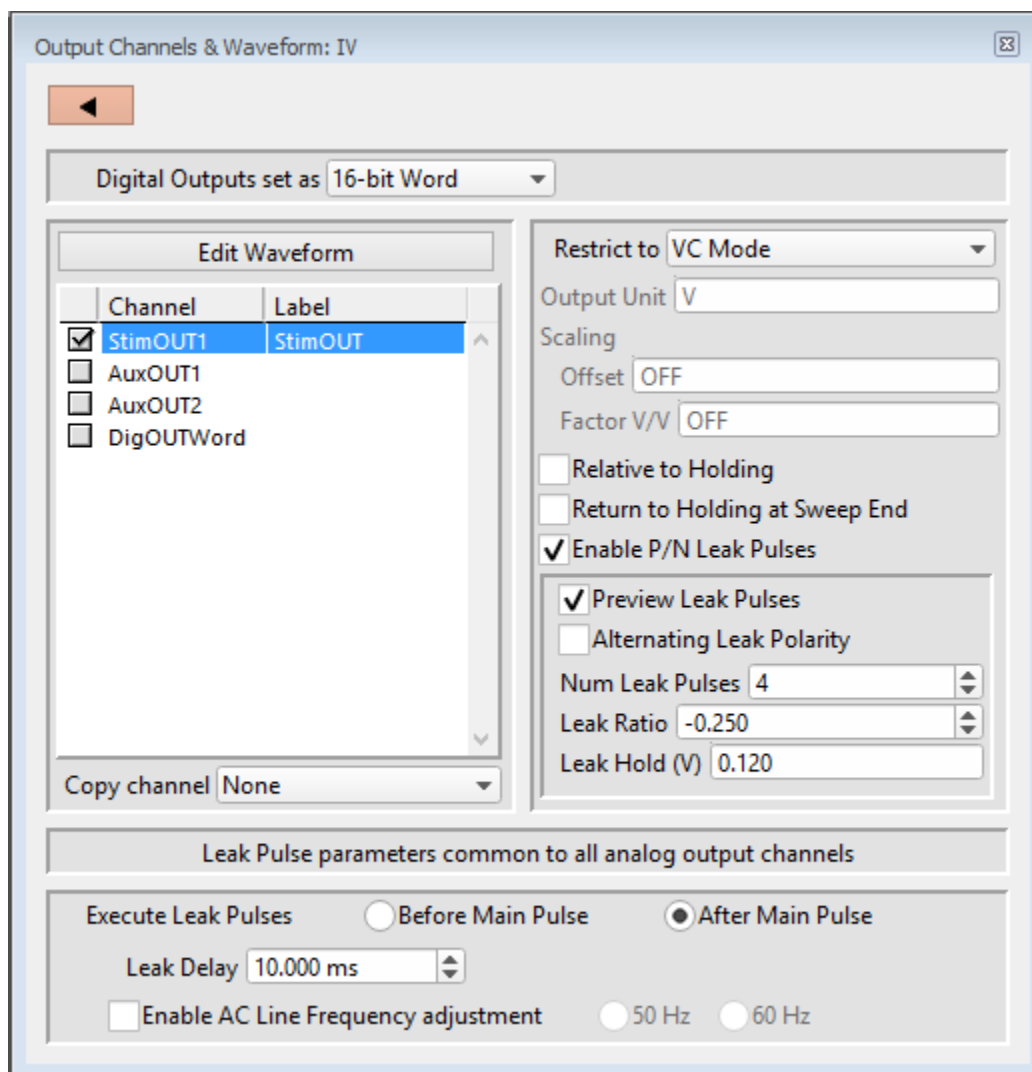


Figure 4-41. Output Channels & Waveform with Leak

Enable P/N Leak Pulses

[The P/N Leak Pulses section is only displayed if this field is enabled.]

Online P/N leak subtraction automates the removal of leakage currents from the data.

Endogenous leak currents can flow, even while a cell is in its resting state, from conditions such as an imperfect or leaky seal, or via existing ion channels, and affect response amplitudes. If endogenous leak conductance is an issue with your cell type, and/or high temporal resolution is required along with a need to reduce capacitive transients (e.g., with voltage-gated sodium currents), click 'Enable P/N Leak Pulses' and configure its settings below.

A "leak pulse" is a replica of the stimulus waveform, and is used to record a fraction of the leakage current. In this technique, leak pulses are generated, and the responses are averaged, scaled, and subtracted from the main response to remove

the effects of leakage.

Note: The sub-pulses are stored as part of the sweep. This ensures that if any events occur during the sub-pulses or between the sub- and main pulses and causes unexpected or hard-to-interpret effects, the full original recording condition can be examined.

Preview Leak Pulses

Display the leak subtraction pulses in the Routine Editor Waveform Preview panel. A leak subtraction pulse is a scaled copy of the main stimulus waveform.

Alternating Leak Polarity

You can reduce directional bias in the leak conductance by alternating the polarity of the leak subtraction pulses on a sweep-by-sweep basis, as long as no ion channels are activated.

Num Leak Pulses

Set the number of leak pulses used to average out noise and leak conductance. Adjust this number in accordance with the amount of noise in the signal. With the high precision of modern 16-bit digitizers, this number can sometimes be reduced to less than 4 leak sub-pulses.

Note: As each leak pulse replicates the stimulus waveform, larger numbers of leak pulses is not recommended, as this can greatly increase the total duration of a sweep during acquisition, and the noise in the sub- and main pulses can add up and actually increase.

The default setting of '4' Leak Pulses, when used with the default Leak Ratio (-0.250) operates equivalently to pCLAMP's default P/N setting (4 subsweeps for P/4).

Leak Ratio

Set the leak subtraction pulse size relative to the main waveform pulse, using a ratio between +1 and -1. The setting should be low enough that no electrically-gated ion channels are activated. For instance, a Leak Ratio setting of 0.25 will generate leak pulses at $\frac{1}{4}$ the amplitude of the main stimulus waveform, while a Leak Ratio of 0.2 will generate leak pulses at $\frac{1}{5}$ the main pulse amplitude.

Note: The program scales the leak subtraction pulses based upon the Leak Ratio setting, not the number of Leak Pulses. This means that the Leak Ratio can be set independently from Num Leak Pulses, instead of those settings being interdependent.

Tip: As an alternate way to avoid electrical activation of ion channels, use a negative ratio to reverse the polarity of the leak pulses relative to the main pulse.

Leak Hold

The leak pulses holding level can be set differently from the Routine main holding level, for flexibility in finding a suitable leak pulse voltage range. The scaled waveform amplitudes are measured relative to the Leak Hold level, but are subtracted relative to the IPA holding level.

Set to a fixed value, or enter as an equation.

(See the Equation Editor section for more details.)

Check Equations	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports that the syntax is “ok”.
Insert special identifier	Acquisition, amplifier and reference settings for use in equations. (See list in Equation Editor.)
Undo	All changes in the equation editing session are discarded.

Leak Pulse parameters common to all D/A channels

Leak Delay [‘s’, or ‘ms’ if < 1 s]

If a settling time is needed between the leak pulses and the main waveform pulse, Leak Delay will insert a time delay between the execution of the leak pulses and the main pulse. Provide enough time to avoid interference of the leak pulses with any active currents or inactivation of ion channels.

When leak pulses occur before the main pulse, Leak Delay uses the IPA Holding level; when leak pulses occur after the main pulse, Leak Delay uses the Leak Pulses ‘Leak Hold’ level.

Enable A/C Line Frequency adjustment

The effect of AC line-frequency noise (hum) can be automatically reduced during P/N leak subtraction recordings.

50 Hz Enable the reduction of 50 cycle AC line noise.

60 Hz Enable the reduction of 60 cycle AC line noise.

This Line Frequency adjustment automatically calculates the proper interpulse interval for the P/N pulses, so that they are counter-phased to the line frequency of the output signal, which reduces hum without filtering the signal.

Execute Leak Pulses

The leak pulses can be set to run before or after the main waveform pulse.

- **Before Main Pulse** Sub-pulses are output relative to the Leak Hold level. After the sub-pulses complete, the signal goes to the IPA Holding level for the duration of the Leak Delay before the main pulse.
- **After Main Pulse** After the main pulse completes, the signal goes to the Leak Hold level for the duration of the Leak Delay setting, and then outputs sub-pulses relative to the Leak Hold level.

Routine Editor: Waveform Editor

Click the 'Edit Waveform' button to open the Waveform Editor and design a command waveform for the selected output channel.

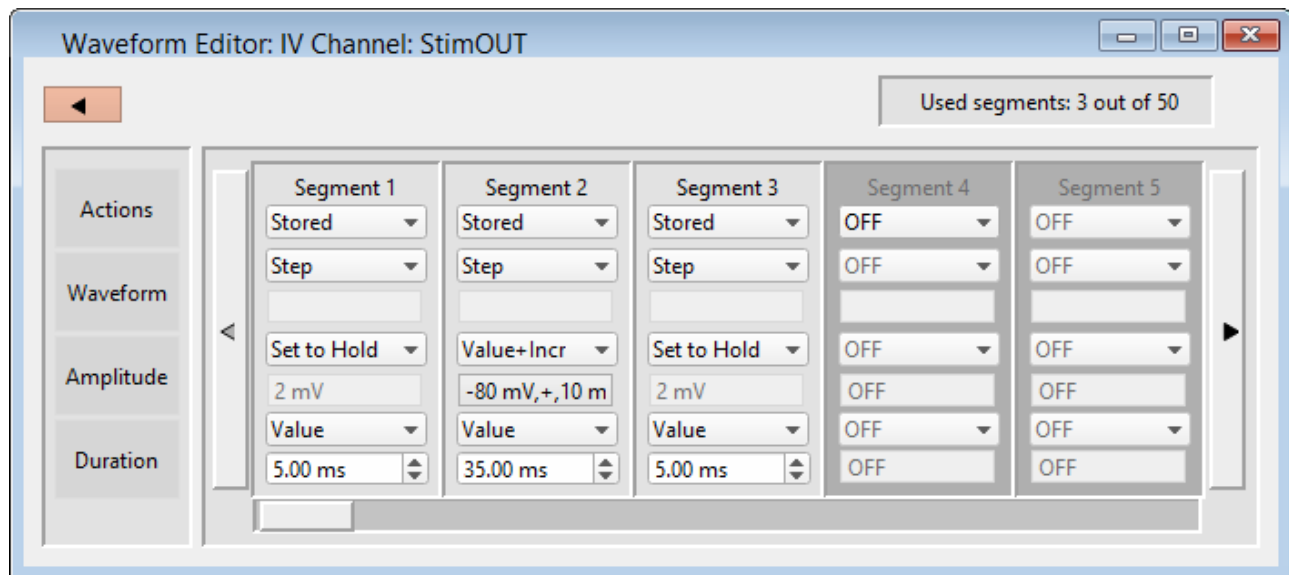


Figure 4-42. Waveform Editor

Close button  Use this button to close the Waveform Editor window.

Used segments: [# out of 50] Up to 50 contiguous segments can be configured in a waveform.

Actions

OFF Unused segments are labeled as 'OFF'.

Tip: A segment with a Duration of '0' ms is equivalent to 'OFF'. This is a convenient way to skip a segment instead of deleting it.

Stored	Enable a segment for stimulation and recording.
Not In Leak	<p>If P/N LeakPulses are enabled, this will optionally exclude the segment from being generated in the P/N Leak Subtraction output wave.</p> <p>This is useful for inactivation or recovery studies, when commands do not change for long periods of time.</p>
Insert	Insert a default Segment into the current position, and increment the position of the following Segments, i.e. move them to the right.
Copy	<p>To copy a segment, click the segment's Actions list and select 'Copy'. A copy is inserted as the next segment.</p> <p>To copy multiple segments, select the segments to be copied. Then, for the segment to be inserted before, click its Actions list, select 'Copy', and enter the number of times to copy the segments - the selected segments are inserted before the "Copy" segment.</p>
Delete	<p>To remove a segment, select its 'Delete' Action.</p> <p>Note: If there is only one segment, it cannot be deleted - there is always at least one segment enabled.</p> <p>To remove multiple segments, select the desired segments. Then, click any segment's Actions list and select 'Delete'. All selected segments are deleted.</p> <p>Tip: To select multiple segments, use the Windows CTRL-click / macOS ⌘-click to highlight segments, or SHIFT-click to highlight a range of segments.</p> <p>Any following segments shift their Segment #'s down by the number of deleted segments.</p>
Waveform	Select the waveform shape.
Step	The waveform amplitude rapidly jumps from a pre-existing level to the new level within one sample point, and stays at the new level for the duration of the segment. The resulting waveform shape looks like a step.
Ramp	The waveform amplitude goes from the previous amplitude to the new amplitude as a smooth straight line - the sloping line looks like a ramp.
Tip:	To create a sawtooth pattern, follow a ramp segment

with a zero duration Step segment. This sharply resets the ramp amplitude to the baseline level – the next ramp will start from the baseline level again.

Sine

The waveform is a sinusoidal wave.

Sine Wave Cycles	Multiple	One or more cycles
	Single	One cycle
	For LockIn	For capacitance measurements in a virtual input channel.
		The Cycle Duration is equal to the Segment Duration.
		Cell Compensation can be enabled in the Amplifier Control Panel for Lock-in recording without the need of manual Lock-in adjustments.

Note: For a single cycle, the Cycle Duration is equal to the Segment Duration.

Cycle Amplitude	First peak amplitude from baseline.
-----------------	-------------------------------------

Tip: To offset a sine wave from the default baseline (0 units), set the segment Amplitude, or enable Routine Editor / Output Channel 'Relative to Holding'.

Cycle Duration	One cycle length (ms)
----------------	-----------------------

[For Lock-in only]

A preset list of frequencies are available:

2.0 ms	(500 Hz)
1.0 ms	(1 kHz)

	Segment Duration	Sine wave duration (ms)	
Squarewave	The waveform generates a train of rectangular pulses		
	Base Amplitude Increment	Increment the baseline amplitude for each succesive pulse.	
	Step1 Amplitude	Amplitude of first pulse.	
	Step1 Width	Duration of first pulse (ms).	
	Step2 Amplitude	Amplitude of second pulse.	
	Step2 Width	Duration of second pulse (ms)	
	Segment Duration	Square wave duration (ms)	
Chirp	This waveform generates a sinusoidal wave that changes its frequency over time.		
	Chirp Type	Linear	A linear change in frequency.
		Geometric	A geometric change in frequency.
	Note: For a geometric chirp, a minimal frequency spread is enforced: the End Frequency has to be at least double the Start Frequency, or half or less than the Start Frequency.		
	Amplitude	[±1000.00 mV, ±20000.00 pA]	
	Start Frequency	[1 – 50000 Hz]	
	End Frequency	[1 – 50000 Hz]	
	Segment Duration	Chirp wave duration (ms)	

Template Assign arbitrary waveforms to a segment.

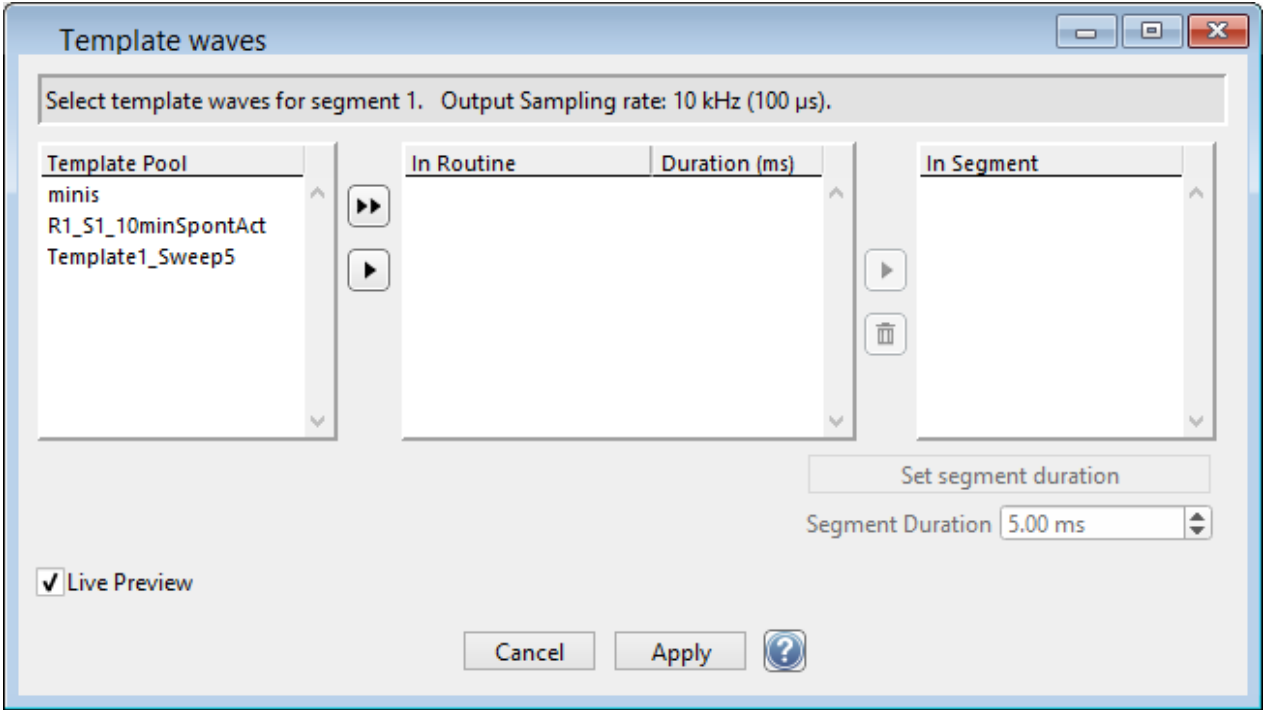


Figure 4-43. Template Waves

[Status field] The Routine segment # and output sampling rate are displayed

If the template sampling rate does not match a Routine sampling rate, the template is interpolated to match the Routine sampling rate.

Template Pool Templates loaded in the Template Editor Pool plus extracted templates.



Copy the selected template wave from the Template Pool into a Routine and Segment.



Copy the selected template wave from the Template Pool into a Routine.

In Routine Templates stored in the Routine.

Each output channel can have a maximum of 16 template waves stored in its routine. The same template can be used in multiple segments. Each segment can have one or multiple templates assigned to it.

Probably the most used case will simply be a single template paired with a single segment, but the possibilities are endless.

Note: To avoid unnecessary increase in the sizes of routines and Routine Pool files, only include templates that are actually used by segments.

Duration (ms)

The duration of the template trace.



Copy the selected template from a Routine to a Segment.



Delete the template wave from a Segment or an unused Routine.

In Segment

Each segment can have one or multiple templates assigned to it.

If only one template is listed, then for any number of sweeps, the segment output wave will be the same for all sweeps.

If more than one template is assigned to a segment, and the number of sweeps is greater than 1, then an output wave is created as:

1st sweep with the 1st template on the list,
2nd sweep with the 2nd template,
etc.

If there are more sweeps than templates, then the templates are reused (round robin) for each subsequent sweep.

Set segment duration

Set the segment duration to match the selected Segment template duration (also shown in the Routine Duration.)

Segment Duration (ms)

The Segment Duration can be manually adjusted here.

Amplitude (*analog*) Set the waveform amplitude for a Segment. For the Sine, Squarewave and Chirp waveforms, this is used as an initial offset.

Set to Hold

Use the Amplifier Control Panel holding level for the Segment amplitude.

Tip:	When used in Segment 1 for voltage-clamp experiments, this allows you to record the leak current along with the actual holding voltage. For current-clamp experiments, this allows you to record the actual cell potential along with the actual holding current.	
	Avoid using the last Segment for this, as post-stimulation data can be recorded, such as from tail currents.	
Value	Use a single number for the segment amplitude.	
Value List	Set an arbitrary segment amplitude for each sweep.	
	[Sweep Value]	For each sweep, enter a number.
	Fill Remaining List	Copy the active value to all remaining sweeps in the list.
	Segment Duration	Adjust the duration of the Segment.
	Number of Sweeps	Adjust the number of sweeps in the Routine.
Value+Increment	Increment the segment amplitude for each sweep.	
	Start Value	<ul style="list-style-type: none"> • Holding • Value [± 1000.00 mV, ± 20000 pA]
	Increment Value	[± 1000.00 mV, ± 20000 pA]
	Segment Duration	Adjust the duration of the Segment.
	Number of Sweeps	Adjust the number of sweeps in the Routine.
Equation	Specify the segment amplitude as an equation.	
	[Equation field]	A free-form text field. Errors are reported under this field.
	Check Equations	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports that the

syntax is “ok”.

Insert special identifier Acquisition, amplifier and reference settings are available for use in equations.
(See list in Equation Editor.)

Undo All changes in the equation editing session are discarded.

(See the Equation Editor for more details.)

Note: Computing an equation for an output wave consumes significant computing power, as every data point needs to be computed by the CPU. While a slight update delay in such operations is expected, for computers with marginal computing power, the “beach ball” icon displays while the computer is unresponsive and busy processing.

Var_r[1 – 16] Variable labels are displayed if the Routine Variables table is enabled.

Amplitude (*digital*) Digital settings are displayed if digital outputs are enabled.

Bit Set the digital level for an individual bit.

- LOW = 0
- HIGH = 1

Bit Word Values are the decimal number of an 8-bit word (0 – 255), also displayed as a bit pattern.

Duration Set the segment duration.

Set to Hold Use the Amplifier Control Panel to set the output holding levels.

Value Use a single number for the segment duration.
[0 – 12 ks]

Value List Set an arbitrary segment duration for each sweep from a list of numbers.

[Sweep | Value] For each sweep, enter a number.
[0 – 12 ks]

(Blank lines are removed.)

Fill Remaining List	Copy the active value to all remaining sweeps in the list.
Number of Sweeps	Adjust the number of sweeps in the Routine.
Value+Increment	Increment the segment duration for each sweep.
Start Value	[ms]
Increment Value	[ms]
Number of Sweeps	Adjust the number of sweeps in the Routine.
Equation	
Specify segment duration as an equation	
[Equation field]	A free-form text field.
[Errors are reported under this field.]	
Check Equation	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports that the syntax is “ok”.
Insert special identifier	Acquisition, amplifier and reference settings are available for use in equations. (See list in Equation Editor.)
Undo	All changes in the equation editing session are discarded

(See the Equation Editor for more details.)

Var_r[1 – 16]	Variable labels are displayed if the Routine Variables table is enabled.
-----------------	--

Segment Controls

To copy or delete multiple Segments, click on the background area of used Segment(s) - the background color turns gold, and enables the Actions items: Copy and Delete.

Standard mouse behaviour is used to select multiple Segments:

- Individual Segments: Windows: CTRL-click

- | | | |
|---------------------|--------|-------------------|
| | macOS: | command (⌘)-click |
| • Range of Segments | Both: | SHIFT-click |

To step through the Segments, click on the left or right Segment arrow buttons.

To scroll through the Segments, use the slider at the bottom of the Waveform Editor.

Routine Editor: Real Time Measurements & Graphs

Online analyses are configured in the Real Time Measurements & Graphs dialog. Measurement regions display in the Scope window, and their associated analyses are plotted in an Analysis sub-window during acquisition.

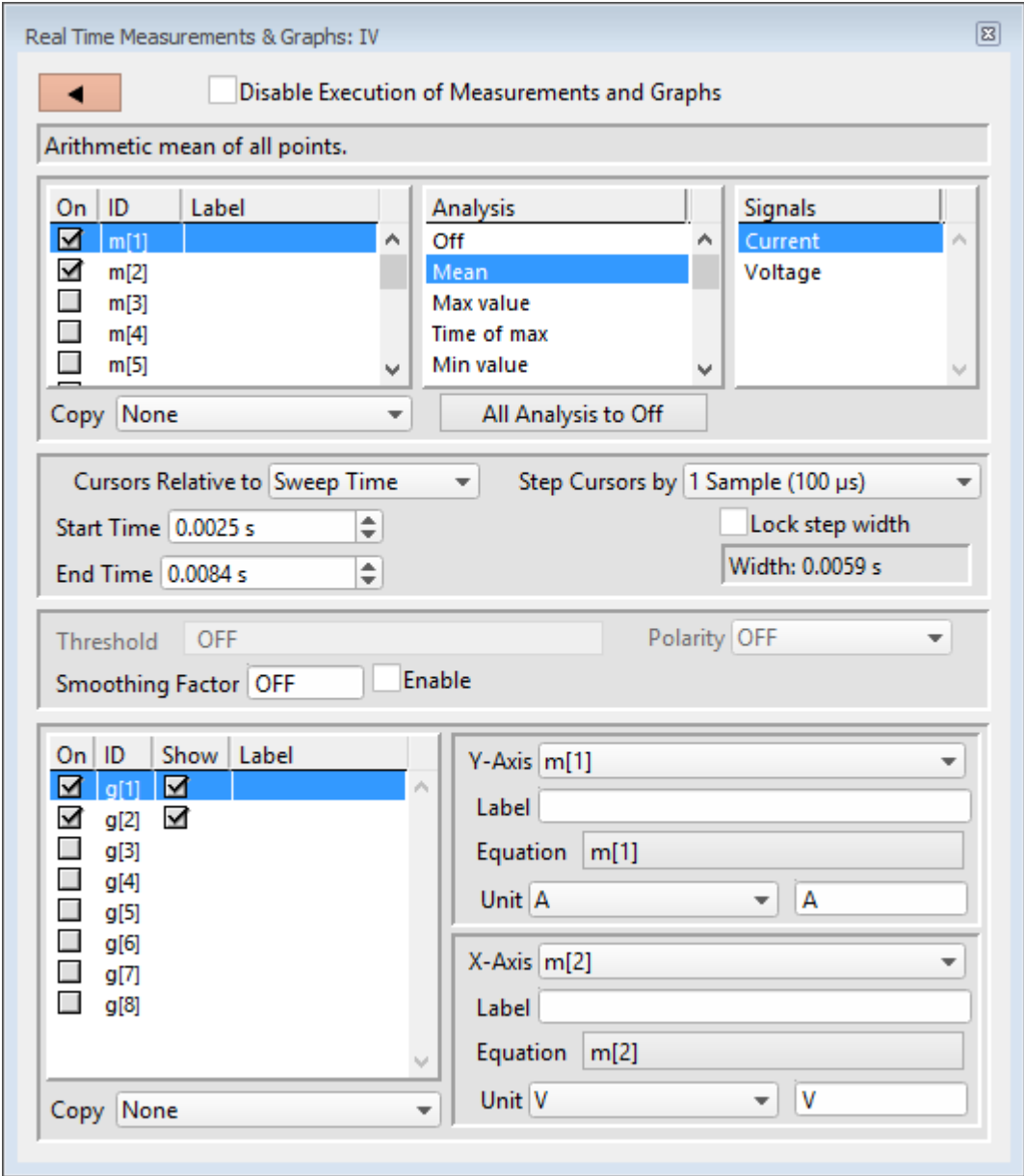


Figure 4-44. Real Time Measurements & Graphs

Disable Execution of Measurements and Graphs

Block all measurements and analyses with one click.

[Status field] A short description of the selected Analysis.

On Enable an analysis to run.

ID Measurement regions are identified with an ID number: m[#]
[1 – 16]

Label Replace the default measurement ID with your own measurement name, to display in the Waveform Preview and Scope windows, and for use in equations.

Analysis Choose from 16 predefined Analysis statistics for each measurement:

Absolute area	Absolute area (negative values are converted to positive).
Absolute peak	Largest absolute value.
Area	Signed area (negative values negate positive values).
Decay time	10 - 90% decay (fall) time.
Decay Tau	Time constant of 'Decay time'.
Frequency	Number of threshold crossings per second (Hz).
Max Slope	Maximum slope of simple linear regression fit.
Max value	Value of largest sample.
Mean	Arithmetic mean of the samples.
Min value	Value of smallest sample.
Rise Tau	Time constant of 'Rise time'.
Rise time	10 - 90% rise time.
RMS noise	Root-Mean-Square noise.
Segment amplitude	Maximum amplitude of the selected segment.
Segment duration	Duration of the selected segment.
Slope	Slope of simple linear regression fit.
Std deviation	Standard deviation of the samples: $\sqrt{(\text{variance})}$
Time of absolute peak	Time from sweep start to largest absolute value.
Time of max	Time from sweep start to largest sample.
Time of min	Time from sweep start to minimum sample.
Time to threshold	Time from sweep start to first threshold crossing.
Variance	Variance of the samples.
Weighted tau	Weighted time constant.

These analyses can be directly plotted, or used in more complex equations.
(See the Equation Editor section for more details.)

Signals For each enabled Analysis measurement, select the signal to be measured from the list of Input Channels. A measurement is only made on one input channel, but it can be used in multiple graphs.

Cursors Relative to Set the measurement boundaries with left/right cursors.

- Trace Encompasses the entire trace.
 Width: # s The width of the cursors in seconds.

- Sweep Time Set relative to the start time of a sweep (time zero).
 Start time Set the left cursor relative to the Sweep start time (s).
 End time Set the right cursor relative to the Sweep start time (s)
 Note: Cursor Start times are always before their End times.
 Step Cursors by: [1, 2, 5, 10, 20 Samples (# ms)]
 Set the step size of the 'Start time' and 'End time' spinners by a number of samples.
 Lock step width Fix the width of the measurement region.
 The measurement width is maintained at a constant value when the 'Start time' is updated.
 Width: # s The width of the cursors in seconds. The minimum width size is 2 sample points.

- Segment # Set relative to the start time of a Segment. Uses the segment timing from the input signal's "Parent Output Channel".
 Start ratio [0 = beginning of Segment]
 Set the left cursor as a ratio of the Segment duration.
 End ratio [1 = end of Segment]
 Set the right cursor as a ratio of the Segment duration.
 Note: If the Start/End ratios extend past the boundary of a Segment, and the measurement is switched to a beginning or ending Segment, the Start/End ratios are reset to '0' and '1' respectively.
 Reset Start / End Ratio [0.00] / [1.00]
 Reset the cursors to span the entire segment.

- Lock step width Fix the relative width of the measurement region.
 The measurement width is maintained at a constant ratio value when 'Start ratio' is updated.

	Width: # s	The width of the cursors in seconds. The minimum width size is 2 sample points.
Threshold	This amplitude level needs to be crossed by the data to trigger measurements for: <ul style="list-style-type: none">• Rise/Decay time• Rise/Decay Tau• Frequency• Time to threshold	
Polarity	The direction of a Threshold crossing. <ul style="list-style-type: none">• Positive Positive direction threshold crossing.• Negative Negative direction threshold crossing.• Largest Change Use the polarity direction of the largest change for Rise / Decay analyses.	
Smoothing Factor	Smoothen noisy data to reduce the effects of high-frequency noise on measurements by averaging the data sample points with an unweighted sliding average. [2 – 200] Note: Smoothing is not applied to the analyses ‘Segment duration’ and ‘Segment amplitude’. These are fixed values not subject to modification.	
Enable	When disabled, this field is set to ‘OFF’ (i.e. zero); when re-enabled, this field is reset to a value of ‘2’.	
<u>Graphs</u>		
On	Enable a graph to configure its settings.	
ID	Graphs have a default ID (identification): g[1] - g[8]	
Show	Display this graph in an Analysis window during acquisition and analysis. Note: If the Y-Axis ‘Equation’ field is blank, the graph will also be blank.	
Label	Double-click and enter a graph label to display in an Analysis graph.	
Copy	Use to transfer graph settings to a new graph. Highlight a disabled Graph ID, (but do not enable it), then select from the drop-down list of enabled graphs.	
<u>Y-Axis</u>	Select a measurement ID to plot against the graph Y-Axis, or select a Y-Equation for a customized Y-axis plot.	
	Label	Enter a customized name for the graph Y-Axis.
	Equation	Click to edit with the Equation Editor.

For example, to monitor cell compensation, use the Special ID 'RsComp'.

(See the Equation Editor for more details.)

If running from a planned Paradigm, see the 'For Each' step example to track cell changes sweep-by-sweep.

Unit Select a standard unit from the drop-down list, or enter a custom unit type.

Note: Standard unit solutions, such as 'pA' or 'mV', are automatically calculated and displayed in the graph.

X-Axis

Display the graph X-axis with a standard Time base, or select a measurement ID to plot against the X-axis, or use an X-Equation for a customized X-axis plot.

Label Enter a customized name for the X-Axis.

Equation Click to edit with the Equation Editor.

(See the Equation Editor for details.)

Unit Select a standard unit from the drop-down list, or enter a custom unit type.

Note: Standard unit resolutions, such as 'pA' or 'mV', are automatically calculated and displayed in the graph.

Routine Editor: Routine Variables

Up to 16 Routine Variables can be configured for stimulus control. These variables allow interactive manual control of the waveform amplitude and duration, or automatically via Paradigms.

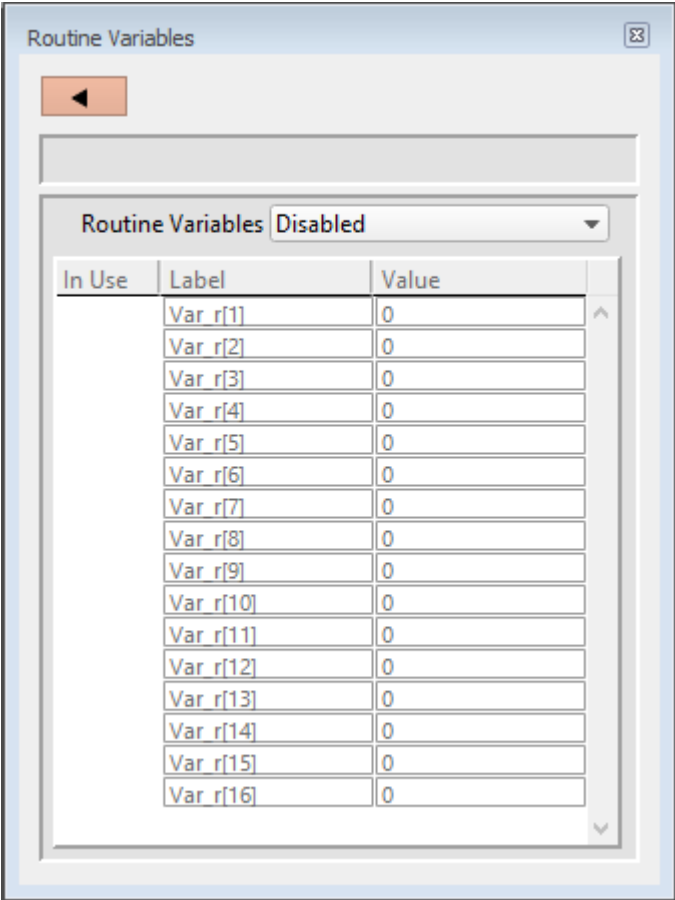


Figure 4-45. Routine Variables

Routine Variables

- Disabled
- Enabled

Once Routine Variables are enabled, they become visible in the Waveform Editor Amplitude and Duration lists.

In Use	A checkmark means this Routine Variable is in use by the Waveform Editor.	
Label	Var_r[1 – 16]	Edit the default variable name if desired.
Value	Numeric values can be manually entered or set by a Paradigm.	
Note:	If Routine Variables are enabled and then disabled, the Waveform Editor converts its 'Var_r' settings to 'Value' settings using the last enabled values.	

4.1.6 Real Time Measurements & Graphs (Scope)

This special version of the 'Real Time Measurements & Graphs' dialog allows for interactive analysis changes during data acquisition. Changes instantly override the loaded Routine settings for fast response. Input channels, measurement regions and graphing options can all be modified and saved back to the Routine.

To access, click on the Scope (Acquisition) window Measurements button.

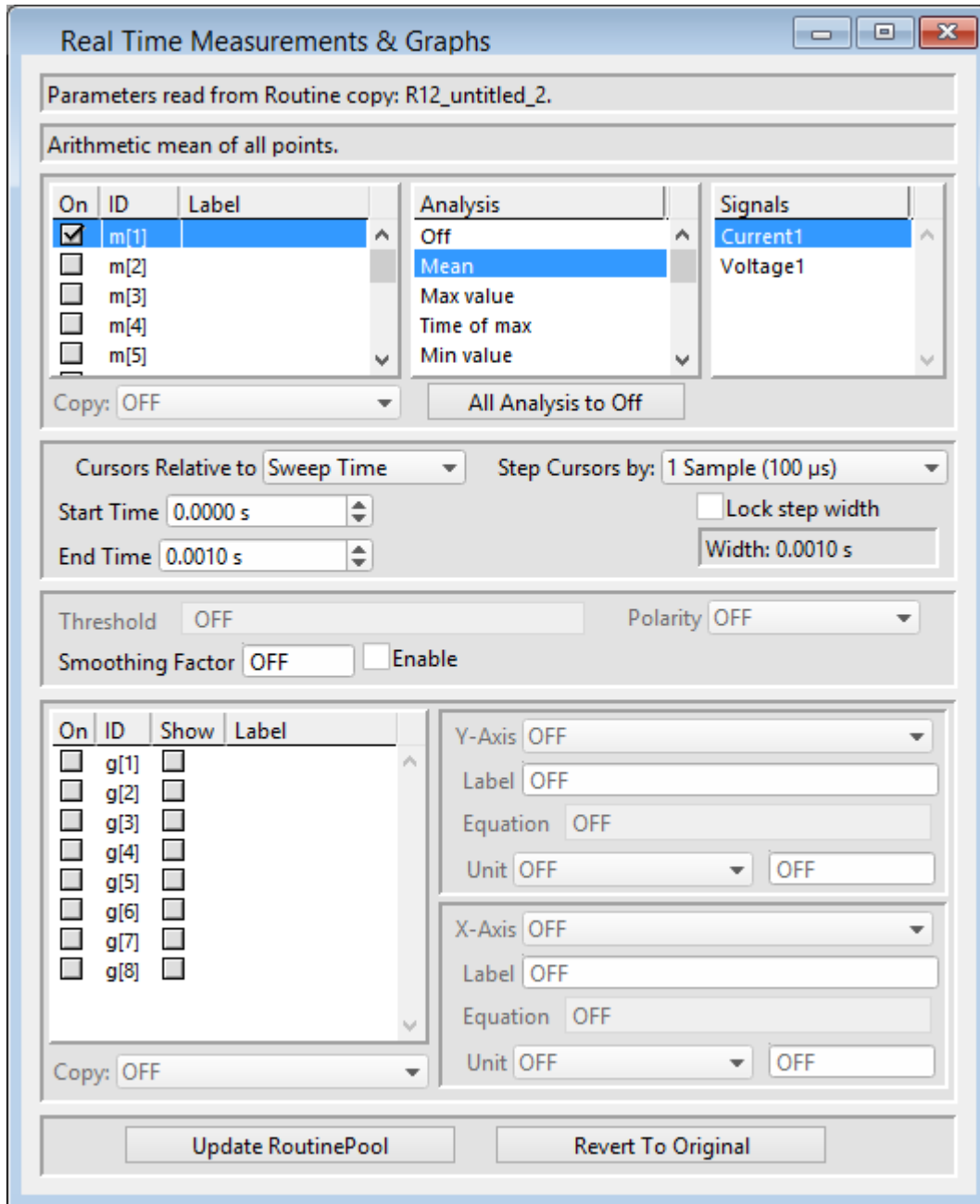


Figure 4-46. Real Time Measurements & Graphs (Acquisition)

Otherwise, this Scope version of the dialog is very similar to the Routine Editor version, with a few additions:

Extra buttons

Update Routine Pool	The stored routine is updated with any changes
Revert to Original	Any changes are reverted when the dialog is closed or the main routine is re-activated.

4.1.7 Scope (Acquisition)

Scope windows provide the central view of your time-series data, whether for data acquisition or data analysis. The data is displayed as a smooth interpolated line. While many of the window controls are common to all Scope windows, each type of Scope window has its own set of buttons.

Only one Scope window can be open at a time. For example, if a Scope window is open for data acquisition, then opening it for the Membrane Test, Free-Run, or data analysis, will close the acquisition Scope window and re-open it as the new type of Scope window.

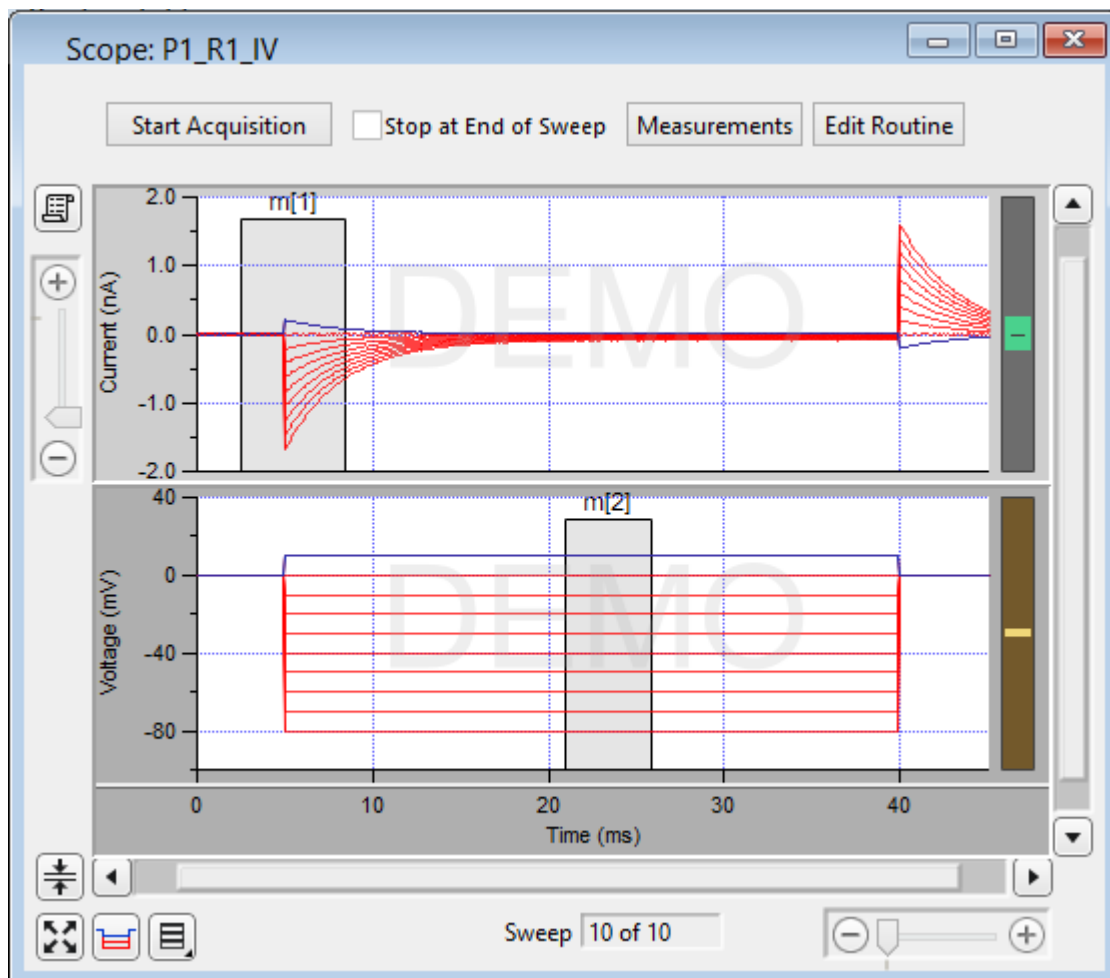


Figure 4-47 Scope (Acquisition) Window

The Scope window is titled with the active Routine name.

Controls

Signals The central area of the Scope window graphically displays data in up to 16 signal panes. There is a separate pane for each enabled input signal. The “active” pane receives the focus of the Y-axis controls (magnify, scroll), and its border area is displayed in a lighter color. When multiple panes are displayed, the inactive panes are displayed with darker axis border areas. Click on a signal pane to make it active.

If multiple signals are displayed stacked on top of each other, their panes can be vertically resized by clicking and dragging the horizontal area between panes, using the resizing mouse cursor (a horizontal line with a vertical double-headed arrow.) The standard mouse cursor will change to the resizing cursor when positioned over a pane separator.

Note: Two additional data points are appended to the sweep data to support post-sweep holding levels and segment boundary rounding issues.

Signal Display Mode

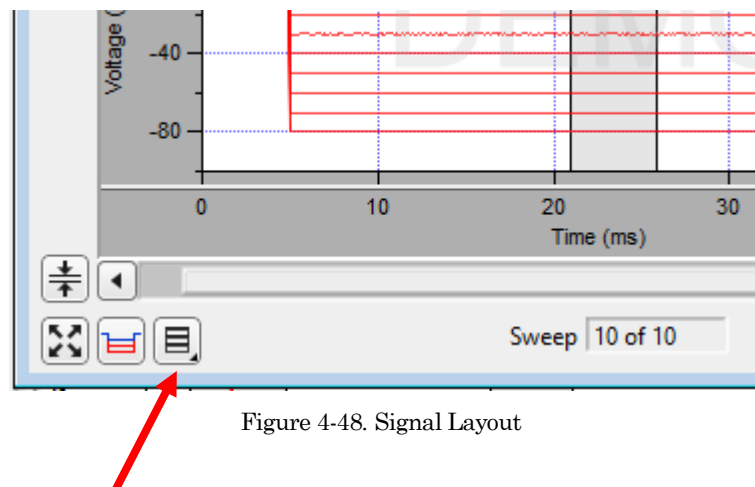



Figure 4-48. Signal Layout

Signal pane arrangements are set by the ‘Select signal layout’ button  in the lower left corner of the Scope window. Select how the input signals are to be graphically arranged:

- Stack Vertical column of signals
- Single Only the active signal
- [1 x 2] Tiled configuration of signals in rows and columns

Cursors Vertical measurement regions can be set up for input signals. Measurement regions display as light gray vertical bars in the signal panes. Each measurement

region has a measurement width, bounded by a start-time cursor and an end-time cursor - cursors are the left and right edges of these regions.

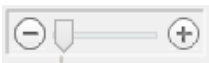
To graphically move a measurement region, click and drag them with the mouse (when selected, the region turns dark.)

To resize a measurement region, click and drag a start- or end-time cursor (the left or right edge of a region.)

Magnification and Scrolling

Signals can be magnified or unmagnified using several X- and Y-axis display controls in the Scope window. Any magnification applied to the signals persists during acquisition.

- Magnification Combo



Click on the “+” and “-” buttons to magnify/unmagnify by steps, or click and drag the slider to smoothly zoom/unzoom the active signal. The X- and Y-axes have their own combo display controls. However, the Y-axis magnification only controls the active pane.

- Axis Zoom

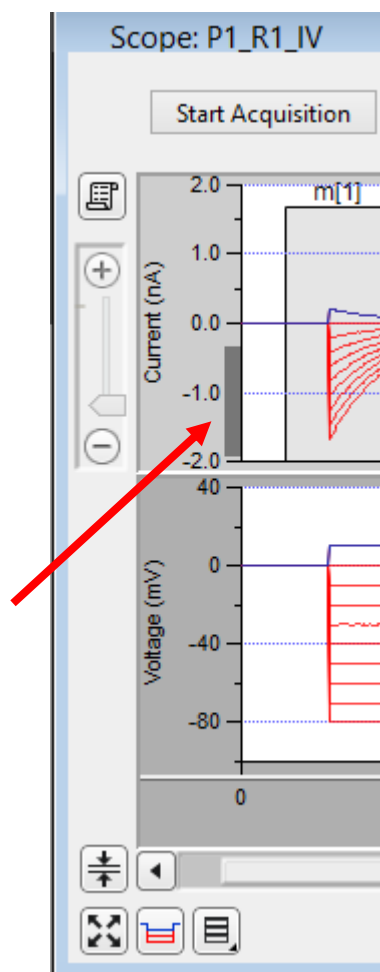


Figure 4-49. Axis Magnification

When the mouse is moved into the X- or Y- axis areas, the cursor changes to a double-headed arrow. As you click and drag the mouse cursor, a dark bar displays in the axis showing the magnification area; or scroll the mouse wheel up/down to expand/shrink the X- or highlighted Y-axis

- Area Zoom:

An area in a signal pane, such as an interesting part of the data, can be arbitrarily selected and expanded.

1. Move the mouse cursor into a signal pane, and it changes into a large “+”.
2. Click and drag a bounding box around a region of interest. The box is also referred to as a “marquee”.
3. Right-click in the marquee and select ‘Expand’ or ‘Shrink’.

Note: For signal panes without an X-axis bar (i.e., all but the bottom-most signal), the zoomed data are displayed independently of any X-axis,

and the time synchronization between signals gets lost. Click the 'Auto-scale All' button to restore synchrony.

- Axis Scroll Bars

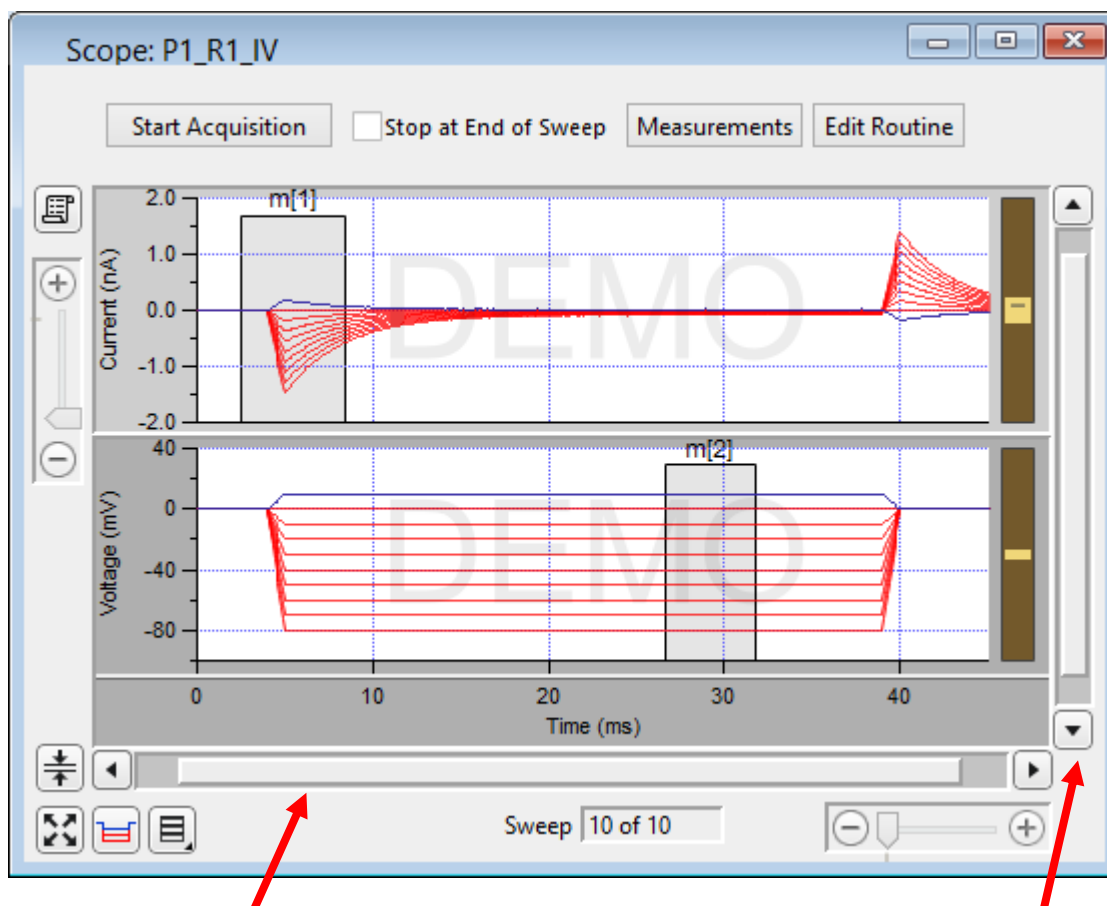


Figure 4-50. Axis Scroll Bars

The X-axis scroll bar is directly underneath the X-axis, while the Y-axis scroll bar is on the far right-edge of the Scope window. The amount of applied magnification is reflected in the size of the X- and Y-axis scroll bar slider buttons. Click and drag the offset bar slider button, or use their outer directional buttons to move the displayed signals in the desired direction. The Y-axis scroll bar controls the active signal pane.

- Center:

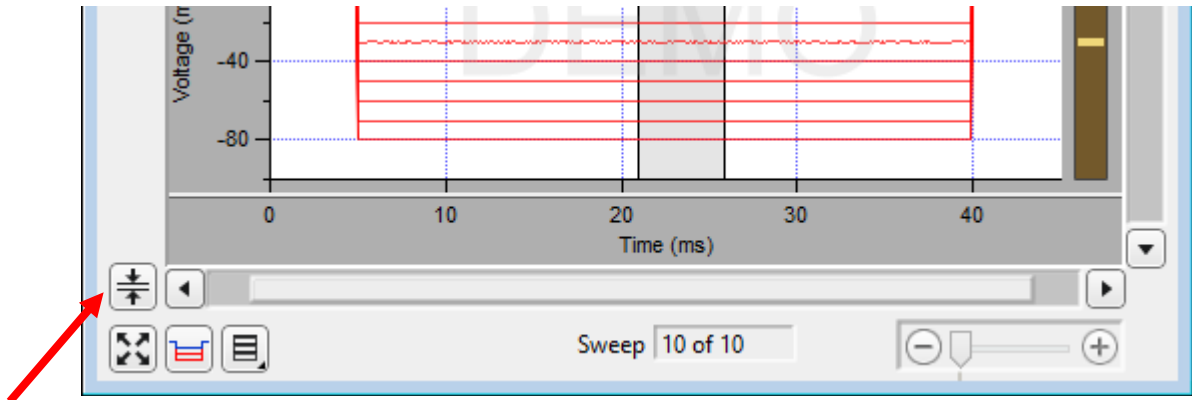


Figure 4-51. Center Button

Center the active signal. If the data are displayed off-center or off-screen, the Center button repositions the active sweep so that the mean of the data is vertically centered in the signal pane. The Y-axis offset is automatically adjusted, while the Y-axis scaling is kept unchanged; the X-axis remains unchanged.

- Autoscale Axes

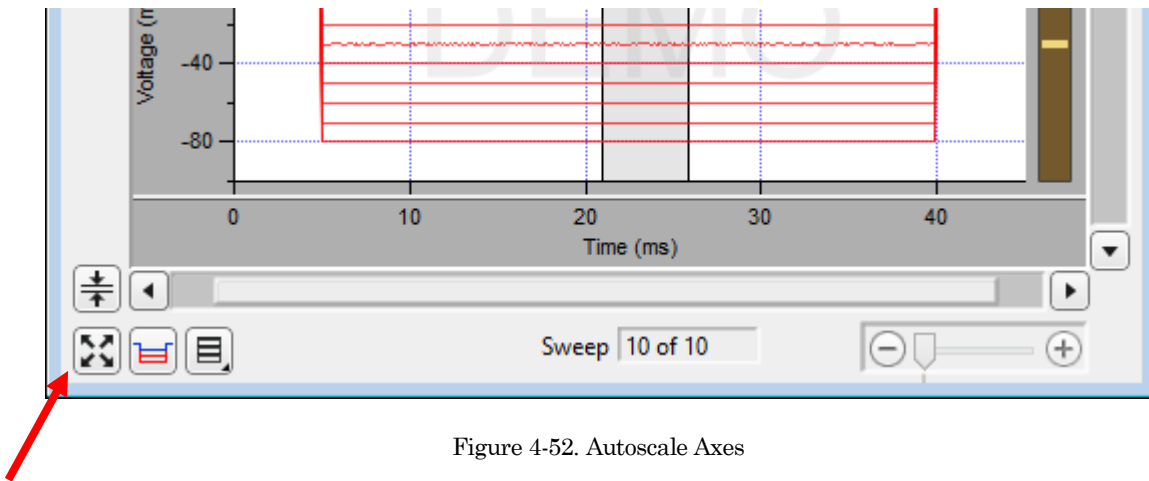


Figure 4-52. Autoscale Axes

Click for a one-time of the Y-axes of all visible signals to their incoming data, i.e., to their visible sweeps data limits, and to reset all X-axes to the full sweep duration.

To continuously Autoscale the Y-axes of all visible signals to their incoming data for each sweep, and to reset all X-axes to the full sweep duration, right-click in Windows, or Control-click in macOS. The continuous Autoscale button remains depressed in this state.

Note: During an Experiment, the state of the Autoscale button is not remembered for Scope (Acquisition) windows, but is remembered between Scope (Membrane Test) windows.

■ Amplitude Meters

for

Amplitude meters are displayed on the right border of signal panes for physical (non-virtual) channels. They provide visual feedback on the integrity of your data recordings, similarly to how audiometers monitor audio signals.

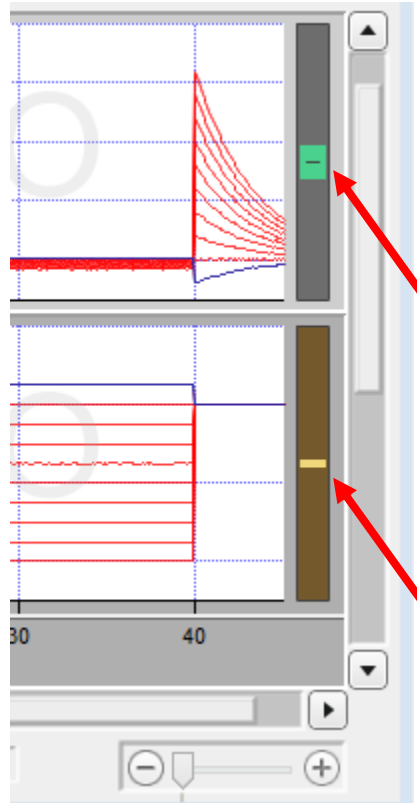


Figure 4-53. Amplitude Meters

For the Triggered Sweeps acquisition mode, each displayed signal has its own Y-axis amplitude meter on the inner-right side of the associated Scope pane.

For the Continuous Sweep acquisition mode, or if acquisition has not yet started, these meters are completely black.

The height of the colored meter bars represents a Signal's data range vs. the full recording range of the IPA digitizer. The color of the meter bar corresponds to the data "health":

- Green: Good Signal within appropriate range

- When the recorded data are within acceptable amplitude limits, the amplitude meter is green.
- Yellow: Caution Signal approaching limits (within 10% of range limits)

When there is a danger that saturation will occur, as the data are near the upper limit, the amplitude meter is yellow, as a warning sign to decrease your hardware gain.

If too little hardware gain is applied, and the recorded signal has insufficient amplitude resolution, the amplitude meter will be a thin yellow line. When this occurs, the digitizer is not utilizing enough bits to accurately represent the data, and your signal of interest might be contaminated with noise. In this case, consider increasing your hardware gain.
- Red: Danger Signal too large or small (within 1% of, or at range limits)

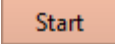
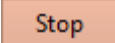

When an amplitude meter is displayed in red, it indicates that the data might have gone out of range and be invalid.

If too much hardware gain is applied, the recorded signal will be in danger of saturating, i.e. your data will exceed the amplitude limits of the digitizer. In this case, those data points are substituted with the maximum amplitude of their input channel.

[IPA only]

If too little hardware gain is applied, the signal is too small for accurate measurement, and the hardware gain needs to be increased.

Other Buttons

	<p>Start recording and displaying digitized analog data in the Scope window input signals.</p> <p>When you click the ‘Start’ button, the Scope is cleared, and data recording starts (after ~300 ms).</p> <p>When acquisition is running, the Scope window updates every 200 ms.</p> <p>If the Sweep Start-to-Start time is ≥ 5 s, the “Time to next sweep: # s” is reported below the Start / Stop buttons.</p> <p>Metadata prompts are set for Routines or Paradigms, the Confirm Metadata Settings dialog displays just before recording occurs.</p> <p>If measurement graphs are enabled, a docked “child” Analysis window plots sweep-by-sweep measurements.</p> <p>If no paradigm is running, an “Auto-triggered Paradigm” is generated and assigned a Paradigm name with the current Date/Time.</p>
	<p>Stop recording data.</p> <p>When you click the ‘Stop’ button, if ‘Stop at End of Sweep’ is enabled, the message ‘Waiting to stop’ displays below the Start / Stop buttons, until the last sweep completes and acquisition stops.</p>
<input type="checkbox"/> Stop at End of Sweep	<p>Determines how data acquisition is terminated. When the ‘Stop Acquisition’ button is clicked, data acquisition is immediately halted, even if this occurs in the middle of a sweep. In this case, any partial sweep that was in progress is not saved with the data.</p> <p>However, if the ‘Stop at End of Sweep’ checkbox is enabled, then the current sweep will complete before data acquisition is stopped, and the last recorded sweep will be a complete sweep of data. If no sweep is currently in progress, acquisition will stop at the end of the next sweep to be recorded.</p>
	<p>Show Cursors: Display measurement cursors in the Scope window.</p> <p>Hide Cursors: Do not display cursors in the Scope window. Button state displays as “Measurements(H)”.</p> <p>Lock Cursors: Prevent cursors from being moved or altered. Button state displays as “Measurements(L)”.</p> <p>Edit Measurements: Open a special Real Time Measurements & Graphs dialog, where all edits apply instantly to the measurements and graphs, even during acquisition. These changes (temporarily) override the loaded routine for quick interactive analysis.</p>

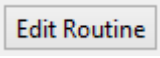


	<p>Edit Virtual Signals: Open the virtual input signals panel for editing.</p>
	<p>Open the Routine Editor dialog, for configuration, management and activation of Routines. Routine configuration includes:</p> <ul style="list-style-type: none"> Data acquisition parameters Input channels Output channels Stimulus waveforms Analysis measurements
<p>Layout</p> 	<p>Paste all visible Scope signals and analyses into a Layout window. If no Layout window is open, a new one is created.</p>
<p>Persistence Display</p> 	<p>When enabled, sweeps are not cleared from the display until a new acquisition is started. When disabled, all sweeps are cleared and only the next acquired sweep is displayed.</p>
<p>Sweep #:</p>	<p>The active sweep number vs. the total 'Number of Sweeps'; if multiple cycles are set, the active sweep cycle number is inserted between these.</p>

Table 4-3. Other Scope Buttons

Right-click Menus

Scope (Acquisition) main window

Note: If you don't click far enough away from the data, the data menu will display instead.

Autoscale All Axes

Add Annotation

Export Graphics

Colors

Hide Signal 'Signal Name'

Show Signal 'Signal Name' Only

Stack All Signals

Y-Axis

Autoscale All Axes

Scale all signals X- and Y-axes

Continuous Autoscale Axis	Continuously scale a signal's Y-axis.
Autoscale Axis	Scale a signal's Y-axis, no change to the X-axis
Full-scale Y Axis	Set a signal's Y-axis to the full-scale range
Axis Properties	Modify the axis style and components
Hide Signal <name>	Remove the signal from the Scope
Show Signal <name> only	Remove all other signals from the Scope
Stack All Signals	Display all signals in a single column

X-Axis

Autoscale All Axes	Scale all signals X- and Y-axes
Full-scale X Axis	Set a signal's X-axis to the full-scale range
Axis Properties	Modify the axis style and components

Data area

An Igor menu displays with numerous options to modify sweeps, such as marker symbols and lines.

Marquee

Click and drag the mouse to surround a region of interest:

Expand	Set the marquee axes limits as the new limits for all axes in all channels.
Horiz Expand	Set the marquee X-axis limits as the new limits for the X-axes in all channels.
Vert Expand	Set the marquee Y-axis limits as the new limits for the Y-axis in the active channel.
Shrink	Adjust all axes so that their current limits are used by the marquee.
Horiz Shrink	Adjust all X-axes so that their current limits are used by the marquee.
Vert Shrink	Adjust the Y-axis in the active channel so that its current limits are used by the marquee.
Extract Template	Copy the (last) sweep to the Template Editor

4.1.8 Solution Editor

Control perfusion systems with “named” solution control settings.

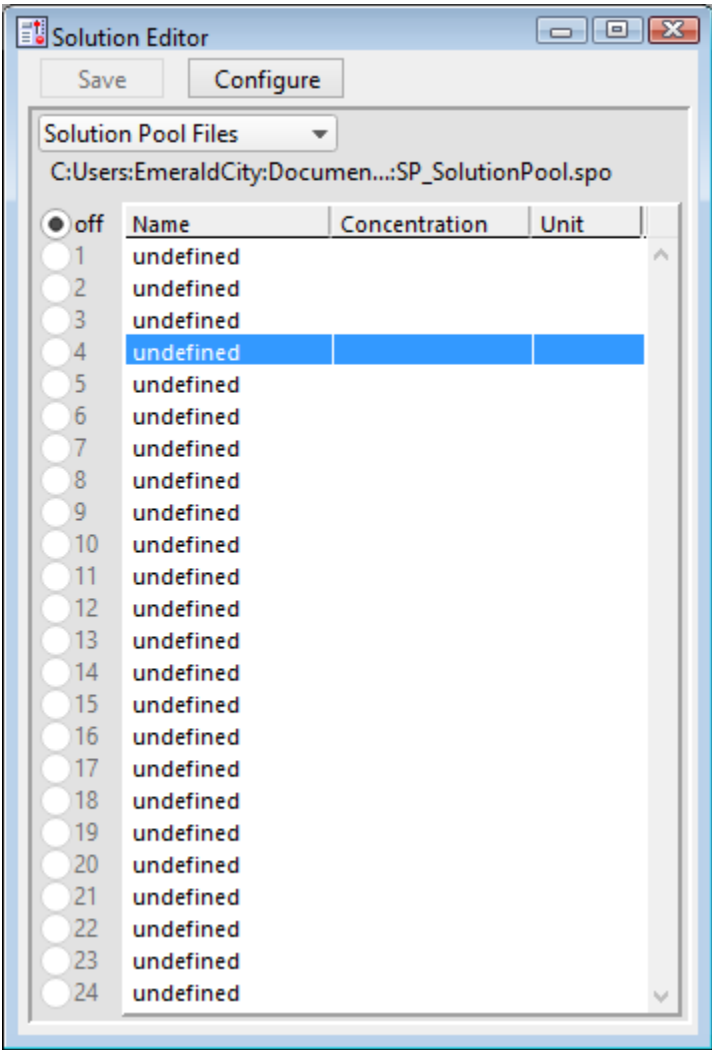


Figure 4-54. Solution Editor

You can create a named list of solution control “valves” to manage your analog and digital commands to physical valves and solution changers.

- Save

Save the solution list as a “*.spo” file.
- Configure

Opens the Configure Hardware Controls dialog to set solution types, channels, and outputs.
- Solution Pool Files

Drop-down list operations affect the entire “Solution Pool”.
- New Solution Pool

Create a blank Solution Pool.
- Load Solution Pool

Load the Solutions of a previously saved Solution Pool file into the Solution Pool.
- Revert to Last Saved

Undo any unsaved changes to the Solution Pool.
- Save Solution Pool

Save the Solution Pool using its existing file name and path.

Save Solution Pool As	Save the Solution Pool to a new file, and switch to the new file.
Save Solution Pool Copy	Save the Solution Pool to a new file, but do not switch to the new file.

Note: Default file names are auto-incremented from the previously loaded Solution Pool name.

The file path and file name of the loaded Solution Pool file is displayed.

[Off, 1 – #]	Manually select a radio button to activate a solution configuration. Only one solution can be active at a time. The number of radio buttons is set by the number of “Configured” solutions with outputs.
Name	Double-click on a field to edit it, or click-and-drag to move it up or down in the table.
Concentration	Enter a concentration value for the solution, including units, such as “0.5 nM”. Access the concentration value from the last-used Solution valve with the Special ID ‘Solutions’ (for ‘Chemical Stimulant’ solution types only).
Unit	The unit type of the concentration.

Configure Hardware Controls

Close Dialog button

[# Solutions]	Set the number of solution configurations (valves). Changing this number creates a new solution pool.
-----------------	---

[4, 8, 12, 16, 20, 24]

With the loading of other Solution Pool files, this allows a virtually unlimited number of solutions to be defined and accessed in an Experiment.

Note: When the maximum 24 solutions are displayed, some Windows 8 computers might not display the last two solution lines.

[Solution Type list]	A list of predefined solution types.
------------------------	--------------------------------------

- Bath Solution
- Pipette Solution
- Chemical Stimulant
- Rinsing Solution

[Output Channel] Select a physical output channel and set its value.

- No Output
- AuxOUT1 [± 10.000 V] Analog output voltage.
- AuxOUT2 [± 10.000 V] Analog output voltage.
- DigOUTWord [0 – 255] Decimal value of an 8-bit digital word.
- DigOUT1 – 8 A single digital bit is set.

4.1.9 Template Editor

Manipulate and manage templates for use in command waveforms.

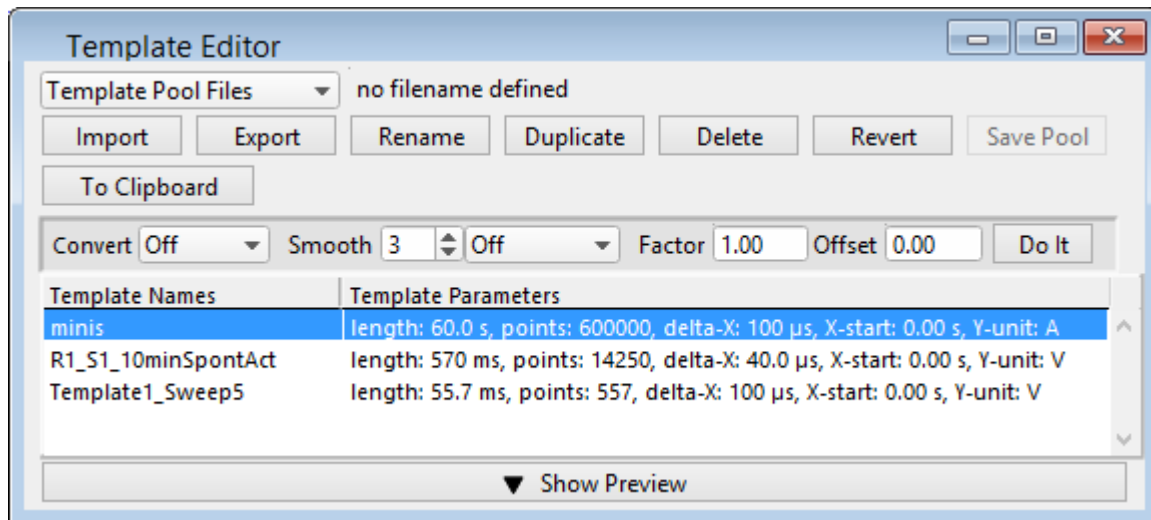


Figure 4-55. Template Editor

Template Pool Files

New Template Pool	Create a blank Template Pool
Load Template Pool	Load the Templates of a previously saved Template Pool
Revert to Last Saved	Undo any unsaved changes to the Template Pool
Save Template Pool	Save the Template Pool using its existing file name and path
Save Template Pool As	Save the Template Pool to a new file, and switch to the new file. The default file name is the same as the original file name.
Save Template Pool Copy	Save the Template Pool to a new file, but do not switch to the new file. The default file name increments.

Merge Template Pools	Insert the Template from a previously saved Template Pool file into the loaded Template Pool
----------------------	--

The file path and file name of the loaded Template Pool file is displayed.

Import	<p>Select a template file (*.ibw).</p> <p>Alternatively, in a Scope window or preview pane, click and drag the mouse to surround a region of interest with a bounding box (the “marquee”). Right-click in the box and select ‘Extract Template’. A template with the signal name is added to the template list.</p> <p>An extracted template is composed of a single sweep:</p> <ul style="list-style-type: none"> • Scope window (Acquisition): last sweep • Scope window (Analysis): selected sweep • Preview pane: last or selected sweep <p>Note: ‘Extract Template’ is not implemented for graphs or the Data Navigator preview pane. Also, it is only valid with monotonically increasing or decreasing X-axes.</p>
Export	Export the selected template to a separate file.
Rename	Edit the name of the selected template. Allowable characters are A-Z, a-z, 0-9, and “_”. Special characters are not allowed; spaces are replaced by an underscore.
Duplicate	Add a copy of the selected template to the list. The new template name’s number is appended or incremented.
Delete	Remove the selected template from the list.
Revert	Discard any unsaved changes to the selected template.
Save Pool	Save the template pool using its existing file name.
To Clipboard	Copy the template to the clipboard.
To Layout	Copy/append the template preview to the Layout window.
Define Template Properties	Update a data wave’s X- and Y-axis parameters to be compatible with SutterPatch templates.
Enter X-increment	The X-axis data point interval.

Enter X-start	The X-axis starting time for the data.
Enter Y-unit	The Y-axis base unit (enclose in double quotes.)
Convert	Adjust the template sampling (enclose between double quotes.)
Smooth	<p>Apply smoothing to the template.</p> <ul style="list-style-type: none"> • Off • Boxcar A fast time-domain filter with excellent 0 – 100% rise-time response. • Gaussian A standard filter with excellent 10 – 90% rise-time response.
Factor	<p>Adjust the template scaling factor.</p> <p>Values are displayed with SI unit prefixes.</p>
Offset	<p>Adjust the template offset.</p> <p>Values are displayed with SI unit prefixes.</p>
Do It	Apply the adjustments to the template parameters.
Template Names	A list of the loaded templates.
Click on a Template entry to make it the active one.	
Double-click on a Template Name to rename it.	
Click-and-drag a Template entry to reposition it in the list.	
Template Parameters	Parameter settings description.
Show Preview	Display the selected template in a preview pane.
The preview pane X- and Y-axes can be controlled in two ways:	
<ul style="list-style-type: none"> • Hover the mouse over an axis line until the cursor turns into a double-headed arrow, then scroll up or down to contract/expand the axis. • In the preview, click and drag the mouse to surround the region of interest with a bounding box (the “marquee”). Right-click in the box and select one of the expand/shrink options. 	

4.2 Analysis

Routines contain measurement settings that are run during online data analysis, and which can be re-applied during offline data analysis. Specialized analyses can also be applied to data via the Data Navigator.

For complete flexibility in controlling how analyses are performed, Paradigms can run virtually any SutterPatch command, Igor analysis or user-defined function. To execute such commands “per sweep”, use the paradigm Execute step in conjunction with ForEachSweep loops.

4.2.1 Action Potential Analysis

Action potentials (AP) are automatically analyzed with this dialog. Select a data signal in the Data Navigator, then click the Available Actions button (or right-click the signal).

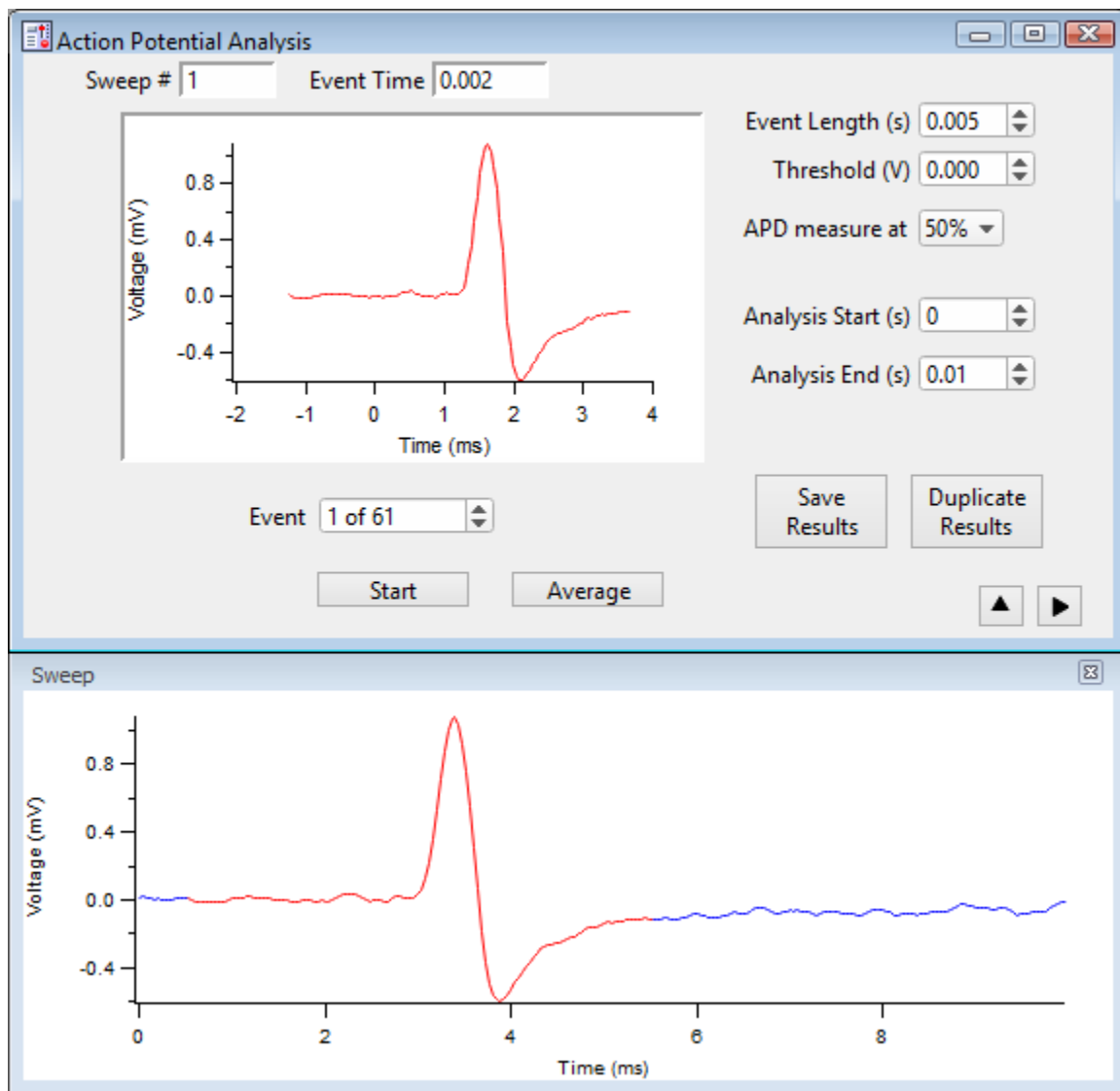


Figure 4-56. Action Potential Analysis

Sweep #	The sweep number of the selected event. When set to '0', this indicates that averaged Results measurements are being displayed.
Event Time	Timepoint when the potential of the selected event crossed the threshold.
[Event pane]	A graph of the selected event.
Event [# of #]	Selected event number vs. total events.
Start	Run the Action Potential Analysis.
Average	Display the averaged event in the Event pane, and its measurements in the Results panel, and set the 'Sweep #' to '0'.
Event Length (s)	Event duration in the Event pane (also highlighted in red in the Sweep sub-window.)

Threshold (V)	This voltage level needs to be reached or exceeded for analysis of an event to be triggered.	
APD measure at:	Set the Action Potential Duration percentile. This measures the duration of an event based on a percentile of the event repolarization duration. <u>APD at % repolarization</u> <ul style="list-style-type: none"> • 50% • 80% • 90% • 100% (same as the AP Duration) 	
Analysis Start (s)	Start time for data analysis in a sweep.	
Analysis End (s)	End time for data analysis in a sweep.	
Save Results	Results are displayed in both a Layout window and a table - prior Results from the loaded data signal are overwritten.	
Duplicate Results	Results are copied to a new Layout window and table, so prior Results are not overwritten.	
Results	A table of all events results.	
	[]	Row number, one row per event.
	Sweep Number	Sweep number the event is in.
	Event Time (s)	Time point of the event start.
	Threshold (V)	Amplitude of the event threshold.
	Threshold Time (s)	Time point of the “trigger” threshold time.
	Peak (V)	Amplitude of the event peak.
	Peak Time (s)	Time point of the event peak.
	AP Duration (s)	Duration of the action potential.
	AHP (V)	Peak amplitude of the ‘After Hyper-Polarization phase.
	AHP Time (s)	Time point of ‘After Hyper-Polarization; the event re-crosses the threshold amplitude at this time.
Action Potential Analysis Results	A Layout window report is created.	
	Total time analyzed	Includes the Start/End times for all sweeps (s).
	Number of events detected	Total number of events found.

Event Frequency (Hz)
[Event graph]
[Phase plot] dV/dt (V/s) vs. mV



Show/hide the sweep sub-window (below).



Displays a graph of the sweep containing the selected event.



Show/hide the results sub-window (on the right).



Displays the sub-window Action Potential Measurements.

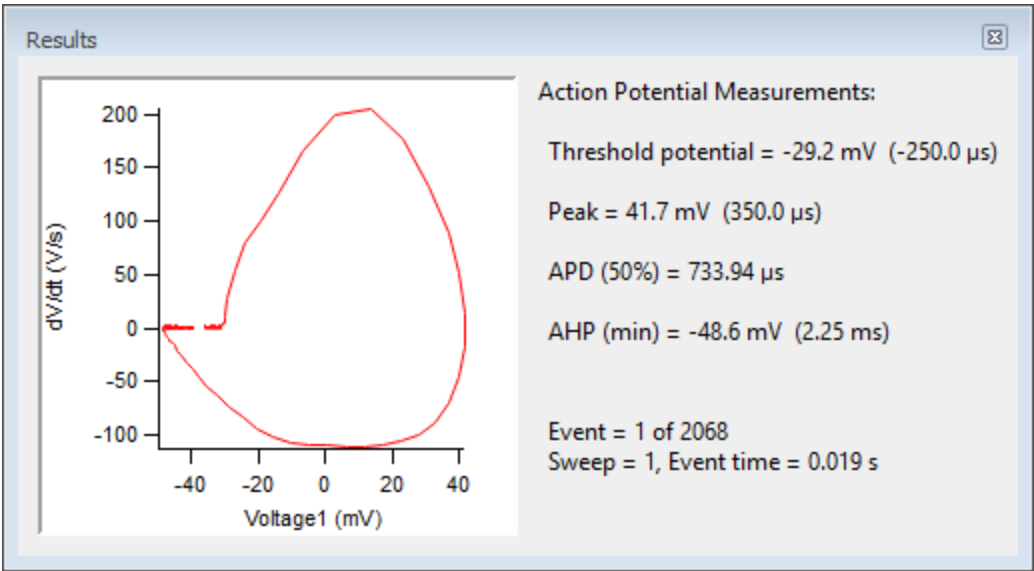


Figure 4-57. Action Potential Measurements

[Phase plot] dV/dt (V/s) vs. mV
[Y-axis vs. X-axis]

Threshold potential = Event starting amplitude (time from Threshold setting).

[mV (μ s)]

Two measures of the biological start of an event are reported, voltage and time. An event occurs when ‘signal slope’ > 1 mV/100 μ s.

The timepoint is reported relative to the ‘Threshold’ setting timepoint.

Note: The exact ‘Threshold potential’ timepoint is based upon differentials using a central differences algorithm:

Peak = [mV (ms)]

The largest amplitude excursion of the event (time from ‘Threshold’ setting).

Two measures of an event’s peak amplitude are reported, voltage and time. Time is reported relative to the ‘Threshold’ timepoint.

APD (%) = [%, ms]

Action Potential Duration of the event at (%) of amplitude repolarization.

AHP (min) = [mV (ms)]

The largest amplitude excursion of the “After HyperPolarization” phase of the event (time from ‘Threshold’ setting).

Two measures of the AHP amplitude are reported, voltage and time. AHP is when the action potential repolarization phase drops to its lowest point below the resting membrane potential, i.e. during the hyperpolarized refractory period of the cell.

Events found = The total number of events found in the analyzed data

Event frequency = The average frequency of events found in the analyzed data

4.2.2 Analysis Editor

Various manipulations can be applied to your experiment analyses and graphs.

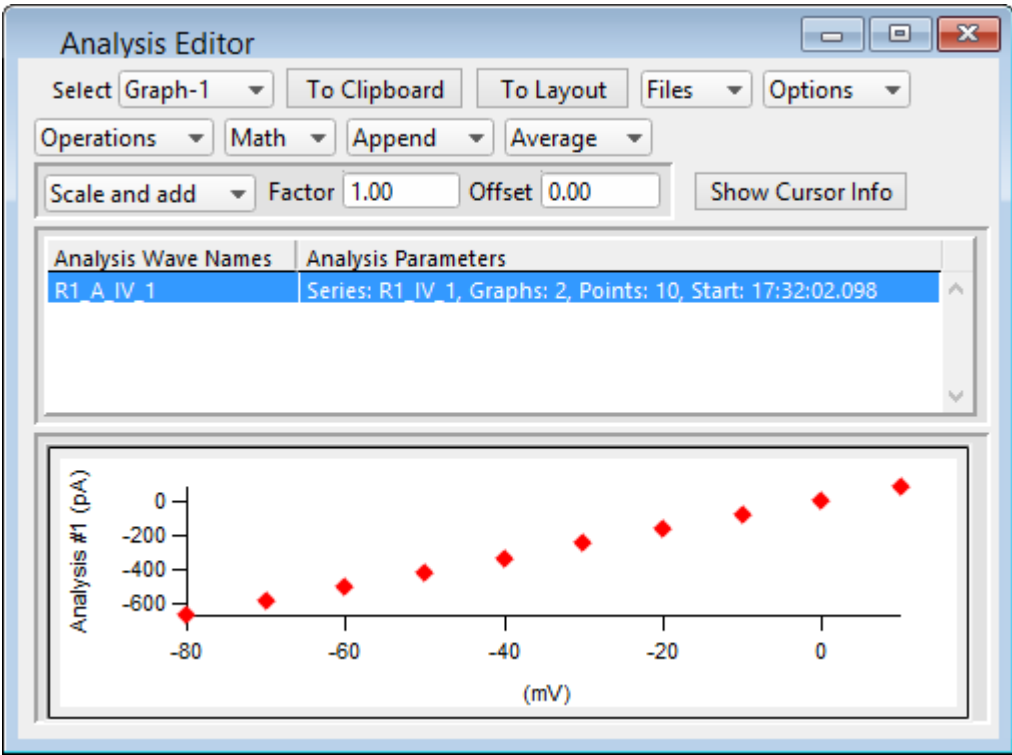


Figure 4-58. Analysis Editor

Select	Choose how to view the data:
Table	View a spreadsheet of the Analysis data
	Column 1: Row number.
	Column 2: X-data for the first graph.
	Column 3: Y-data for the first graph.
	Column m – n: Pairs of X- and Y-data columns repeat for each additional graph.
	The first graph (X-Y-pair) that uses X-axis time units populates its X-data column with time values; subsequent graphs that use X-axis time units have blank X-data columns, as they use the first time-column created.
Note:	The first data point is in row 0, so the last data point is in row [N – 1].
	Row N is a blank row that contains grayed-out cells. It is used to manually add extra rows of data to the table. Once a number is entered into one of these cells, the blank (gray) row is automatically moved to the row beneath it.

Warning! Editing the table will permanently alter the data.

	Graph-[1 – 8]	Select an Analysis graph for the selected data wave. The graph number refers to its original Analysis window position.
To Clipboard	Copy the selected table or graph to the operating system (OS) clipboard.	
To Layout	Copy the selected table or graph to the Layout window.	
Files	Save or open an analysis graph file.	
	Export Graphs	Save the entire graph as a multidimensional Igor Binary Wave file (*.ibw).
	Export Graph X-column	Save the X-column data, including labels, as a 1-D wave file (*.ibw).
	Export Graph Y-column	Save the Y-column data, including labels, as a 1-D wave file (*.ibw).
	Import Graphs	Open and display a saved graph.
	Note: There is no import of 1-D wave data.	
Options	Show Axes Color	Display a background color for the axes.
	Show Grid	Display X & Y grid lines in the graph.
	Show Error Bars	Display SEM error bars for averaged data.
	Show Markers	Display data points with marker symbols.
	Show Lines	Display a line between data points. (Toggle between 'markers' and 'lines', or both.)
	Cell Separator: TAB	Use tab separators when exporting a table (e.g. to Excel).
	Cell Separator: Comma	Use comma separators when exporting a table (e.g. to MS-Works).
	Include Column Labels	Column labels appear on the first line of an exported table.
Operations	Duplicate	Append a copy of the graph.
	Delete Graph or Table	Delete the entire analysis wave.
	Delete Single Graph	Delete the selected graph.
	Note: An analysis cannot be deleted if it exists in a graph or analysis window - it must first be closed.	
Math	Normalize: zero to maximum	Rescale the largest point to 1.0.
	Normalize: minimum to maximum	Rescale the smallest point to zero, and the

largest point to 1.0.

Invert Reverse the Y-axis sign of the data.

Append Displays available analysis wave names for appending.

Select an analysis wave for appending with the loaded wave. Time-course data are plotted relative to the loaded analysis wave's "time zero".

Average Displays available analysis wave names for averaging.

Select an analysis wave to be averaged with the loaded wave. A weighted average is performed, i.e., the number of data sets is accounted for when averaging in new data.

Two new entries are inserted into the wave list after the loaded wave:

1. The averaged wave.
2. The SEM (Standard Error of Means) data points wave.

If Options / Show Error Bars is enabled, the SEM data are used to display error bars in the corresponding averaged-data graph.

Standard Error of the Mean (SEM) Algorithm

$$\text{SEM} = \sqrt{(\text{SumSq} - \text{Mean}^2 * N) / (N-1)}$$

SumSq = sum of all squared samples

Mean = sum of all samples / N

Note: The SEM algorithm is similar to the Standard Deviation " $\sqrt{(\text{variance})}$ ", but using 'Mean' vs. 'sum of all samples'.

Scale and add List of available analysis wave names for scaling.

Use to combine waves, with optional scaling and offset applied.

When the Factor is '1.00' and the Offset is '0.00', this operation will simply add the selected wave to the displayed wave.

Factor Set a scaling factor for the selected wave to be added.

Values are displayed with SI unit prefixes.

To subtract a wave, change the Factor to a negative number, such as '-1.00'.

Offset Set an offset for the selected wave to be added.

Values are displayed with SI unit prefixes.

Values less than one are displayed with unit conversions.

Show Cursor Info Open the Cursor bar to see X & Y data values, and to set a fitting range.

A: pnt: The fit beginning data-point symbol, and its point number field.

B: pnt: The fit ending data-point symbol, and its point number field.

1. Select symbol A and drag it onto the beginning data point (or enter the data-point number in the 'pnt' field.)
2. Select symbol B and drag it onto the ending data point (or enter the data-point number in the 'pnt' field.)
3. The data points X- and Y-coordinates are displayed.
4. Right-click in the graph and select 'Quick Fit' for a list of built-in Igor fitting functions.
5. The fit is displayed in the graph, and the fitting information is written to the Log and Command windows.



Options menu

- One Mover Moves All
- All Styles Change the symbol graphics.
- Show Cursor Pairs Display up to 5 sets of cursor symbol pairs.

After the cursor endpoints are set, right-click in the graph and select 'Quick Fit' for a list of built-in Igor fitting functions to apply to the cursor range. The fit will be displayed in the graph, and the fitting information is written to the Log and Command windows.

Analysis Wave Names Loaded analyses available for manipulation.

Analysis Parameters

- Series: R#_ Name of the Series.
- Graphs: # Number of graphs in the analysis wave.
- Points: # Number of data points in the graph.
- Start: # Start time of analysis wave.
- Average: # Number of graphs averaged or appended.

Tip: If the Analysis Parameters text is not fully visible, increase the width of the Analysis Editor window.

[Graph & Table pane] Data point markers are plotted, or a numeric table is displayed.

X- and Y-axes can be magnified to be larger or smaller. Place the mouse cursor in the axis ticks region, then scroll the mouse wheel up or down. The axis ticks region does not include the tick label (numbers) area.

4.2.3 Analysis Window

Scope measurements are plotted in an Analysis window docked on the right side of the Scope window. An Analysis window can be resized or closed, but not undocked from the Scope window.

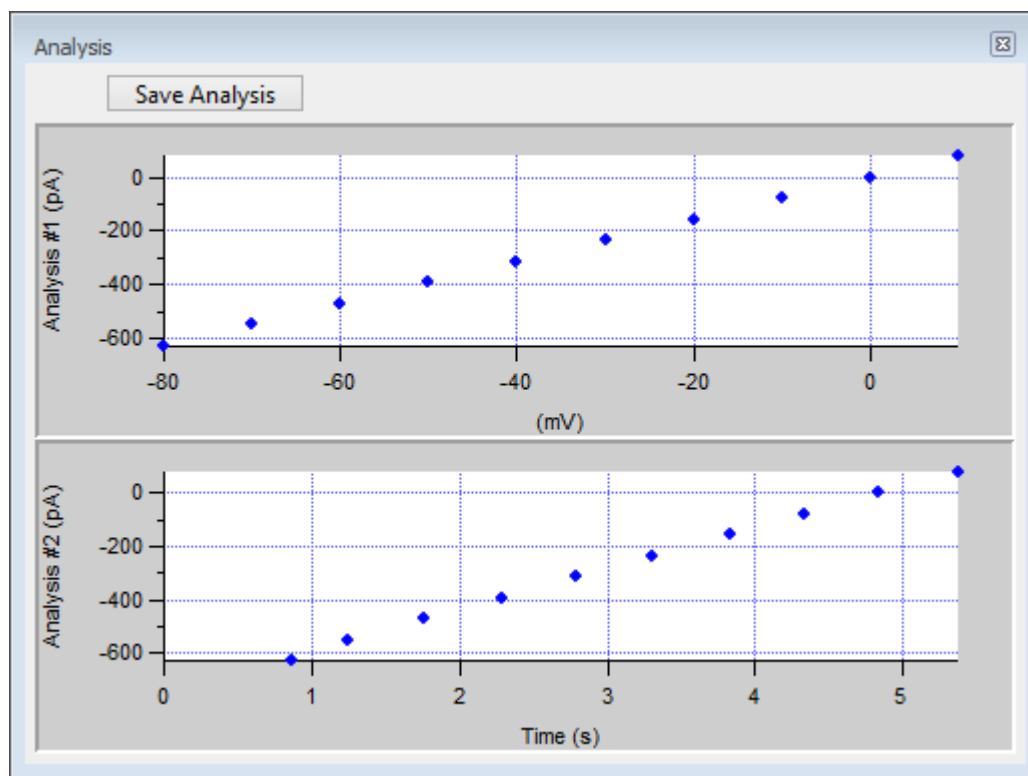


Figure 4-59. Analysis Window

Analysis measurements are configured in the Routine Editor Real Time Measurements & Graphs section. A separate pane is created in the Analysis window for each enabled Measurement graph.

Online measurements are plotted during data acquisition in real-time.

Data can be selected for offline review or analysis via the Data Navigator. When stored data are rerun for analysis, the data displays in a Scope window, and the analyses are graphed in an accompanying Analysis window. The last measurements applied to the data are automatically used to reanalyze the data.

Save Analysis This button saves the displayed analyses with the Experiment. Saved analyses are viewable in the Analysis Editor or from the Data / Data Browser: Data / Analysis folder.

[Graph pane] X- and Y-axes can be magnified to be larger or smaller. Place the mouse cursor in the axis ticks region, then scroll the mouse wheel up or down.

Note: The axis ticks region does not include the tick labels (numbers).

Symbols

Symbols can be manually overlaid onto Analysis window data points.

1. Select the Analysis window and click CTRL-I, to display the symbols tool bar.
2. Select symbol A or B.
3. Drag it onto a data point, or enter the data point number in the 'pnt' field.
4. The data point X- and Y-coordinates are displayed. When two symbols are placed into the graph, the delta X-Y value is also displayed.



'Options' menu

- One Mover Moves All Move all symbols together with a control.
- All Styles Change the symbol graphics.
- Show Cursor Pairs Display up to 5 sets of cursor (symbol) pairs.

Note: If the Analysis window is closed when a Layout graph is created, Analysis graphs will not be included in the Layout graph.

4.2.4 Data Browser

Igor's Data / Data Browser can be used to display all of the Experiment's data objects, such as data waves, analysis graphs, layouts, images, metadata, Paradigms and Routines.

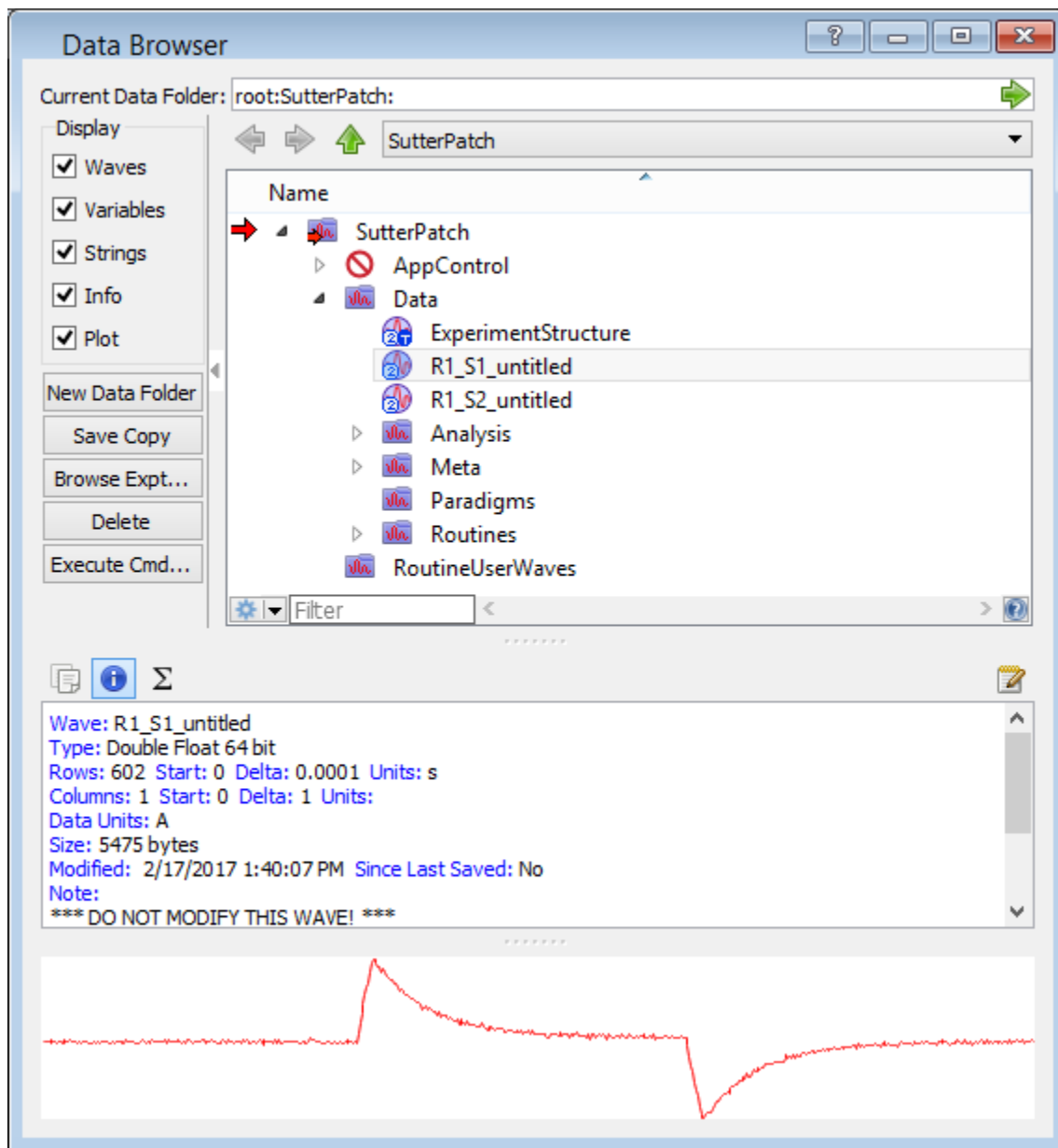


Figure 4-60. Data Browser

Objects are displayed in a tree structure, using a path “root” of ‘SutterPatch’.

Recorded data are listed in the ‘Data’ subfolder, arranged per Signal.

Right-click Menu

Display	Display the Analysis data in a visual graph.
Edit	Display the Analysis data in a numerical table.

SutterPatch signal data are stored in two-dimensional data waves, with one column per trace, and one row per sample point.

Warning! Editing data here will permanently alter the raw data. Modify at your own risk!

Copy Full Path Copy the object's path to the clipboard. This refers to Igor's internal data folder in the Data Browser, not the computer OS file system. This path can be used by advanced Igor users in user functions and executable commands.

4.2.5 Data Navigator

The Data Navigator window organizes and displays all levels of data and controls for the loaded Experiment.

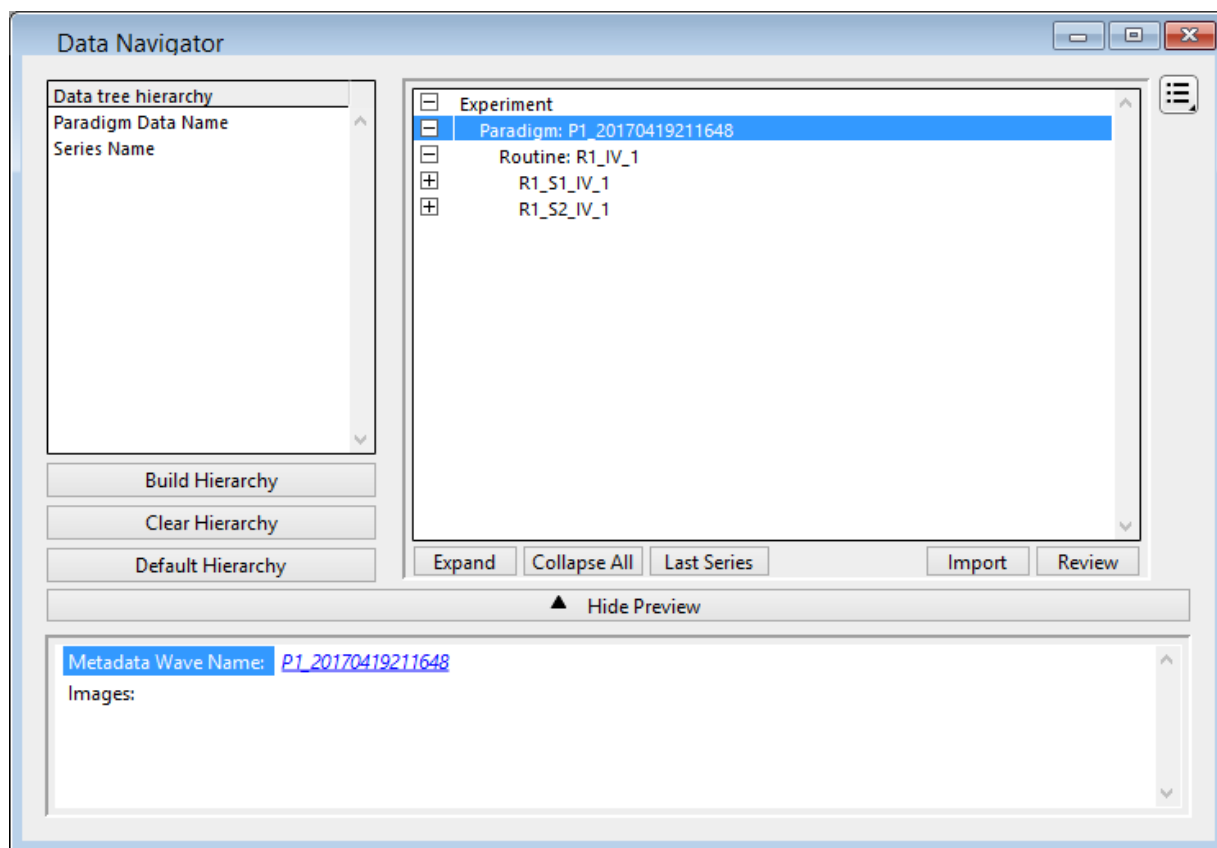




Figure 4-61. Data Navigator

Data tree hierarchy The hierarchy of sorting levels is displayed in this pane.

Build Hierarchy Re-organize the data tree using custom settings.

Select parameter group Organize by metadata parameters:
(availability depends on Preferences)

	<p> All Categories Frequently Used Experiment Hierarchy Tag Operator Preparation - Animal Preparation - Tissue Preparation - Cell Experiment Amplifier Data Acquisition Settings Imaging Stimulus </p>
Available parameter	Click on a specific parameter to select it.
	Click on the “copy” button to insert the selected parameter into the data hierarchy.
Hierarchy	The Hierarchy pane displays the new sorting hierarchy. The entries can be re-organized by selecting an entry and clicking on the Up/Down keys to reposition them, or using the ‘Del’ key to remove them.
	
Clear Hierarchy	Remove the existing hierarchy. The current Experiment’s raw data are organized in a simple time sequence.
Default Hierarchy	Restore the default experimental hierarchy: Experiment > Paradigm > Routine > Series > Sweeps
Do It	Click on the ‘Do It’ button to apply these changes to the data tree.
Data Tree Window	The current Experiment’s data are arranged in the data tree (down to the Sweep level.)
Expand	All closed nodes of the data tree are expanded down to the Signal level.
Collapse All	All nodes of the data tree are collapsed up to the

Paradigm level.

Last Series	The last Routine's first signal is highlighted in the data tree and its data are displayed in the Preview pane below it.
Import	Select a previously saved SutterPatch experiment to incorporate into the current experiment.
Analyze] For Routine and Series] The selected signals are opened in the Scope window for analysis.
Review	[For Paradigms] The selected signals and data are opened in the Scope window for review.


Note: Depending upon the data level, the same button displays 'Analyze' or 'Review'.

Show Preview / Hide Preview The display is based upon the data level.

Note: The Preview pane does not support mouse operations.

Paradigm:	Metadata Wave Name:	Click to display the Paradigm's metadata.
	Images:	Open any saved images.
	[Preview sub-pane]	Displays the first signal of the first Routine.
Routine:	Metadata Wave Name:	Click to display the Routine metadata.
	Signals:	Number of signals.
	Sweeps:	Number of sweeps.
	Routine Data Name:	Click to display the Routine parameters.
	Analysis:	Open any saved analyses into the Analysis Editor.
	Images:	Open any saved images.
Signal:	[Preview sub-pane]	Displays the first signal of the selected Routine.
	[Preview]	Displays the selected Signal.

Sweep:	[Preview]	Displays the selected Sweep.
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Available Actions button		Various actions can be performed on the selected data level.
--------------------------	---	--

These actions are also available via a right-click on the selected data level.

Paradigm:	Review	Display the Series data in the Paradigm Review window.
	View Metadata	Display the metadata in the Metadata Review window.
	Discard Paradigm Data	Remove the selected Paradigms and their Series from the Experiment. If the last Paradigm is discarded, then acquiring another Paradigm in the same Experiment session will increment the new Paradigm name. Otherwise, if the Experiment is closed and re-opened after the discard, then acquiring another Paradigm will use the last discarded Paradigm number.
	Save as Igor Binary File	Save the signal formatted as an “Igor Binary Wave” file (*.ibw).
	Export as ATF File	Save the signal formatted as an “Axon Text File” (*.atf).
	Export as ABF File ignoring unselected sweeps	Save each signal of the routine formatted as an “Axon Binary File” v.1.8 (*.abf).
	Export as ABF File replacing unselected sweeps with NaNs	Save each signal of the routine formatted as an “Axon Binary File” v.1.8 (*.abf).

Note: This file type is not supported by pCLAMP.

Routine:	Analyze	Display the Series data in a Scope (reanalysis) window.
	View Metadata	Display the metadata in the Metadata Review sub-window.
	View Routine	Display the Routine parameters in the Routine Review window.
	Show Data in Data Browser	Open Igor's Data Browser window.
	Discard Series	Delete the selected Series and remove from the Experiment. If the last Series is discarded, then acquiring another Series in the same Experiment session will increment the new Series name. Otherwise, if the Experiment is closed and re-opened after the discard, then acquiring another Series will use the last discarded Series number.
	Save as Igor Binary File	Save the signal formatted as an "Igor Binary Wave" file (*.ibw).
	Export as ATF File	Save the routine formatted as an "Axon Text File" (*.atf).
	Export as ABF File ignoring unselected sweeps	Save each signal of the routine formatted as an "Axon Binary File" v.1.8 (*.abf).
	Export as ABF File replacing unselected sweeps with NaNs	Save each signal of the routine formatted as an "Axon Binary File" v.1.8 (*.abf).
		Note: This file type is not supported by pCLAMP.

Signal:	Analyze	Display the Series data in a
---------	---------	------------------------------

		Scope (reanalysis) window. All signals and sweeps display.
	Event Detection	Open to analyze synaptic events (minis, etc.)
	Action Potential Analysis	Open to analyze action potentials.
	Edit	Display the Series signals as numeric columns in an editable table.
	Average Signal	Average all sweeps in the signal and display in the Analysis Editor.
	View Metadata	Display the Series metadata in the Metadata Review sub-window.
	Copy Full Path	Copy the Series internal Igor path to the clipboard. (root:SutterPatch:Data:Series_name)
	Save as Igor Binary File	Save the signal formatted as an “Igor Binary Wave” file (*.ibw).
	Show Data in Data Browser	Open Igor’s Data Browser window.
	Export as ATF File	Save the signal formatted as an “Axon Text File” (*.atf).
	Export as ABF File ignoring unselected sweeps	Save each signal of the routine formatted as an “Axon Binary File” v.1.8 (*.abf).
	Export as ABF File replacing unselected sweeps with NaNs	Save each signal of the routine formatted as an “Axon Binary File” v.1.8 (*.abf).
		Note: This file type is not supported by pCLAMP.
Sweep:	Analyze	Display the Series data in a Scope (reanalysis) window.
	Edit	Display the Series data as

	numeric columns in a table.
Extract Sweep	When a single sweep is selected, creates a graph of the sweep in the Analysis Editor.
Average Selected Sweeps	When multiple sweeps are selected, creates a graph of the averaged sweep in the Analysis Editor.
Show Data in Data Browser	Open Igor's Data Browser window.
Export as ATF File	Save the entire signal formatted as an "Axon Text File" (*.atf).
Export as ABF File ignoring unselected sweeps	Save each signal of the routine formatted as an "Axon Binary File" v.1.8 (*.abf).
Export as ABF File replacing unselected sweeps with NaNs	Save each signal of the routine formatted as an "Axon Binary File" v.1.8 (*.abf).
	Note: This file type is not supported by pCLAMP.

4.2.6 Data Table

The Data Table provides direct access to the sample points in a data Series, using a spreadsheet-style presentation.

Row	R1_S1_IV[][0]	R1_S1_IV[][1]	R1_S1_IV[][2]	R1_S1_IV[][3]	R1_S1_IV[][4]	F
	0	1	2	3	4	
0	5.23321e-12	-5.63541e-14	1.63961e-12	3.32488e-12	8.47496e-13	
1	-9.90098e-13	1.71653e-12	1.4612e-12	-2.72349e-12	-4.44117e-12	
2	-3.81879e-12	3.93903e-13	-2.90443e-12	4.29801e-13	6.7005e-13	
3	-7.61568e-12	-4.25361e-12	5.03512e-13	-2.13725e-12	-2.38818e-13	
4	1.77125e-12	-2.23609e-12	-9.99009e-13	5.83818e-13	-1.80796e-12	
5	-8.78851e-13	-1.72464e-12	5.22561e-12	-4.20016e-12	-2.72047e-12	
6	1.95622e-12	-4.37322e-12	-2.92145e-12	2.1944e-12	-1.13872e-13	
7	-2.17838e-12	-3.97302e-12	-1.08042e-12	-4.92174e-13	1.49426e-12	
8	3.4504e-12	-6.02635e-12	9.20459e-13	-2.37966e-12	-8.44283e-13	

Figure 4-62. Data Table

Data Tables are accessed from the Data Navigator by highlighting a data Series, right-clicking on it, and selecting the 'Edit' command. Alternatively, use the menu item Data / Data Browser and select a Series from the Data folder; then use the right-click menu item 'Display'.

To allow adding data to the table, the last row of data in the table is followed by a final row of blank (gray) cells. Manually entering data into the final blank row causes a new last row of data to be created in the table, followed by a new final blank row.

4.2.7 Equation Editor

The Equation Editor manages simple or complex expressions that evaluate to a value. Such math equations can be used to create stimulus waveforms, and for data analysis.

Access the Equation Editor from the SutterPatch menu.

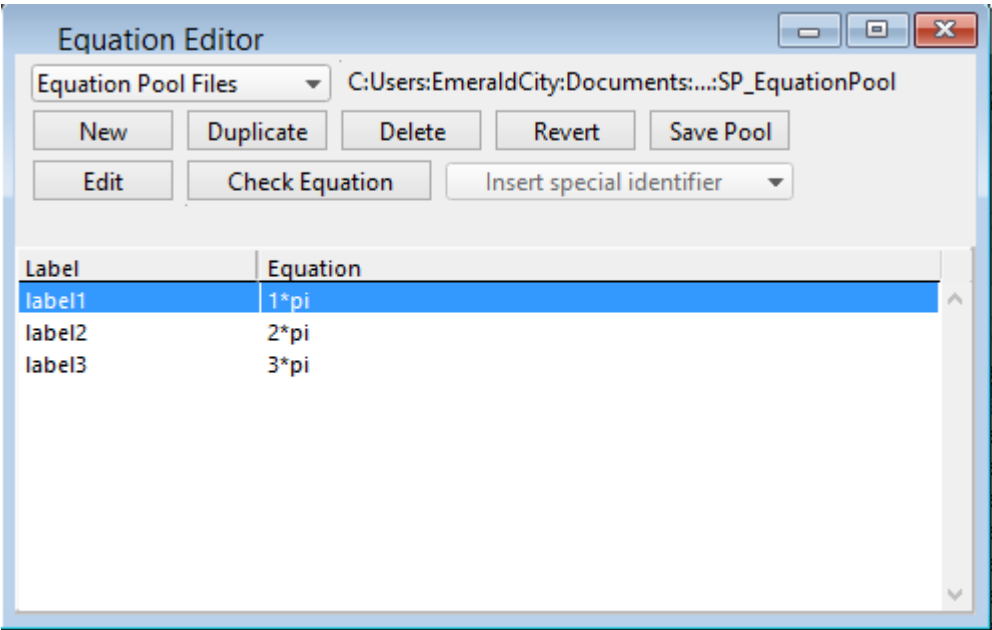


Figure 4-63. Equation Editor

Equation Pool Files Equations are created and saved in an Equation Pool file

New Equation Pool	Create a blank Equation Pool file.
Load Equation Pool	Load the Equations of a previously saved Equation Pool file into the Equation Pool.
Revert to Last Saved	Undo any unsaved changes to the Equation Pool.
Save Equation Pool	Save the Equation Pool using its existing file name and path.
Save Equation Pool As...	Save the Equation Pool to a new file, and switch to the new file. The default file name is the original file name.
Save Equation Pool Copy...	Save the Equation Pool to a new file, but do not switch to the new file. The default file name has 'Copy of' prepended to it.
Merge Equation Pools	Insert the Equation from a previously saved Equation Pool file into the loaded Equation Pool.

Note: Equation Pool files are simple text files that can be directly edited.

- | | |
|-----------|---|
| New | Create a blank Equation. |
| Duplicate | Add a copy of the selected Equation to the Equation Pool. |

Delete	Remove the selected Equation from the Equation Pool.
Revert	Select an Equation and click the Revert button. All editable steps are reset to their last saved settings.
Save Pool	Save the Equation Pool using its existing file name.
Edit	Make edits to the 'Equation' field.
Check Equation	Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports that the syntax is "ok".
Label	Column of editable equation names, for quick usage in place of the equation.
Equation	Column of equations in free-form text fields.

Insert special identifier

The following acquisition, amplifier and reference settings are available for use in equations:

Loop	(active paradigm ForLoop count)
Sweep	(active paradigm EachSweep count)
LastSweep	(active paradigm sweep count of last sweep)

Processing can occur before or after the last sweep of a series.

Example: In a Paradigm 'If' step, compare 'sweep' numbers in a ForEachSweep loop.

```

ForEachSweep
  EachSweep, Target=IV
  If, Left=sweep, Operation='=', Right=LastSweep-1
    Alert, Text=LastSweep, DoBeep=true
  EndIf
ForEachEnd

```

AqStopped	(last acquisition was stopped)
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The last Routine-Series did not complete by itself.

ParadigmTime	(time at start of paradigm, s)
RoutineTime	(time at start of routine, s)
Stimulant	(last applied stimulant concentration)

From the Solution Editor ‘Concentration’ setting, for solutions configured as a ‘Chemical Stimulant’.

Time	(present date-time, s)
Timer	(timer time, s)

m[1..16]	(n’t analysis measurement value)
gx[1..8]	(n’t analysis graph x value)
gy[1..8]	(n’t analysis graph y value)
r[1..16]	(n’t routine stimulus variable)
p[1..16]	(n’t paradigm variable)
Hold[1..8]	(holding of n’t output channel)
Input	(Input variable on paradigm window)

AuxIN[1..8]	(reading of auxiliary input, V)
-------------	---------------------------------

A single-point voltage reading from an Auxiliary Input channel of the IPA system, such as from a slowly changing temperature probe.

Note: This usage does not require setting up a Routine Input Channel.

Imon	(amplifier current reading, A)
	In the Amplifier Control Panel (pA).

Vmon	(amplifier voltage reading, V)
	In the Amplifier Control Panel (mV).

Mean[name or count, start,width]	(mean of given input signal)
----------------------------------	------------------------------

ActiveProbe	(active probe)
-------------	----------------

[1 – 4]

The “active” probe number is the Sutter headstage

presently controlled by the Amplifier Control Panel.

For a single headstage system, the active probe is always headstage number "1".

NumProbes (number of probes)
[1 – 4]
The number of IPA headstages attached to the system.

CCMode (amplifier current clamp)

VCMode (amplifier voltage clamp)

Hold (IHold in CC-mode, VHold in VC-mode)

[$\pm 0.000,000,020$ A ($\pm 20,000$ pA), or ± 1.000 V (± 1000 mV)]

IHold (amplifier holding current, A)

[$\pm 0.000,000,020$ ($\pm 20,000$ pA)]

IHoldOn (amplifier holding current On)

VHold (amplifier holding voltage, V)

[± 1.000 V (± 1000 mV)]

VHoldOn (amplifier holding voltage On)

IGain (amplifier current gain, V/A)

The gain of the active voltage-clamp 'Current' input channel.

- 0.5 mV/pA
- 1 mV/pA
- 2.5 mV/pA
- 5 mV/pA
- 10 mV/pA
- 25 mV/pA

VGain	(amplifier voltage gain, V/V)
The gain of the active current-clamp 'Voltage' input channel.	
<ul style="list-style-type: none"> • 10 mV/mV • 20 mV/mV • 50 mV/mV • 100 mV/mV • 200 mV/mV • 500 mV/mV 	
Filter	(amplifier input filter, Hz)
Apply a filter to the appropriate input channel in either VC- or CC-modes.	
Use a preset value, or a 10% threshold between the preset values is applied to the equation (to avoid over filtering).	
<ul style="list-style-type: none"> • 500 (500 Hz) • 1000 (1 kHz) • 2000 (2 kHz) • 5000 (5 kHz) • 10000 (10 kHz) • 20000 (20 kHz) 	
Offset	(amplifier pipette offset, V)
OffsetLock	(amplifier pipette offset lock)
VTrack	(amplifier tracking potential, V)
VTrackOn	(amplifier tracking potential On)

ECompMag	(amplifier electrode compensation magnitude, F)
ECompPhase	(amplifier electrode compensation phase, fraction)
ECompOn	(amplifier electrode compensation On in CC mode)
CmComp	(amplifier cell compensation Cm, F)

RsComp	(amplifier cell compensation Rs, Ohm)
RsCompOn	(amplifier cell compensation Rs On)
RsCorr	(amplifier Rs correction, fraction)
RsLag	(amplifier Rs correction lag, s)
RsCorrOn	(amplifier Rs correction On)
Bridge	(amplifier bridge balance, Ohm)

Relectr[1..4]	(electrode/seal/access resistance, Ohm)
Rmemb[1..4]	(membrane resistance (Cell mode), Ohm)
Cmemb[1..4]	(membrane capacitance (Cell mode), F)
RMSNoise[1..4]	(membrane test RMS noise, A)

Other identifiers are forwarded to Igor Pro's 'Execute' command.

Equation Extras

Constants

true	1
false	0
ON	1
OFF	0

The following constants have 27-digit precision:

e	2.71...	(Euler's number)
pi	3.14...	(π)

Parsing and Operators

Equation parsing is executed from left to right, processing the highest precedence level operators first:

Precedence	Type	Operator
8	Comment	;
7	Exponentiation	^
6	Negation (logical) operations: Unary Negation, Logical	-, !

	Negation	
5	Multiplication, Division, Remainder	*, /, %
4	Addition, Subtraction	+, -
3	Bitwise comparisons: And, Or, Nor, Xor	&, , nor, xor
2	Logical operations: And, Or, Conditional If	&&, , If(? :)
1	Relational operations	>, >=, <, <=, !=
0	All other operations	Round, trunc, ceil, floor, exp, sqrt, ln, log, sin, cos, tan, asin, acos, atan, abs, rad, deg, noise, random

Table 4-4. Equation Parser

Warning: There are several differences in operator processing between the SutterPatch equation parser and the Igor Pro command parser.

Comment: ;

SutterPatch All characters to the left of a semi-colon are ignored

Igor Pro: A semi-colon separates multiple commands on the same command line.

Negation (unary): -, !

SutterPatch: Precedence:
Exponentiation > Negation

Igor Pro: Precedence:
Negation > Exponentiation

except when used in combination,
i.e., $-1^2 = -(1^2)$.

Logical: &&, ||, If(? :)

SutterPatch: Precedence:
Logical > Relational

Igor Pro: Precedence:
Relational > Logical.

Given these conflicting priorities, we recommend using parentheses to explicitly define the order of execution for expressions using Negation, Exponential, Logical and Relational operators.

The ‘Conditional’ operator “If(? :)” is evaluated as:

If(statement ? Result-when-True : Result-when-False)

Note: The " : " is a colon with 2 blank spaces around it.

Example: Set a threshold (lower limit) on cursor measurement values of 2 pA

If(m[1] > 2p ? m[1] : NaN)

While the bitwise left shift (<<) and right shift (>>) operators in Igor Pro are not supported in SutterPatch, they can be easily constructed from existing operators:

Example: Shift number (#) left by “n” bits

* 2^n

Example: Shift # right by “n” bits

/ 2^n

Syntax

All equations use the same syntax as Igor Pro, with a few additions:

Three kinds of brackets [], { }, (), can be used equivalently to improve the clarity of nested expressions.

Numeric values can be written in scientific E-notation using exponents:

5e-12 (5 picoamps)

or in equivalent engineering notation using unit prefixes:

5p (5 picoamps)

Prefix	Exponent	Prefix Name		Prefix	Exponent	Prefix Name
k	10 ³	Kilo		m	10 ⁻³	milli
M	10 ⁶	Mega		μ (or u)	10 ⁻⁶	micro
G	10 ⁹	Giga		n	10 ⁻⁹	nano
T	10 ¹²	Tera		p	10 ⁻¹²	pico
P	10 ¹⁵	Peta		f	10 ⁻¹⁵	femto
E	10 ¹⁸	Exa		a	10 ⁻¹⁸	atto
Z	10 ²¹	Zetta		z	10 ⁻²¹	zepto
Y	10 ²⁴	Yotta		y	10 ⁻²⁴	yokto

Table 4-5. Engineering Notation

Insert an equation from the Equation Editor Pool into an Equation field by entering “#” followed by the label of the equation, e.g. “#MyLabel(5)”. This passes the argument “5” to the equation labeled “MyLabel” for evaluation.

Example: Using an LED light source

To stimulate in increments of light intensity, use an equation to transform light intensity values in Routine variables into actual stimulus values with amplitudes in volts.

Build an equation in the equation pool as follows:

equation	=	$\ln(r[1]) * 2.55 + 3$	The natural log of the Routine Variable r[1] is multiplied by 2.55 and added to 3.
label	=	power_to_volts	In the Waveform Editor, set a Segment Amplitude field to ‘Equation’, and enter the equation as “#power_to_volts”.

A simplified version of the Equation Editor allows Equations (and equation labels) to be used in the following areas:

Paradigm Editor

Steps: Amplifier, Checkbox, Set Variable, Sound, Write Log, If, Else If

Routine Editor

Input Channels: Virtual Channels: Math Type

Output Channels: Leak Hold, Waveform Editor: Amplitude, Duration

Measurements: Time to Threshold, X-Axis, Y-Axis

Note: Computing an equation for a data wave consumes significant computing power, as every data point needs to be computed by the CPU. While a slight update delay in such operations is expected, for computers with marginal computing power, the “beach ball” icon displays while the computer is unresponsive and busy processing.

4.2.8 Event Detection (synaptic events)

Post-synaptic potentials and currents from excitatory and inhibitory events (EPSPs, EPSCs, IPSPs, IPSCs) are analyzed with this application module. Spontaneous miniature events (“minis”), which generate small and/or overlapping events, are also often detectable with this technique. It uses an innovative deconvolution algorithm to find events with high temporal fidelity, while also improving the signal-to-noise ratio (SNR).

Sweep #	The sweep number of the displayed data.
Event Time	The time of the event’s threshold crossing (s).
Amplitude	The amplitude averaged around the peak by ± 1 ms.
concatenate sweeps	Combine all sweeps into a single pseudo-sweep before processing. This may be useful if data was collected using the continuous acquisition mode.
display template	Display the ideal event’s template on top of the selected event in the graph - its Y-axis displays on the right edge of the graph.
Tip:	To match the template to the data, hover the mouse cursor over the right Y-axis, and use the mouse wheel to rescale the template.
Threshold (xSD)	<p>A detection threshold representing the “Event Strength”, where a lower (“weaker”) number finds more events, while a higher (“stronger”) number finds fewer events. Adjust this threshold based on empirical testing of your data.</p> <p>Lower # = more noise-based events (false-positives) Higher # = more missed events (false negatives)</p>
Note:	The default threshold is set to 4 times the standard deviation of a Gaussian fit to an all-points histogram of the (Fourier) deconvolved data signal.
Ampl Threshold	Set an amplitude threshold for the minimum size of events.
Analysis Start (s)	Sweep time to start looking for an event threshold..
Analysis End (s)	Sweep time to stop looking for an event threshold..
Event	The current event number vs. total number of events.
Remove	Delete the current event from the analysis.
Template	Open the Template sub-panel to configure a template.
Start	Run the Event Detection analysis.
Average	The averaged event displays in the graph.
Threshold Analysis	A scatter plot of the Event Strength vs. Current is displayed in a sub-panel.

Save Results

Event Detection Results A Layout window report is created.

Total time analyzed = Includes the Start/End times for all sweeps (s).

Number of events detected = Total number of events found.

Event Frequency = (Hz)

Average Event Amplitude = ± 1 ms peak average (pA)

Standard Deviation of Event Amplitude = (pA)

Graphs: Cumulative probability vs. Amplitude
 Amplitude (Average) vs. Time
 Frequency vs. Sweep Number
 Amplitude vs. Sweep Number

Results Table A table of columns is created.

[blank] Row number with one row per Event.

Sweep Number The sweep number the Event is in.

Event Time (s) 'Time to event' from the start of sweep.

Event Strength (xSD) A measure of how well the signal matches the template. (Lower is weaker, higher is stronger.)

Event Amplitude (A) The Event peak amplitude ± 1 ms average

Event Integral (A*s)

10-90% Rise Time (s)

Event Decay Tau (s)

Absolute Event Time (s) 'Time to event' from start of Routine.

Inter Event Interval (s) Time between two contiguous events.

Template

Create a template of a typical event as a double-exponential curve. The data will be deconvolved to this template for further analysis.

Event Polarity 1 = positive
 -1 = negative

Rise Time (μ s) Time constant (τ) for the rising phase of the template event.

Decay time (μ s) Time constant (τ) for the falling phase of the template event.

Create Template Click this button to create the template.

References

Deconvolution paper:

Pernía-Andrade AJ, Goswami SP, Stickler Y, Fröbe U, Schlögl A, Jonas P. A Deconvolution-Based Method with High Sensitivity and Temporal Resolution for Detection of Spontaneous Synaptic Currents In Vitro and In Vivo. *Biophys J.* 2012 Oct;103(7):1429–39.

4.2.9 Igor Analyses

Curve fitting options are located in the Analysis main menu:

- Curve Fitting Create your own fitting equation
- Quick Fit Use a pre-defined equation:

line

poly

poly_XOffset

gauss

lor

exp_XOffset

dblexp_XOffset

exp

dblexp

sin

HillEquation

Sigmoid

Power

LogNormal

poly2D

Gauss2D

FitBetweenCursors

Weight from Error Bar Wave

Textbox Preferences

Example: Perform a fit on a section of a sweep:

1. In the Data Navigator, select the sweep, and use the 'Extract Sweep' command on it.
2. In the Analysis Editor, click the 'To Layout' button.
3. In the Layout window, right-click for the "Show Window" command.

4. With the Graph window active, select the main menu item Graph > Show Info.
5. In the Graph window cursor pane, drag cursors 'A' and 'B' onto the data.
6. Select main menu Analysis / Quick Fit and the fit of your choice.

Built-in Igor analyses are documented in the Igor Pro Help:

Fourier Transforms

Convolve

Correlate

Differentiate

Integrate

Smooth

Interpolate

Filter

Resample

Sort

Histogram

Compose Expression

Packages

Average Waves

Batch Curve Fitting

Function Grapher

Global Fit

Median XY Smoothing

MultiPeak Fitting

Percentiles and Box Plot

Wave Arithmetic

4.2.10 Layout Window

The Layout window is used to prepare your data for publication. Scope window input signals and associated Analysis window graphs are displayed in a Layout window for graphical arrangement and editing.

Layout windows can be manually created from a Scope window 'Layout' button



Layout windows can also be automatically created by a Paradigm running an Export step.

Note: The Layout window is often created behind other windows, and if blank, needs to be clicked on to update.



When a Layout window is open, the main menu “Layout” item displays. The ‘Append to Layout’ command allows you to append any graph object in the Experiment into the active Layout window.

Once a Layout window is created and filled, additional graphs are automatically appended to additional pages. The default arrangement is “2 x 4” graphs per page.

New Layout windows allow page layout configuration for the number of graphs per page (‘column’ x ‘row’). Control this setting in Preferences / Export Graphics or the Paradigm ‘Export’ step.

- 1 single pane
- 2 stacked panes
- 3 stacked panes
- 2 x 2 matrix
- 2 x 3 matrix
- 2 x 4 matrix

A toolbar displays in the upper-right corner of the Layout window. There are two configuration buttons at the top, which display different sets of buttons:

-  Operate Mode Selection tools and object insertion
-  Draw Mode Drawing tools

When you close a Layout window, you can choose to save it as a “macro”. To restore a Layout window, go to the Igor Pro menu Windows / Layout Macros and select the macro name. Refer to the Igor Pro documentation for further details about macros.

4.2.11 Metadata Review

Metadata describes the system environment (computer and amplifier) and user settings of Paradigms and Routines.

Metadata parameters can be retrieved several different ways:

Data Navigator

- Select a Paradigm or Routine, and then its Metadata Wave Name in the Preview pane.
- Select a Paradigm or Routine, then click the 'Available actions' button and select View Metadata.

- Right-click a Paradigm or Routine and select View Metadata.

Scope (Reanalysis) Use the View Metadata button in this window.

Note: User-defined parameters from all levels of the Set Preferences / Metadata / 'Metadata detail level' are displayed along with the automatic metadata.

Paradigm Data Displays the name of the Paradigm.

Routine Data Displays the name of the Routine.

- By Event System settings, routine settings, and defined metadata events are displayed per Signal. Highlighted fields are editable.
- By Parameter Includes an expanded set of software parameters displayed per category

Expand All All parameter settings are displayed.

Collapse All All parameter settings are hidden.

Automatic Metadata Parameters “By Parameter”

Tag

Tag Number

Tag Creation Timestamp

Timer Time at Tag Creation

Tag Signals

Tag Source Event

Operator

Login Name

Experiment

Experiment Timestamp

Amplifier

Amplifier Sequence Number

Amplifier Manufacturer

Amplifier Model

Amplifier Serial Number

Amplifier Channel

Number of Available Headstages

Headstage Sequence Number

(not displayed for a 1 headstage system)

Headstage Model

Headstage Serial Number

(not displayed in Demo mode)

Instrumentation and Software

Interface Sequence Number
 Interface Manufacturer
 Interface Model
 Interface Serial Number
 Interface Input Channel (physical)
 Interface Out. Ch. (physical or logical)
 Interface Signal Type
 Interface Number of Digital Outputs
 Computer Name
 Operating System Platform
 Operating System
 Software Environment
 Software Environment Version
 Software Environment Build
 Software Environment Kind
 Software Environment Serial Number
 Data Acquisition Software
 Data Acquisition Software Version
 Data Acquisition Software Build

Paradigm

Paradigm Data Sequence Number
 Paradigm Data Base Name
 Paradigm Name
 Paradigm Data Start Timestamp

Series (= Routine Data)

Series Sequence Number
 Series Base Name
 Routine Name
 Routine Acquisition Mode
 Routine Data Start Timestamp
 Routn. Completed / Terminated Early
 Number of Input Signals

Sweep

Sweep Number

Data Acquisition Settings

Active Headstage
 Recording Mode
 Current Gain
 Voltage Gain
 Headstage Gain
 Headstage Feedback Mode
 Filter Cutoff Frequency
 Filter Type
 Voltage Clamp Offset
 Voltage Clamp Offset Lock On/Off

Current Clamp Offset	
Current Clamp Offset Lock On/Off	
Input Signal Name	
Input Signal Units	
Input Scaling Factor	
Virtual Signal Scaling Offset	
Input Full-scale Minimum	
Input Full-scale Maximum	
Virtual Signal Math Type	
Virtual Signal Equation	
Virtual Signal Source Channel	
Virtual Signal Smoothing Factor	
Electrode Fast Magnitude	
Electrode Fast Time Constant	
Whole-cell Compensation On/Off	
Series Resistance Value	
Membrane Capacitance	
Series Resistance Prediction Value	
Series Resistance Correction On/Off	
Series Resistance Correction Value	
Series Resistance Corr. Lag Time	
Capacitance Neutralization On/Off	(only displayed in CC mode)
Capacitance Neutralization Magnitude	(only displayed in CC mode)
Capacitance Neutralization Phase	(only displayed in CC mode)
Bridge Balance Resistance	
Current Clamp Slow Tracking On/Off	(only displayed in CC mode)
Current Clamp Slow Tracking Potential	(only displayed in CC mode)
Command Signal Name 1	
Command Signal Units 1	
Command Full-scale Minimum 1	
Command Full-scale Maximum 1	
Command Holding Enabled 1	
Command Holding Value 1	(if disabled, equals zero)
Command Signal Name 2	(hidden for a 1 headstage system)
Command Signal Units 2	(hidden for 1 headstage system)
Command Full-scale Minimum 2	(hidden for 1 headstage system)
Command Full-scale Maximum 2	(hidden for 1 headstage system)
Command Holding Enabled 2	(hidden for 1 headstage system)
Command Holding Value 2	(if disabled, equals zero)
Digital Holding Pattern (1 → 8)	
Amplifier Seal Test On/Off	

4.2.12 Paradigm Review

The ‘Paradigm Review’ Scope window displays a time-course of all data recorded with the Paradigm, including all executed Routines and their Series data.

This view also displays the tags that occur between Routines.

See the Scope Window for usage of the window controls.

4.2.13 Reanalysis Measurements & Graphs

The Scope (Analysis) window Measurements button allows editing of the 'Reanalysis Measurements & Graphs' panel. Use it to apply different analysis scenarios to recorded data by configuring measurements on input channels and analysis graphs.

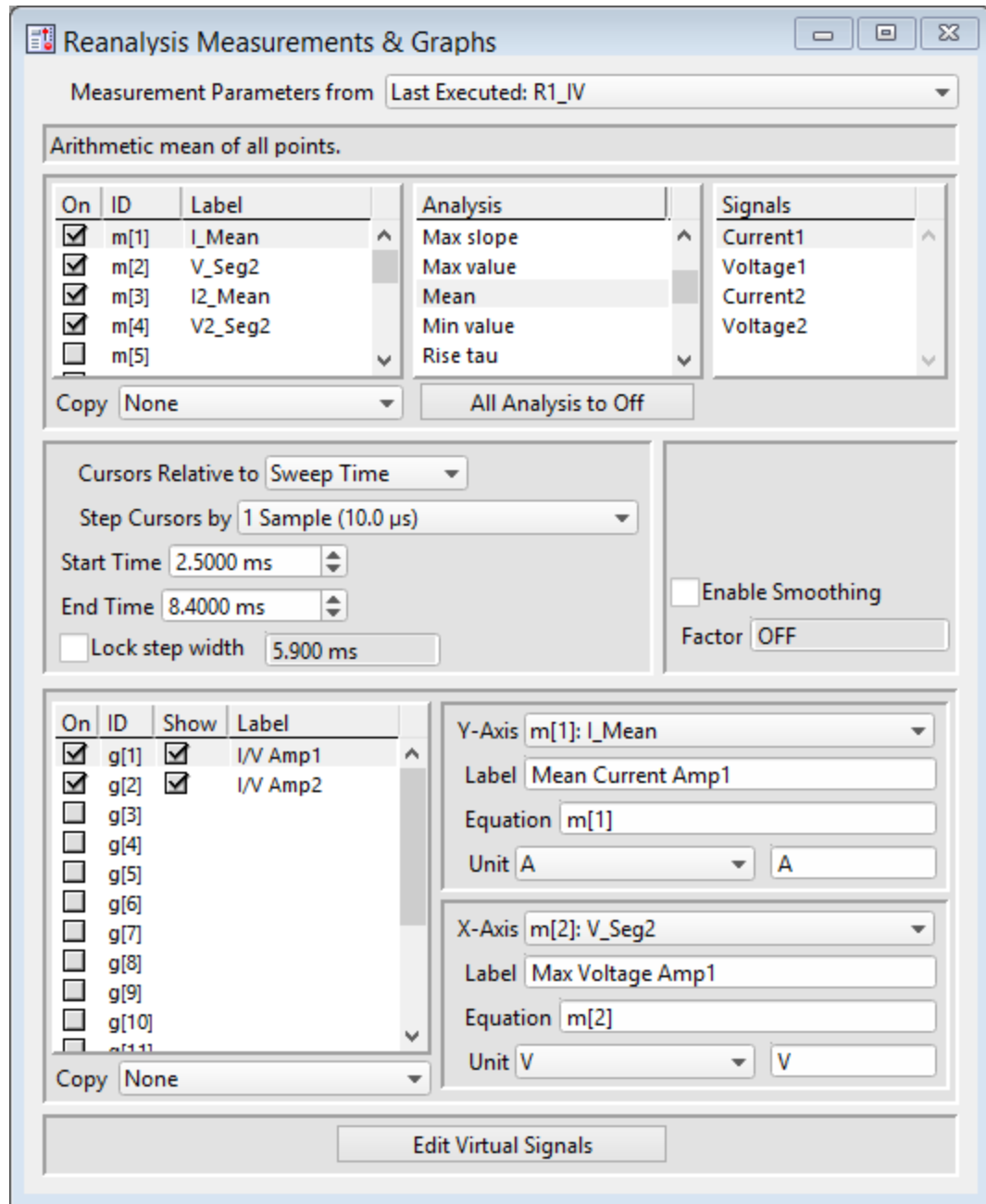


Figure 4-64. Reanalysis Measurements & Graphs

This dialog operates similarly to the Routine Editor: Real Time Measurements & Graphs dialog, with a few additions:

Measurement parameters from	Select which Routine settings to record with:
<ul style="list-style-type: none">• Current Settings	(newly modified settings)
<ul style="list-style-type: none">• Last Executed	(last used version)
<ul style="list-style-type: none">• Original Routine	(original loaded version)

Edit Virtual Signals A virtual signal dialog allows modification of these “pseudo” input signals.

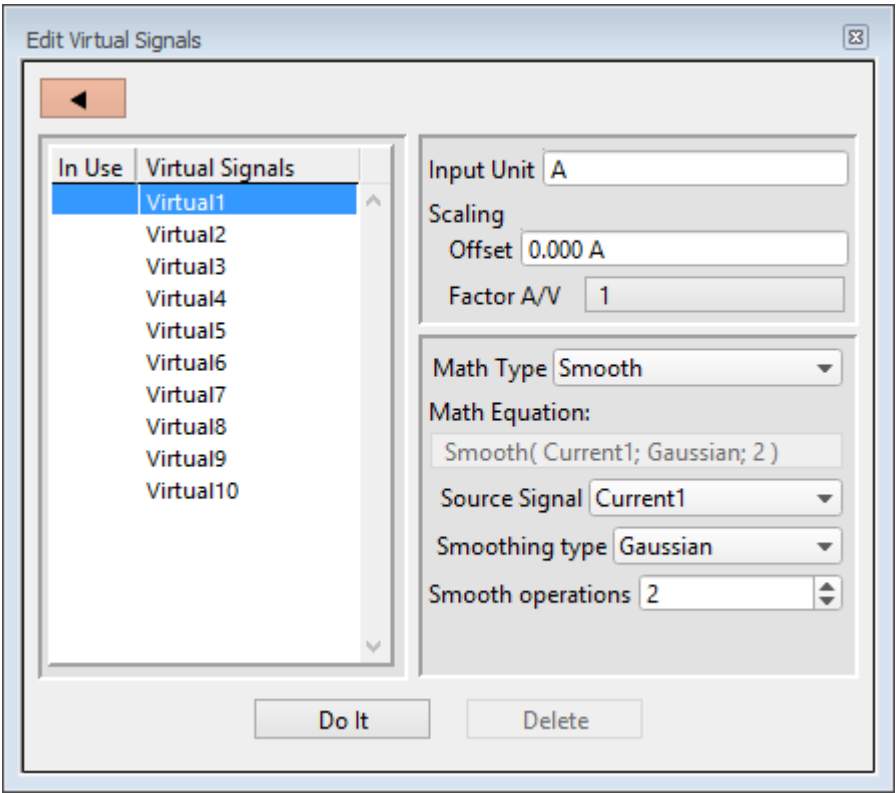


Figure 4-65. Edit Virtual Signals

Virtual signals support a wide variety of data transformations. To enable a virtual signal, highlight a signal name, then click the ‘Do It’ button. Changes to the highlighted signal are saved when you click ‘Do It’, and changes in unhighlighted signals are discarded.

Input Unit The base unit of measurement from its Source signal. The resolution of the unit is automatically adjusted in the signal.

Scaling

Offset Apply an amplitude offset to the input signal (after any scaling)

For “mV” units, append with ‘m’ or ‘e-3’

For “pA” units, append with ‘p’ or ‘e-12’

Example: 5 picoamps using engineering notation: 5p
or in equivalent scientific E-notation: 5e-12

Factor Apply scaling to interpret the input signal data.
Specify as a numeric value or an equation.

Note: The IPA data acquisition system uses a high-resolution 16-bit ADC with 64-bit data, so data resolution is not an issue when scaling input signals.

Math Type Apply a data transformation to a virtual input signal.

- BaselineSubtract Subtract a value from an input trace.

Source channel Select an input channel to process.

Baseline From Select the value to subtract.

- Value Subtract a fixed value from the input trace.

Value Spinner adjusts in 1 pA or 1 mV increments.

- Trace Subtract from the input trace.
- Sweep Time Subtract the average of the Start/End Time data from the input trace.

Start Time Set the starting time of the data to be averaged.

End Time Set the ending time of the data to be averaged.

- Segment #s Subtract the average of a Segment from the input trace.

Start Ratio Set the starting time of the data to be averaged, as a ratio relative to the Segment duration.

Start Time [reported value]

End Ratio Set the ending time of the data to be averaged, as a ratio relative to the

- Segment duration.
- End Time [reported value]
- **BesselFilter** A frequency-domain filter.
 - Source channel Select an input channel to filter.
 - Filter Band Select a frequency range.
 - **LowPass** Allow signal frequencies less than the cutoff frequency.
 - **HighPass** Allow signal frequencies greater than the cutoff frequency.
 - Filter Order [1, 2, 4, 8]
 - Number of “poles” in the filter
 - Cutoff Frequency (Hz) [100 to < ½ the sampling rate]
 - Restrict frequencies from this boundary point onwards.
 - **Differentiate** Data transformation using differentiation.
 - Source Signal Select an input signal to differentiate.
 - **Equation** Specify a math equation for the virtual signal.
 - [Equation field] A free-form text field.
 - < Syntax status message >
 - Check Equation Check the equation syntax. The equation is evaluated for sweep #1, and if valid, it reports “Syntax is “ok”.
 - Insert special identifier
 - Special references can also be used within commands:
 - **p[#]** n'th Paradigm variable.
 - **s[series-count, sweep-count, trace-count, routine name]**
 - Reference an input trace (data

wave) via counts of Series #, Sweep #, Trace # (Scope position), and the Routine name.

The “current” item is the “active” trace in the Scope window, and has a count value of zero.

If a “count” number is non-zero, it is used as an offset from the current count value of zero. Any fractions in count numbers are truncated to integers.

If the routine name is left blank, the current routine name is used.

Ex: s[0,0,0,]

The current series, current sweep, current trace, of the current routine.

- t[#]

Reference the input trace (data wave) in Scope position “n” for the last sweep of the current Series.

Undo

All changes in the equation editing session are discarded.

(See the Equation Editor for more details.)

- Integrate Data transformation using integration.
Source Signal Select an input signal to integrate.
- Leak Leak subtraction.
Source Signal Select an input signal to transform.
Show averaged leak Display the average of the leak sub pulses.
Leak zero segment Select a data segment without leakage, i.e. at "holding".
- LineFreq AC line frequency reduction.
Source Signal Select an input signal for noise reduction.

- | | | |
|--|----------------|----------------|
| | Line Frequency | 50 Hz
60 Hz |
|--|----------------|----------------|
- Smooth Data filtering.

Source Signal	Select an input signal to smooth.
Smoothing type	
Gaussian	Smooth operations [1 – 32767] # of smoothing operations to perform
Boxcar	Smooth repetitions [1 – 32767] # of smoothing repetitions to perform Boxcar window points [1 – 99] (odd values only) # of points in boxcar “window”
 - Stimulus Replicate the command waveform.

Source channel	Select an input channel – the waveform from its Parent Out Chan is used.
----------------	--
 - SweepAverage Average the input traces.

Source channel	Select an input channel to process.
Average Type	Cumulative RunningAvg Number of Sweeps
 - SweepSubtract Subtract a sweep from the input trace.

Source channel	Select an input channel to process.
Reference Sweep	The sweep that is subtracted from all other sweeps. If the sweep does not yet exist, no subtraction occurs.

Analysis Examples

Example 1: Plot the mean of the data (using sample routine IV)

1. Set measurement m[5] to the 'Mean' analysis and select signal Current1.
2. Enable graph [g5].
3. From the graph's Y-Axis list, select m[5]. The Equation field displays:

$$m[5]$$
4. Set 'X-Axis' to 'time'.
5. Run the analysis.
6. An Analysis window displays a graph of the mean vs. time.

Example 2: Plot the difference between two measurements

1. Set measurement m[5] to the 'Mean' analysis and select signal Current1.
2. Set measurement m[6] to the 'Mean' analysis, using the same signal.
3. Adjust the m[6] cursors Start/End times so they do not overlap with the m[5] cursors.
4. Enable graph [g6].
5. For the graph's Y-Axis, select 'Y-Equation' and enter the equation as:

$$m[5] - m[6]$$
6. Set the X-Axis to 'time'.
7. Run the analysis.
8. An Analysis window displays a graph of the difference vs. time.

4.2.14 Routine Review

The Routine Review window is similar to the Routine Editor Routine Settings and Preview pane sections, except that this Preview pane does not include sweep- and region-selection controls.

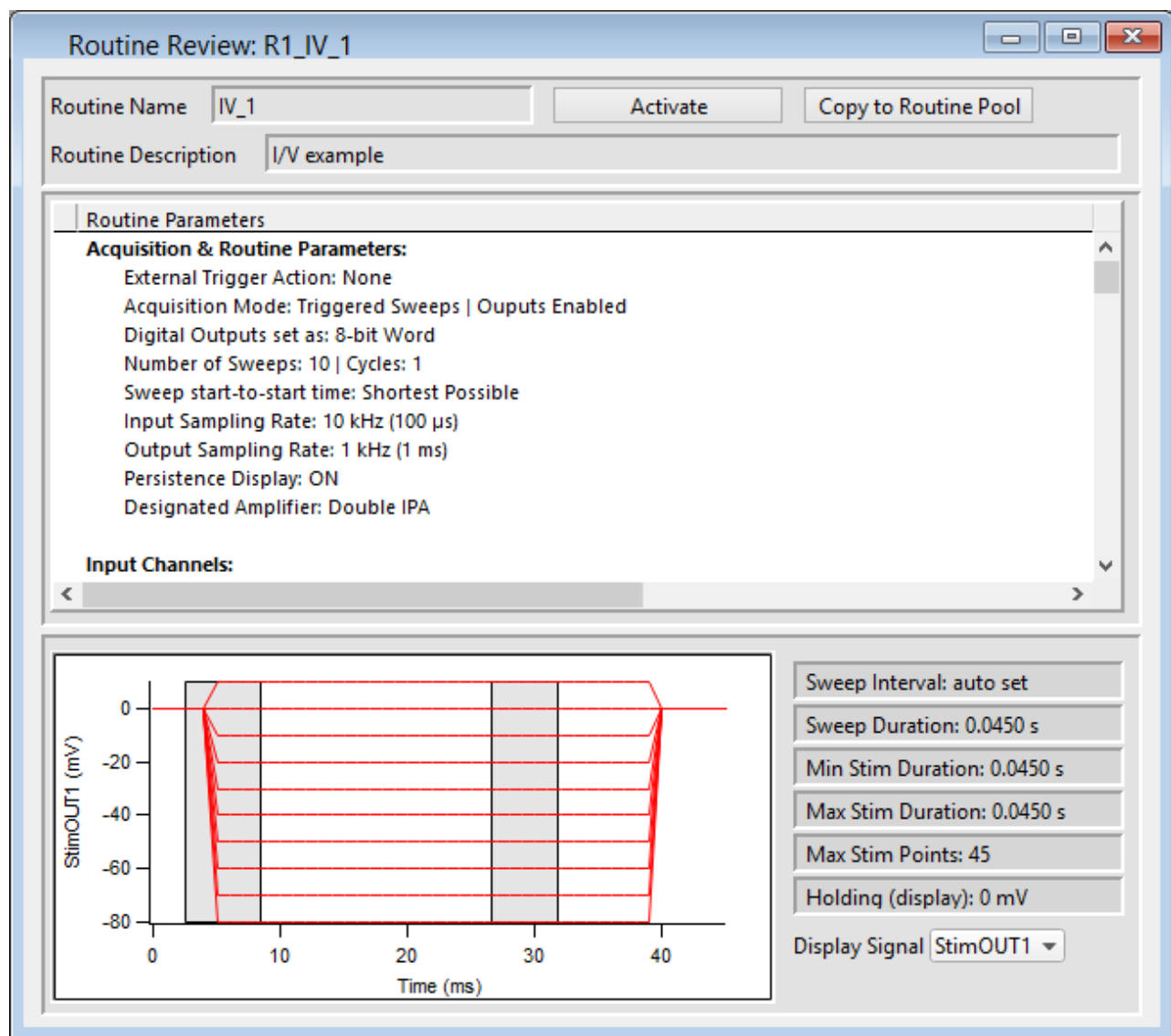


Figure 4-66. Routine Review

Open this window from the Data Navigator by highlighting a Routine, and selecting the 'View Routine' command from a right-click menu or the 'Available actions' button, or by selecting the Routine Data Name in the Data Navigator Preview pane.

Routine Name	Displays the Routine name.
Activate	Opens the Scope window loaded with these settings.
Copy to Routine Pool	Adds this routine to the loaded Routine Pool.
Routine Description	Displays the Routine description.
Display Signal	Select the output signal to display.

4.2.15 Scope (Analysis)

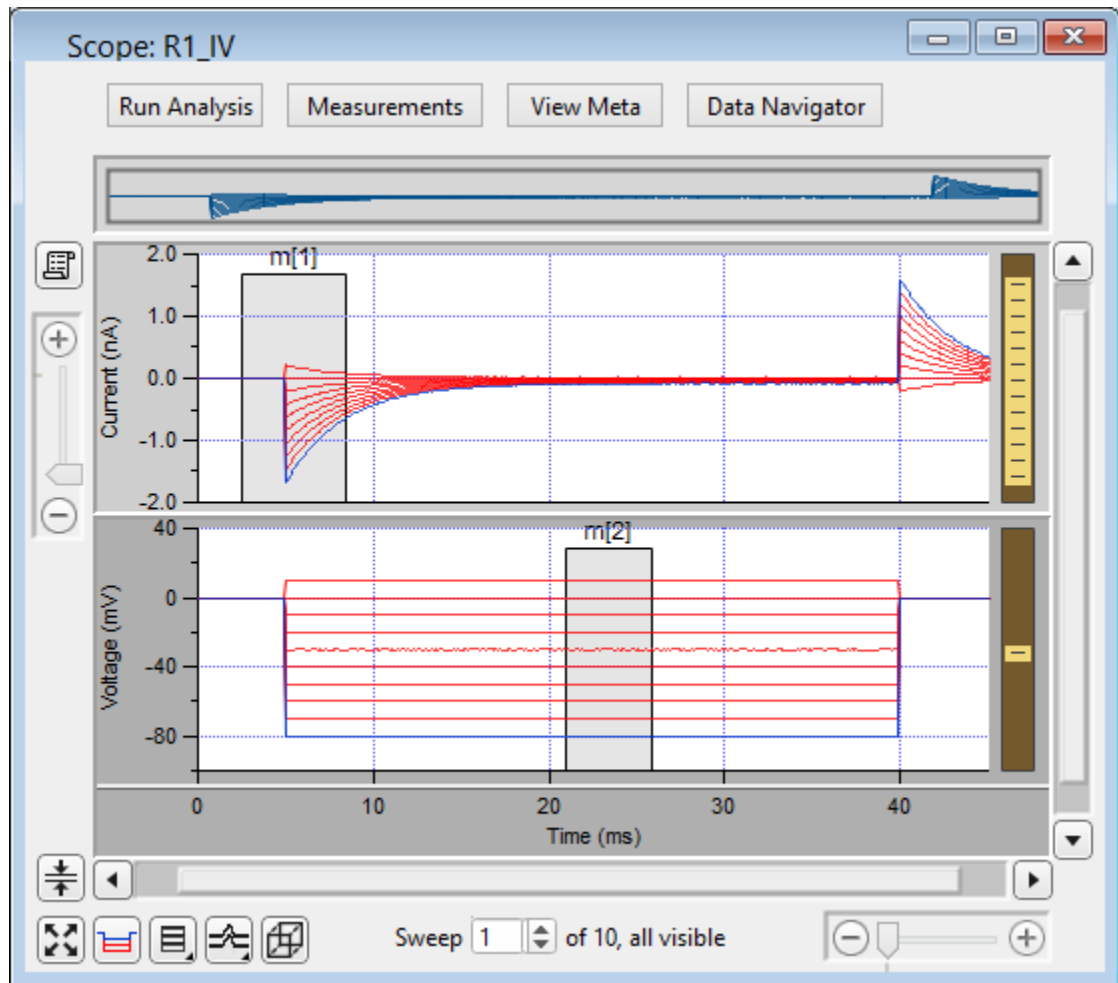


Figure 4-67. Scope (Reanalysis) Window

This analysis version of the Scope window is used to display and reanalyze stored data. Both physical and virtual channels can be displayed here. Measurement regions are used as described for the Scope (acquisition) window. The additional window controls are described below:

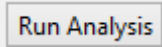
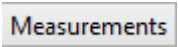
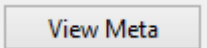
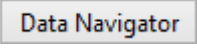


Navigation pane:

The Navigation pane appears at the top of the Scope window. It displays an overview of the active signal's full-scale data, with a gray box surrounding the magnification area.



Figure 4-68. Navigation Pane

The Navigation pane “magnification” box can be used to scroll through the active signal’s data. Place the mouse cursor over the magnification box and it changes into a ‘hand’ icon; click and drag the magnification box to scroll through the data.

	<p>Run the defined analysis for the data series, and graph the results in the Analysis window. To stop a long-running analysis, click on the ‘Abort’ button in the bottom right corner of the main screen.</p>
	<p>Show Cursors: Display measurement cursors in the Scope window.</p> <p>Hide Cursors: Do not display cursors in the Scope window. Button state displays as “Measurements(H)”.</p> <p>Lock Cursors: Prevent cursors from being moved or altered. Button state displays as “Measurements(L)”.</p> <p>Edit Measurements: Open a special Reanalysis Measurements & Graphs dialog, where all changes apply instantly and interactively to the measurements and graphs, even during analysis. These edits (temporarily) override the loaded routine for fast response.</p> <p>Edit Virtual Signals: Open the virtual input signals panel for editing,</p> <p>Single Channel Threshold Analysis: Configure single-channel analysis of ion channels.</p>
	<p>Display any extra information (metadata) associated with the displayed data Series, such as the operator, preparation details, solution information, etc.</p>
	<p>Open a Data Navigator window with all of your Experiment data and metadata available in a tree structure.</p>
<p>Layout</p> 	<p>Create a new Layout window containing all Scope signals and Analysis graphs.</p>
<p>Center</p> 	<p>Center the mean of the data in the selected signal pane. The Y-axis offset is automatically adjusted, while the Y-axis scaling is unchanged.</p>








Autoscale 	Autoscale all visible signals. Rescale the X-axes to their full sweep duration, and the Y-axes to their displayed sweeps data limits.	
Persistence Display 	Display all sweeps (per Preferences settings). Otherwise when disabled, only one sweep is displayed at a time.	
Signal Display 	Graphically arrange the input signals. Stack: A vertical column of signals Single: Only the active signal m x n: A tiled array of signals	
Sweeps Display	This button has 3 modes:	
	Sweeps 	Each trace starts from time zero to the duration of the waveform.
	Time Course 	Display sweeps in time sequence on a single time axis. Portions without data are left blank (such as the time between triggered sweeps.) Note: Emulation mode has a minimum 0.5 s interval between sweeps, both triggered and continuous. If the sweep duration is less than 0.5 s, the time between sweeps will be padded with “blank” time.
	Concatenated 	Display sweeps similarly to the Time Course mode, but any blank portions are replaced by a vertical line.
	The ‘Show 3D view of current signal’ button brings up a separate 3D display window attached to the right of the Analysis window. The Sweep data are color-coded for amplitude, and their 3D graph can be rotated in any direction.	
Sweep #:	The ‘Sweep #’ display at the bottom of the Scope window indicates the ‘active sweep’ number, the total number of sweeps in the Series, and either “all” or the total number of visible sweeps (per Preferences).	

Table 4-6. Scope Window Buttons

X- and Y-Axes	Same as the acquisition Scope
Main window	Additional items:

- 3D View
- Zero Baselines
- Show All Sweeps (with triggered sweeps)
- Select Sweeps (with triggered sweeps)

Data

Additional items:

- Hide Sweep_# (with triggered sweeps)
- Show Sweep_# Only (with triggered sweeps)

4.2.16 3D View Window

The 3D View window creates a 3D representation of your data, color-coded to show amplitude variations.

The axis definition in 3D View is based on the change of a waveform over the course of successive sweeps. In a two-dimensional display, the X-axis represents the Sweep Time, while the Amplitude is plotted on the vertical Y-axis. For consistency, the vertical axis in the SutterPatch 3D view is also defined as the Y-axis. In the default orientation of the 3D View, the Z axis, on which the Sweep Number is plotted, points backward and to the right.

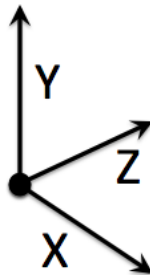


Figure 4-69. 3D Axes Definition

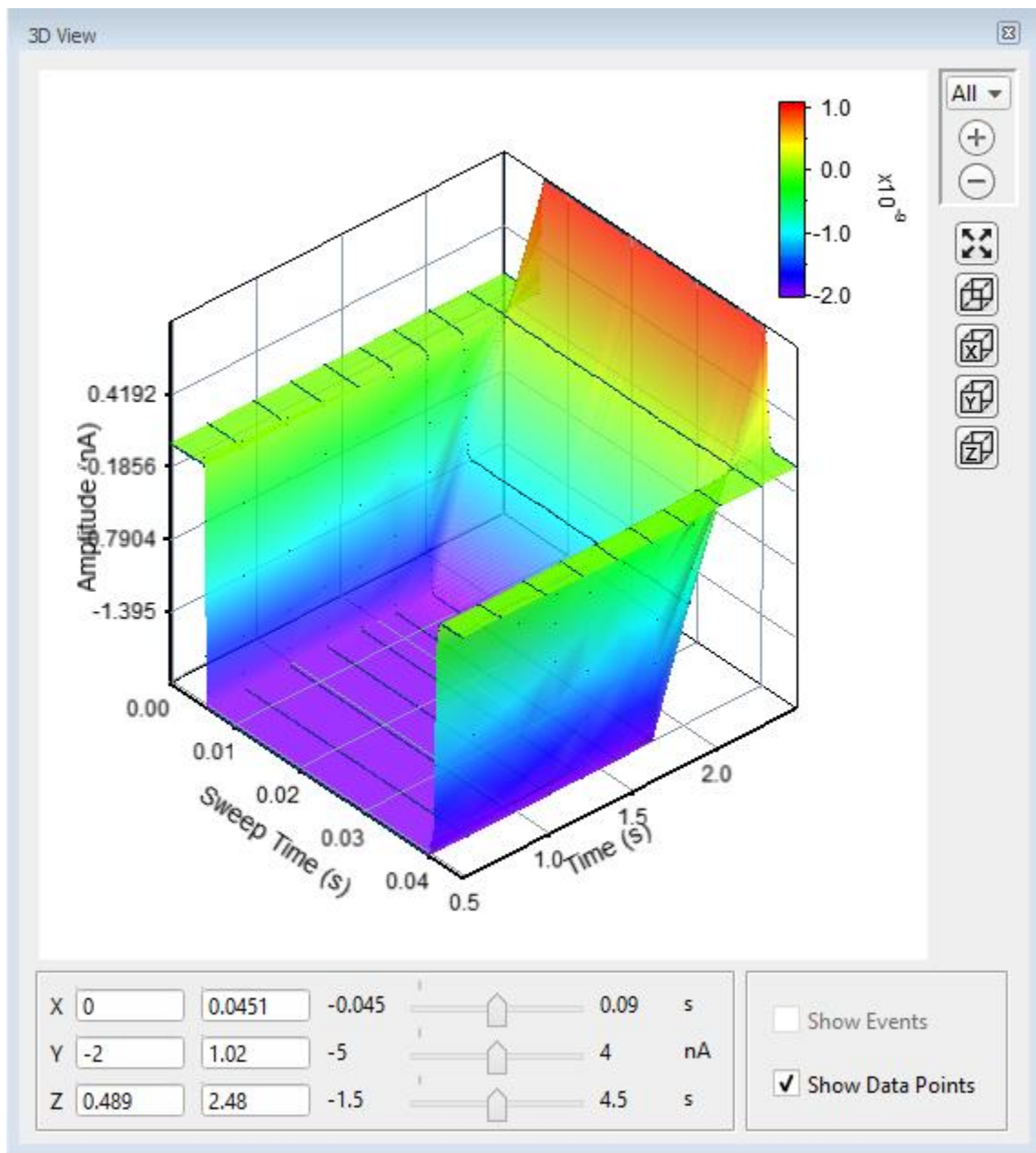


Figure 4-70. 3D View

A “heat map” bar illustrates the color measurement units.



Magnification buttons are located in the upper right corner of the window for the selected axis:

- All (All 3 axes)
- X (Sweep Time)
- Y (Amplitude)
- Z (Time)







Zoom in

(Magnify)

	Zoom out	(Unmagnify)
	Autoscale	(Set to the data limits)

X, Y and Z axis limits can be set in the bottom section of the 3D View window. Their delta value is preserved when using the scroll bars to update the visual graph (and the numeric axes limits.)

The 3D graph viewing angle can be changed with a set of 3D buttons:

	= Default View	(X, Y & Z axes display)
	X = Right View	(Y & Z axes display)
	Y = Top View	(Z & X axes display)
	Z = Front View	(X & Y axes display)

Alternatively, you can rotate the display in any direction by simply clicking and dragging the 3D graph. If you release the mouse button while dragging, the 3D display will rotate in the direction of the mouse drag.

Show Events Display tagged events in the 3D graph.

Show Data Points Display data points as surface dots in the 3D graph.

4.2.17 Set Metadata

Preparation and stimulus parameters can be associated with an Experiment, Paradigm, or Routine as user-configurable “metadata”.

Predefine the Metadata parameter values here. The level of available Metadata parameters can be configured in SutterPatch > Set Preferences > Metadata.

Show Summary An overview of the user-defined metadata parameters.

All parameters defined in any level of the Set Preferences / ‘Metadata detail level’ are displayed .

Metadata Summary dialog

All parameters that have values defined are displayed, even if they would not be displayed based on the current setting of Set Preferences > ‘Metadata detail level’. Double-clicking on any line opens the Set Metadata dialog with the respective parameter selected.

Metadata Parameter	Parameter name.
Current Value	Parameter value.
Increment Enabled	If enabled, double-click to review details.
Prompt before	Display a metadata prompt before running:.
<ul style="list-style-type: none"> ▪ Expt ▪ Pdgm ▪ Routn 	<ul style="list-style-type: none"> Experiment Paradigm Routine
Metadata Group	(Per the Set Preferences > Metadata detail level)
1. BASIC Level	Default metadata groups:
	Preparation – Animal Preparation – Tissue Preparation – Cell Experiment, Stimulus
2. EXTENDED Level	Additional metadata groups provided:
	Electrode Recording Solution
3. FULL Level	Even more metadata groups included:
	Operator Paradigm Cell Health / Quality Control Series (= Routine Data)
Metadata Parameter	Parameters from the selected metadata group are displayed for configuration per the Set Preferences > Metadata detail level. (See table below.)

[Parameter description]

Configuration choices for the selected parameter:

- Do not write this parameter This parameter is not stored.

If this parameter was previously written in this Experiment, then its Previous Value is displayed.
- Use last value The last used parameter is written.

- Use a previous value Select from a drop-down list of previous values.
- Use new value Enter a new value for the metadata parameter.
- Increment Numerically increment the value:

By

- Experiment At the start of each Experiment.
- Paradigm At the start of each Paradigm.
- Routine At the start of each Routine.

Prefix

Enter text to be prepended to the value.

Start value

The initial value (including decimals and negative numbers).

Increment:

Select an arithmetic operator [+, -, *, /]

[]

Enter the incremental amount.

Suffix

Enter text to be appended to the value.

Prompt for confirmation before:

- Experiment At the start of an Experiment.
- Paradigm For “planned” or named Paradigms (i.e., not auto-triggered by Routines).
- Routine At the start of a Routine.

Confirm Metadata Settings

This dialog displays when an Experiment, Paradigm or Routine is started while enabled for prompts.

Write

Write the selected metadata parameter with the Experiment, Paradigm or Routine.

Metadata Parameter

The selected metadata parameter.

Value

The metadata value to write.

Update

Store edited metadata values for future Experiments, Paradigms or Routines. This field is automatically disabled after each execution .

Prompt

Include this metadata parameter in the Confirm Metadata Settings dialog. If no metadata parameters are enabled for Prompt, the Confirm Metadata Settings dialog will not display.

BASIC	EXTENDED	FULL	GROUP / Parameters	NOTES
		F	OPERATOR /	
		F	Full Operator Name	

B	E	F	PREPARATION - ANIMAL /	
B	E	F	Animal Identifier	
B	E	F	Animal Species	Binomial species name
	E	F	Animal Strain	Strain, breed or variety characterizing the animal
	E	F	Animal Genotype	
B	E	F	Animal Age	
B	E	F	Animal Age Units	Ex.: h, d, m
B	E	F	Animal Sex / Gender	Ex.: 1: F, 2: M, 3: Undetermined
B	E	F	Animal Weight	
B	E	F	Animal Weight Units	
	E	F	Animal Preparation Date	ISO Date, Format: YYYY-MM-DD
	E	F	Animal Preparation Time	Time of Day, Format: hh:mm[:ss.000]
	E	F	Animal User Parameter 1 Name	
	E	F	Animal User Parameter 1	
	E	F	Animal User Parameter 2 Name	
	E	F	Animal User Parameter 2	
	E	F	Animal User Parameter 3 Name	
	E	F	Animal User Parameter 3	
	E	F	Animal User Parameter 4 Name	
	E	F	Animal User Parameter 4	
	E	F	Animal User Parameter 5 Name	
	E	F	Animal User Parameter 5	
B	E	F	PREPARATION - TISSUE /	
B	E	F	Tissue Preparation Identifier	
B	E	F	Organ	
	E	F	Organ Region	
	E	F	Preparation Method	
	E	F	Tissue Preparation Date	ISO Date, Format: YYYY-MM-DD
	E	F	Tissue Preparation Time	Time of Day, Format: hh:mm[:ss.000]
	E	F	Tissue Incubation Duration	
	E	F	Tissue Incubation Duration Units	
	E	F	Tissue Incubation Temperature	
	E	F	Tissue Incubation Temperature Units	
	E	F	Tissue Incubation Solution	
	E	F	Tissue User Parameter 1 Name	
	E	F	Tissue User Parameter 1	
	E	F	Tissue User Parameter 2 Name	
	E	F	Tissue User Parameter 2	
	E	F	Tissue User Parameter 3 Name	

	E	F	Tissue User Parameter 3	
	E	F	Tissue User Parameter 4 Name	
	E	F	Tissue User Parameter 4	
	E	F	Tissue User Parameter 5 Name	
	E	F	Tissue User Parameter 5	
B	E	F	PREPARATION - CELL /	
B	E	F	Cell Preparation Identifier	
	E	F	Acutely Dissociated Cells	
	E	F	Cell Line	
	E	F	Slice Preparation	
	E	F	Whole-organ Preparation	
	E	F	In-situ Recording	
	E	F	Stem Cell Preparation	
	E	F	User-defined Preparation	
B	E	F	Cell Type	
B	E	F	Cell Identifier	
B	E	F	Cell Preparation Date	ISO Date, Format: YYYY-MM-DD
B	E	F	Cell Preparation Time	Time of Day, Format: hh:mm[:ss.000]
	E	F	Cell Dissociation Solution	
	E	F	Cell Preparation Dissociation Temperature	
	E	F	Cell Prep. Dissociation Temperature Units	
B	E	F	Cell Preparation Incubation Duration	
B	E	F	Cell Prep. Incubation Duration Units	
B	E	F	Cell Preparation Incubation Temperature	
B	E	F	Cell Prep. Incubation Temperature Units	
B	E	F	Cell Preparation Incubation Solution	
B	E	F	Ion Channel	
	E	F	Cell Fluorescent Marker	
	E	F	Cell Diameter	
	E	F	Cell User Parameter 1 Name	
	E	F	Cell User Parameter 1	
	E	F	Cell User Parameter 2 Name	
	E	F	Cell User Parameter 2	
	E	F	Cell User Parameter 3 Name	
	E	F	Cell User Parameter 3	
	E	F	Cell User Parameter 4 Name	
	E	F	Cell User Parameter 4	
	E	F	Cell User Parameter 5 Name	
	E	F	Cell User Parameter 5	
B	E	F	EXPERIMENT /	
		F	Experiment Category 1 Name	
		F	Experiment Category 1	

		F	Experiment Category 2 Name	
		F	Experiment Category 2	
		F	Experiment Category 3 Name	
		F	Experiment Category 3	
		F	Experiment Category 4 Name	
		F	Experiment Category 4	
		F	Experiment Category 5 Name	
		F	Experiment Category 5	
B	E	F	Experiment User Parameter 1 Name	
B	E	F	Experiment User Parameter 1	
B	E	F	Experiment User Parameter 2 Name	
B	E	F	Experiment User Parameter 2	
B	E	F	Experiment User Parameter 3 Name	
B	E	F	Experiment User Parameter 3	
B	E	F	Experiment User Parameter 4 Name	
B	E	F	Experiment User Parameter 4	
B	E	F	Experiment User Parameter 5 Name	
B	E	F	Experiment User Parameter 5	
		F	Experiment Category Parameter 1 Name	
		F	Experiment Category Parameter 1	
		F	Experiment Category Parameter 2 Name	
		F	Experiment Category Parameter 2	
		F	Experiment Category Parameter 3 Name	
		F	Experiment Category Parameter 3	
		F	Experiment Category Parameter 4 Name	
		F	Experiment Category Parameter 4	
		F	Experiment Category Parameter 5 Name	
		F	Experiment Category Parameter 5	
	E	F	ELECTRODE /	
	E	F	Electrode Identifier	
	E	F	Electrode Glass Manufacturer	
	E	F	Electrode Glass Item Number	
		F	Electrode Glass Lot Number	
		F	Electrode Glass Material	
		F	Electrode Glass Item Outer Diameter	
		F	Electrode Glass Item Inner Diameter	
		F	Filamented Glass	
	E	F	Electrode Glass Ramp Test Value	
	E	F	Pipette Puller Manufacturer	
	E	F	Pipette Puller Model	
		F	Pipette Puller Serial Number	
		F	Puller Filament Type	
		F	Puller Filament Item Number	

		F	Pull Program Number	
		F	Pull Program Parameters	
		F	Pull Program Air Mode	
		F	Pull Program Air Pressure	
		F	Puller Heat-on Enabled	
		F	Puller Heat-on Time	
		F	Electrode Fire-polished	
		F	Electrode Coated	
		F	Electrode Coating Material	
		F	Electrode Beveled	
		F	Electrode Bevel Angle	
	E	F	Electrode User Parameter 1 Name	
	E	F	Electrode User Parameter 1	
	E	F	Electrode User Parameter 2 Name	
	E	F	Electrode User Parameter 2	
	E	F	Electrode User Parameter 3 Name	
	E	F	Electrode User Parameter 3	
	E	F	Electrode User Parameter 4 Name	
	E	F	Electrode User Parameter 4	
	E	F	Electrode User Parameter 5 Name	
	E	F	Electrode User Parameter 5	
	E	F	RECORDING SOLUTIONS	
	E	F	Solution Pair Identifier	
	E	F	Solution Pair Name	
	E	F	Bath Solution Identifier	
	E	F	Bath Solution Name	
		F	Bath Solution Batch	
		F	Bath Solution Composition	
		F	Bath Solution Preparation Date	
		F	Bath Solution Preparation Time	
		F	Bath Solution pH	
		F	Bath Solution pH Adjustment Agent	
		F	Bath Solution Osmolarity	
		F	Bath Solution Osmolarity Adj. Agent	
	E	F	Pipette Solution Identifier	
	E	F	Pipette Solution Name	
		F	Pipette Solution Batch	
		F	Pipette Solution Composition	
		F	Pipette Solution Preparation Date	
		F	Pipette Solution Preparation Time	
	E	F	Pipette Solution pH	
		F	Pipette Solution pH Adjustment Agent	
	E	F	Pipette Solution Osmolarity	

	F	Pipette Solution Osmolarity Adj. Agent	
	F	Liquid Junction Potential, computed	
	F	Liquid Junction Potential, measured	
	F	Solution User Parameter 1 Name	
	F	Solution User Parameter 1	
	F	Solution User Parameter 2 Name	
	F	Solution User Parameter 2	
	F	Solution User Parameter 3 Name	
	F	Solution User Parameter 3	
	F	Solution User Parameter 4 Name	
	F	Solution User Parameter 4	
	F	Solution User Parameter 5 Name	
	F	Solution User Parameter 5	
	F	PARADIGM /	
	F	Bath Temperature	
	F	Bath Temperature Units	
	F	Ambient Temperature	
	F	Ambient Temperature Units	
	F	Atmospheric Composition	
	F	Atmospheric Pressure	
	F	Atmospheric Pressure Units	
	F	Atmospheric Humidity	
	F	Paradigm User Comment	
	F	Paradigm User Parameter 1 Name	
	F	Paradigm User Parameter 1	
	F	Paradigm User Parameter 2 Name	
	F	Paradigm User Parameter 2	
	F	Paradigm User Parameter 3 Name	
	F	Paradigm User Parameter 3	
	F	Paradigm User Parameter 4 Name	
	F	Paradigm User Parameter 4	
	F	Paradigm User Parameter 5 Name	
	F	Paradigm User Parameter 5	
	F	CELL HEALTH / QUALITY CONTROL /	
	F	Cell Health User Parameter 1 Name	
	F	Cell Health User Parameter 1	
	F	Cell Health User Parameter 2 Name	
	F	Cell Health User Parameter 2	
	F	Cell Health User Parameter 3 Name	
	F	Cell Health User Parameter 3	
	F	Cell Health User Parameter 4 Name	
	F	Cell Health User Parameter 4	
	F	Cell Health User Parameter 5 Name	

		F	Cell Health User Parameter 5	
B	E	F	STIMULUS /	
	E	F	Key Stimulus	
	E	F	Stimulus Duration	
	E	F	Compound Group	
	E	F	Compound Group Index	
B	E	F	Compound Identifier	
B	E	F	Compound Name	
B	E	F	Compound Concentration	
B	E	F	Compound Concentration Units	
	E	F	Compound Batch	
	E	F	Compound Lot	
	E	F	Compound Salt Code	
	E	F	Compound Solution	
	E	F	Compound Vehicle / Solubility Enhancer	
	E	F	Compound Vehicle Concentration	
	E	F	Compound Vehicle Concentration Units	
	E	F	Compound Reservoir Identifier	
	E	F	Application Tip Identifier	
	E	F	Compound Plate Identifier	
	E	F	Compound Plate Row	
	E	F	Compound Plate Column	
	E	F	Chem. Stimulus User Parameter 1 Name	
	E	F	Chem. Stimulus User Parameter 1	
	E	F	Chem. Stimulus User Parameter 2 Name	
	E	F	Chem. Stimulus User Parameter 2	
	E	F	Chem. Stimulus User Parameter 3 Name	
	E	F	Chem. Stimulus User Parameter 3	
	E	F	Chem. Stimulus User Parameter 4 Name	
	E	F	Chem. Stimulus User Parameter 4	
	E	F	Chem. Stimulus User Parameter 5 Name	
	E	F	Chem. Stimulus User Parameter 5	
B	E	F	Light Stimulus Wavelength	
B	E	F	Light Stimulus Intensity	
B	E	F	Light Stimulus Intensity Units	
	E	F	Light Stimulus User Parameter 1 Name	
	E	F	Light Stimulus User Parameter 1	
	E	F	Light Stimulus User Parameter 2 Name	
	E	F	Light Stimulus User Parameter 2	
	E	F	Light Stimulus User Parameter 3 Name	
	E	F	Light Stimulus User Parameter 3	
	E	F	Light Stimulus User Parameter 4 Name	
	E	F	Light Stimulus User Parameter 4	

	E	F	Light Stimulus User Parameter 5 Name	
	E	F	Light Stimulus User Parameter 5	
B	E	F	Mechanical Stimulus Intensity	
B	E	F	Mechanical Stimulus Intensity Units	
	E	F	Mechanical Stimulus User Parameter 1 Name	
	E	F	Mechanical Stimulus User Parameter 1	
	E	F	Mechanical Stimulus User Parameter 2 Name	
	E	F	Mechanical Stimulus User Parameter 2	
	E	F	Mechanical Stimulus User Parameter 3 Name	
	E	F	Mechanical Stimulus User Parameter 3	
	E	F	Mechanical Stimulus User Parameter 4 Name	
	E	F	Mechanical Stimulus User Parameter 4	
	E	F	Mechanical Stimulus User Parameter 5 Name	
	E	F	Mechanical Stimulus User Parameter 5	
B	E	F	Acoustic Stimulus Frequency	
B	E	F	Acoustic Stimulus Intensity	
B	E	F	Acoustic Stimulus Intensity Units	
	E	F	Acoust. Stimulus User Parameter 1 Name	
	E	F	Acoust. Stimulus User Parameter 1	
	E	F	Acoust. Stimulus User Parameter 2 Name	
	E	F	Acoust. Stimulus User Parameter 2	
	E	F	Acoust. Stimulus User Parameter 3 Name	
	E	F	Acoust. Stimulus User Parameter 3	
	E	F	Acoust. Stimulus User Parameter 4 Name	
	E	F	Acoust. Stimulus User Parameter 4	
	E	F	Acoust. Stimulus User Parameter 5 Name	
	E	F	Acoust. Stimulus User Parameter 5	
B	E	F	Thermal Stimulus Temperature	
B	E	F	Thermal Stimulus Temperature Units	°C, °F or K
	E	F	Thermal Stimulus User Parameter 1 Name	
	E	F	Thermal Stimulus User Parameter 1	
	E	F	Thermal Stimulus User Parameter 2 Name	
	E	F	Thermal Stimulus User Parameter 2	
	E	F	Thermal Stimulus User Parameter 3 Name	
	E	F	Thermal Stimulus User Parameter 3	
	E	F	Thermal Stimulus User Parameter 4 Name	
	E	F	Thermal Stimulus User Parameter 4	
	E	F	Thermal Stimulus User Parameter 5 Name	
	E	F	Thermal Stimulus User Parameter 5	
B	E	F	Electrical Stimulus Frequency	The frequency of an external electrical stimulus

B	E	F	Electrical Stimulus Intensity	The intensity of an external electrical stimulus
B	E	F	Electrical Stimulus Intensity Units	The intensity units of an external electrical stimulus
	E	F	Electrical Stimulus User Parameter 1 Name	
	E	F	Electrical Stimulus User Parameter 1	
	E	F	Electrical Stimulus User Parameter 2 Name	
	E	F	Electrical Stimulus User Parameter 2	
	E	F	Electrical Stimulus User Parameter 3 Name	
	E	F	Electrical Stimulus User Parameter 3	
	E	F	Electrical Stimulus User Parameter 4 Name	
	E	F	Electrical Stimulus User Parameter 4	
	E	F	Electrical Stimulus User Parameter 5 Name	
	E	F	Electrical Stimulus User Parameter 5	
	E	F	Other Stimulus User Parameter 1 Name	
	E	F	Other Stimulus User Parameter 1	
	E	F	Other Stimulus User Parameter 2 Name	
	E	F	Other Stimulus User Parameter 2	
	E	F	Other Stimulus User Parameter 3 Name	
	E	F	Other Stimulus User Parameter 3	
	E	F	Other Stimulus User Parameter 4 Name	
	E	F	Other Stimulus User Parameter 4	
	E	F	Other Stimulus User Parameter 5 Name	
	E	F	Other Stimulus User Parameter 5	
		F	SERIES (= ROUTINE DATA) /	
		F	Routine User Comment	

Table 4-7. Metadata Parameters

4.3 General

SutterPatch general operations.

Note: Hidden unminimized windows can be brought into view with the menu command Windows / Control / Retrieve All Windows.

4.3.1 Command Window

The Command window is labeled with the currently loaded Experiment filename. A history of commands and responses is displayed in the upper portion of the window. Some warning messages are also displayed here

This window is also an Igor Pro code interpreter, for programmatic interaction with SutterPatch. Commands can be entered into the command buffer in the lower portion of the window. A maximum of 400 characters can be entered into the command buffer, however they

can be spread over multiple lines. Highlighted lines in the history section are transferred into the command section for processing when the Enter key is pressed.

The Command window has a resizing line between the upper history section and the lower command section – the mouse cursor will change to a double-headed arrow.

For more information, see Section II-2 of the Igor Pro manual.

4.3.2 Dashboard Panel

The Dashboard panel provides a convenient gateway to key areas of the SutterPatch program.

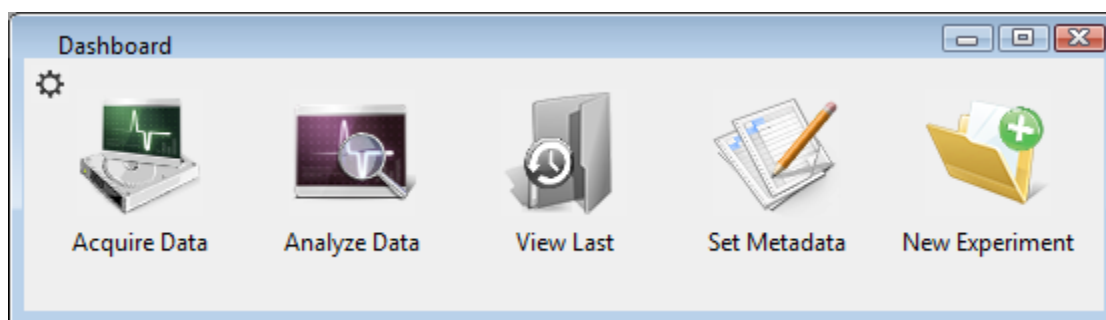



Figure 4-71. Dashboard

-  Preferences

Icon Size	Large Icon Small Icon
Icon Orientation	Vertical Horizontal
- Acquire Data Live recordings and acquisition configuration.
- Analyze Data Review and analyze data in the Data Navigator.
- View Last Data Open the Experiment's last recorded data Series.
- Set Metadata Configure metadata settings and values.
- New Experiment Start a new Experiment, and/or switch the amplifier model or emulation mode.

Clicking the Acquire Data icon opens an adjoining secondary pane:



Figure 4-72. Dashboard – Acquire Data

- Control Panel Hardware control via the Amplifier Control Panel.
- Membrane Test Monitor seal formation and cell health.
- Free Run Run an oscilloscope-style signal monitor.
- Routines Configure Routine acquisition settings.
- Paradigms Control the execution of commands.

4.3.3 File Import/Export

Experiments & Data

There are two formats for saving an experiment. The packed format is recommended for most purposes.

- Packed experiment: (*.pxp file)

A SutterPatch Experiment is saved by default as a “packed” (Igor Pro) experiment, which includes all data, analyses, graphs, routines, paradigms, etc., in one file.

- Unpacked experiment: (*.uxp file, experiment Folder)

A SutterPatch Experiment can also be saved as an “unpacked” (Igor Pro) experiment, which saves all waves, procedure windows, and notebooks as individual files in an experiment or “home” Folder, along with an instruction (*.uxp) file to recreate the experiment.

The advantage of an unpacked experiment is:

- Much faster processing of experiment recordings that include very large numbers of waves (thousands or more), as existing data waves are not resaved with each new recording.

The disadvantages of an unpacked experiment are:

- Much more disk space is used, especially for experiments that have a lot of small waves.
- The UXP format is more “fragile”, as you need to keep the experiment file and its corresponding folder together when you copy or move the experiment.

Saved experiments can be re-opened into the current experiment via the Data Navigator 'Import' button.

Note: If a SutterPatch Experiment file is opened into Igor Pro without SutterPatch running, its graphs will not be visible – display them with the Igor Pro Windows / Graphs' or 'Windows / Layouts' menu items.


pCLAMP ATF Series-level (Routine) data can be exported to the pCLAMP ATF file format via the Data Navigator 'Available Actions' button (or right-click menu). One Series produces one ATF file.

Graphs and Layouts

Import graph files into or export from the current experiment via the Analysis Editor / Files options as Igor Binary Wave (*.ibw) files.

Note: Graph data for each axis can also be saved as Igor Pro 6 one-dimensional wave files, however files using this older format cannot be re-imported back into SutterPatch.

Graphs can also be exported to a Layout window via:

Analysis Editor:	[To Layout] button
Scope window:	"Export a layout" button 
Paradigms:	Export step

Graphs exported to the Layout window use the Preferences / Export Graphics settings.

Individual graphs can be saved as Graph Macros - recall them via the Windows / Graph Macros menu.

Layout windows can be saved as Layout Macros - recall them via the menu Windows / Layout Macros menu.

Routines.

PatchMaster Pulse Generator Files (*.pgf) can be opened in the Routine Editor 'Pools and Files' section and their Sequences merged with the current routine pool.

Templates

Templates can be imported or exported via the Template Editor as Igor Binary Wave files (*.ibw).

4.3.4 Log Window

The Log window collects time-stamped commands, responses, administrative information and error messages in a structured, searchable format. These entries provide a history of the steps having a possible influence on the execution of the experiment and its data.

When SutterPatch starts up, the Log window displays the SutterPatch Version and Build numbers.

Column Names (default) The Log window can be sorted based on a column – click on a column header name, and the Log Window is sorted numerically and alphabetically based on that column. Clicking on the column header a second time reverses the sort order.

Date / Time The Date/Time-stamp is in ISO format:

Year-Month-Day hours:minutes:seconds.milliseconds

Example: 2016-04-25 14:21:34.478

Note: The timestamp has a resolution of 1 ms, and multiple entries can occur within this timespan. This may create an unreliable sort order when sorting by Date/Time.

To sort Log entries in the order they were created, right-click on the column header and select Default View. Alternatively, right-click on a column header, choose 'Select columns to show', enable the Log ID column, and then sort on this column.

Event Type Log entries are assigned an Event Type:

Command Command window execution.

Comment

Data Acquisition Scope acquisition operations.

Data Management

Exit

History

Metadata

Paradigm

Routine Routine Pool operations.

Startup Startup / loading information.

Unknown

Event Description A text description of the log entry.

Paradigm / Routine / Series Paradigm, Routine and Series (data) names associated with the log entry are displayed.

Log ID	A unique sequence number for events. Useful for sorting events that occur within the same 1 ms Time stamp.
Column header	(right-click for menu)
Search for Text:	A Search Log dialog opens with multiple full-text search options.
Find	Enter or select the text string to search on.
Case sensitive	Text capitalization must match.
Whole word	Partial-word matches are ignored.
Select columns to search	
All	Search all columns in the Log window.
Previous	Revert to the initial column selection.
Column list	Select columns by clicking on them. The <Shift> and <Ctrl> or <Cmd> keys for multi-row selection are supported, according to operating system standards.
• Highlighted	Display all rows, with matches highlighted.
• Filtered	Only display rows that match the Find criteria.
Back	Select the previous matching row.
Next	Select the next matching row.
Wrap	Cycle between the first and last search matches when the Back/Next buttons are used.
Clear	Return the Search window to its default settings.
Find	Execute the search.
Sort on this column:	Alphabetically sort the entire window based on the selected column.
Default view:	Restore the Log window default view. All data sorting, filtering, and column selections are cleared.
Paradigm / Routine / Data	(Right-click menu items)
• Paradigm data	
Review Paradigm Data:	Opens the Paradigm data into a Paradigm Review window.
• Series data	
Analyze Series:	Opens the named Series data into a Scope window for analysis.

4.3.5 Menus

The SutterPatch main menu item contains all of the SutterPatch-specific menu items. The rest of the main menu items provide the standard Igor Pro functionality. For documentation of the non-SutterPatch features, refer to the Igor Pro online help or manual.

Window/Dialog Controls

Keyboard “Return” key	=	‘OK/Yes’ buttons
Keyboard ESC key	=	‘Cancel’ button

File

New Experiment	<p>Unload the current Experiment and start a new Experiment.</p> <p>It is recommended that you create one Experiment per cell, to keep file sizes manageable.</p>
	<p>Note: Even if immediately selected after saving an Experiment, you will still be re-asked to save the prior Experiment (due to internal processes.)</p>
Open Experiment	Open a previously saved SutterPatch Experiment (*.pxp, *.uxp) file. If a SutterPatch experiment is opened into an Igor-only session, SutterPatch is automatically loaded.
Save Experiment	If the current experiment is already named, it is immediately saved. Otherwise, a ‘Save experiment as’ file dialog is displayed. If Preferences are enabled for automatic file naming, a default Experiment name is provided.
Save Experiment As	Saves and renames the loaded Experiment to a new file name.and/or format.
Save Experiment Copy	Save the Experiment to another file name and/or format without closing the current session.
Revert Experiment	Undo any unsaved changes to the Experiment.
Recent Experiments	A list of recently used Experiments.
Exit	An Experiment file ‘Save’ dialog is displayed before closing the program. If an Experiment is not saved, global variables and window sizes/positions are lost.

Data

Data Browser	Access all SutterPatch objects contained in the experiment.
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Windows

The Windows menu provides access to graphs, tables and layouts.

Layout

The Layout menu only displays when a Layout is the active window. Use it to modify the Layout window objects and display.

SutterPatch

Dashboard	Display icons for core program functions.
Scope Window	Bring an open Scope window to the front.
Hardware Control	
Amplifier Control Panel	Open the hardware control panel.
Reset Control Panel	Return the Amplifier Control Panel to its default settings.
Lock-In Adjustments	
Enable Manual Adjustments	
Absolute Values	
Phase Delay Adjustment	
Reset	Reset to 0.00 s
Attenuation Adjustment	
Reset	Reset to 1.00
List Results	
Reset USB	Re-initialize USB communication with the computer. If in Demo mode, you need to start a 'New Experiment' to access 'Reset USB'.
Membrane Test	Open and run the Scope window to monitor seal formation and cell health.
Free Run (Scope)	Open and run the Scope window in oscilloscope style.
Reset Acquisition	Stop the Paradigm and/or data acquisition and clear corrupted acquisition settings.
Paradigm Editor	Open the dialog to load, edit and run Paradigms.
Routine Editor	Open the dialog to load and edit Routines.
Template Editor	Open the dialog to manage templates.
Equation Editor	Open the dialog to load and edit Equations.
Solution Editor	Open the dialog to control solutions.
Camera Module	Open the window to capture images.
Data Navigator	Open the window to organize and display the experiment Paradigm, Routine and acquisition data in a tree structure.
Analysis Editor	Open the dialog to manage analysis graphs.

Layout Page	Show
	Delete
Set Metadata	Open the dialog to configure user-specified experimental information.
Set Preferences	Open the dialog to modify the default program settings.
Log Window	Open the window to display a history of program actions.
Shortcuts	Open the Shortcuts Editor dialog to manage keyboard shortcuts.
Available Analysis Modules	Action Potential Analysis
	Synaptic Event Analysis
	Single Channel Analysis

Help

Igor Help Browser	Igor and SutterPatch Help Topics.
About SutterPatch	SutterPatch version and contact information.

Scope Right-click Menus

Different areas of the Scope windows support additional functionality through "right-click" menus in Windows, or "Command-click" menus in macOS.

Scope (Acquisition) main window (right-click a blank area in a signal)

Note: If you don't click far enough away from the data, the Data menu will display instead.

- Autoscale All Axes
- Add Annotation
- Export Graphics
- Colors
- Hide Signal '<signal name>'
- Show Signal '<signal name>' Only
- Stack All Signals

Scope data (right-click on the data)

- Browse '<series signal name>'
- Edit '<series signal name>'
- Remove Sweep_#

- Hide Sweep_#
- Duplicate Sweep_#
- Replace Sweep_#
- Copy
- Modify Sweep_#
- Customize at Point
- Mode
- Line Style
- Line Size
- Markers
- Marker Size
- Color
- Bring to Front
- Send to Back
- Forward
- Backward
- Move to Opposite Axis
- Modify Contour
- Modify Image
- Modify Box Plot
- Modify Violin Plot
- Quick Fit
- Export Graphics
- Hide Signal '<signal name>'
- Show Signal '<signal name>' Only
- Stack All Signals

Scope Y-Axis

- Autoscale All Axes
- Continuous Autoscale Axis
- Autoscale Axis
- Full Scale Y Axis
- Axis Properties...
- Hide Signal 'Signal Name'
- Show Signal 'Signal Name' Only

- Stack All Signals

Scope X-Axis

- Autoscale All Axes
- Full Scale Axis
- Axis Properties...

Scope (Reanalysis) main window (additional items vs. Acquisition)

- 3D View
- Zero Baselines
- Show All Sweeps
- Select Sweeps

The average of the first four data points is subtracted from the sweeps display. Does not affect data values in measurements.

4.3.6 Preferences

Preferences settings customize the default settings for several areas of the SutterPatch program. To access, go to the SutterPatch / Set Preferences menu.

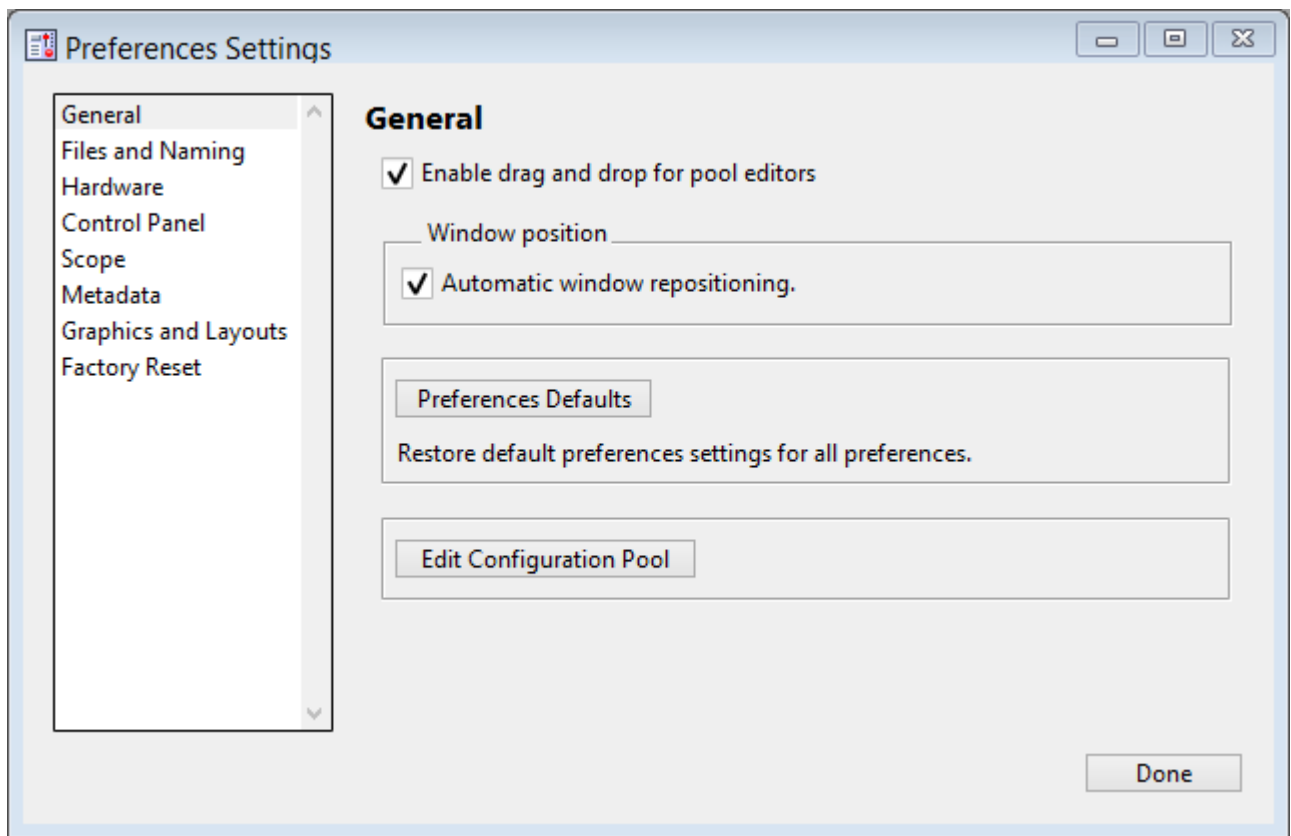


Figure 4-73. Preferences Settings

i. General

Enable drag and drop for pool editors

The Routine Editor loads a “pool” of Routines from a Routine Pool file. These Routines can be re-arranged in the Routine Pool list by clicking and dragging with the mouse.

The Paradigm Editor operates in the same manner, and also displays a list of Paradigm Steps, which can also be re-arranged by dragging-and-dropping.

Window Position

Automatic window repositioning

When SutterPatch windows or dialogs are opened or moved, when the action is done, they are automatically repositioned to be fully visible. If a “child” window is opened, the parent window is moved to the left until the child sub-window is fully visible or the parent window reaches the left edge of the main window/screen.

Dual-monitor option (macOS only)

However, for very long experiments, this can result in delays when saving new data, as the entire experiment is re-saved with each additional recording.

- uxp

Optional “unpacked” experiment.

The experimental information is saved into an instruction file and an experiment folder containing additional files.

When recording thousands of waves, this allows the experimental to proceed without “re-saving” delays.

Text: []
Legal characters are A-Z, a-z, 0-9 and “_”.

Include user text in the file name.

Date: YYMMDD Include the date in the file name.

Time: hhmmss Include the time in the file name.

- Save to temp file after each routine

The raw data are saved into a temporary file after each recording. This can help to speed up file-saving time for large Experiments composed of several smaller recordings.

The temporary file starting size is based on the starting size of the Experiment. The temporary data are re-saved to the main Experiment when the Experiment is closed and/or saved.

- Save entire experiment after each routine

This default option re-saves the entire Experiment after each recording (all data and Experiment information). This is the safest method of operation for data integrity, but can produce significant post-recording file-saving delays in larger Experiments.

- Don't save to temp file after each routine

Data and information are held in memory until the Experiment is explicitly saved; there are no file-saving delays after a recording is stopped. This provides the fastest method of operation when making multiple recordings, but is also the least secure, as data loss can occur if the computer encounters problems.

iii. Hardware

- Prompt for hardware on startup (if no Sutter hardware is found).

When a new Experiment is started, if Sutter patch-clamp hardware is not connected to the computer and turned on, you are prompted to retry the USB connection or select an emulation mode.

- If no Sutter hardware is found, emulate:
 - IPA Integrated Patch Amplifier system.
 - Double IPA Dual-headstage IPA system.
 - dPatch Ultra-fast Digital Patch-clamp system.

When a new Experiment is started, if Sutter patch-clamp hardware is not connected to the computer and turned on, automatically start up in the selected hardware emulation mode.

Stability Control

In CC mode reduce electrode compensation by [0.0 – 3.0] pF.

During whole-cell patching, if the Electrode Compensation control is set too high, the patch-clamp seal will become unstable and will be lost. As the Voltage Clamp mode typically operates with higher electrode compensation values than the Current Clamp mode, this preference promotes “safe” switching between the Voltage Clamp and Current Clamp modes.

iv. Control Panel

Customize the active headstage tab's color in the Control Panel.

Headstage background color

Headstage 1 Color palette displays.

Headstage2 Color palette displays.

v. Scope

To apply these settings, open or re-open the Scope window.

General

Time axis unit:

- Auto-set Sweep duration < 120 s, use “s”
Sweep duration ≥ 120 s, use “min”
Sweep duration ≥ 7200 s, use “h”
- SI unit Always use standard SI base units, such as “s” for time.

Acquisition

Y axis autoscale settings:

- Continuous autoscale The Y-axis limits are rescaled with each sweep so that all data are visible.
- Continuous autoscale from zero

One Y-axis limit is positioned at zero, while the other Y-axis limit is rescaled with each sweep. The Y-axis direction matches the sign of the largest absolute value in the sweep, can change during a sweep, and can be different for each sweep.

This is just a display setting - all data, both positive and negative, are recorded.

If Persistence is enabled, the direction of the first sweep is used for all subsequent sweeps.

- Full scale The full-scale range is used.

Reanalysis

Show tags by default

Y axis autoscale settings:

- Autoscale The Y-axis limits are rescaled with each sweep so that all data are visible.
- Autoscale from zero One Y-axis limit is positioned at zero, while the other Y-axis limit is rescaled with each sweep. The Y-axis direction matches the sign of the largest absolute value in the sweep, and can change during a sweep, and can be different for each sweep.

If Persistence is enabled, the direction of

the first sweep is used for all subsequent sweeps

- Full scale

The full-scale range is used.

Signals Display

- Show all signals
- Use last selection
- Signals []

Enter signal numbers and/or ranges separated by commas.

For example: 1,2,4-5

Sweeps Display

- Show all sweeps
- Use last selection
- Sweeps []

Enter sweep numbers and/or ranges separated by commas.

For example: 1,3,5-20

Appearance Use a color palette to choose colors.

Active signal panel color [light gray]

Inactive signal panel color [dark gray]

Active sweep color [blue]

Inactive sweep color [red]

Drop-down color palette Click on a color square to set it as the active color.

Other Use a color dialog with more options.

Opaque < unused >

[Preview Pane] The selected signal and sweep colors are displayed in a preview pane.

Tip: For dark-room experiments, the window background color can be adjusted by the operating system:

- Windows: In the Control Panel / Appearance / Personalization window, scroll down and select the High Contrast Black theme, or use

the Windows Magnifier tool with option 'Turn on color inversion' enabled.

- macOS:

Press 'Control-Option-Command-8' to set the System Preferences / Accessibility / Display / Invert Display colors option, or open its menu with 'Command-Option-5'.

vi. Metadata

Metadata detail level: Increasing levels of data categorization complexity.

Select which metadata groups and parameters are visible for configuration in Set Metadata and in Data Navigator / Build Hierarchy.

- | | | |
|---|----------|---|
| 1 | Basic | Show only the most essential parameters, |
| 2 | Extended | Show additional detail. |
| 3 | Full | Expose all available metadata parameters. |

Note: This setting does not affect the Metadata Review dialog - all user-defined metadata are displayed irrespective of the metadata detail level.

vii. Graphics and Layout

These settings apply to Layout windows and stand-alone graph windows (not graph files).

Copy "To Clipboard" graph format

Several popular file formats are supported:

- | | |
|--------|----------------------------------|
| • PNG | Portable Network Graphics |
| • PDF | Portable Document Format |
| • TIFF | Tagged Image File Format |
| • JPEG | Joint Photographic Experts Group |

Note: When pasting, not all formats may be supported by other programs.

Layouts

Default graphs per page

- 1
- 2
- 3
- 2 x 2 (Column x Row)
- 2 x 3 (Column x Row)
- 2 x 4 (Column x Row)

Graphs

left (typically the Y-axis)

Tick location:

- Outside
- Crossing
- Inside
- None

Labels:

- On
- Axis only
- Off

Grid:

- Off
- On
- Major only

bottom (typically the X-axis)

Tick location:

- Outside
- Crossing
- Inside
- None

Labels:

- On

- Axis only
- Off

Grid:

- Off
- On
- Major only

viii. Factory Reset

[Factory Reset] Click this button to reset SutterPatch to it default settings. SutterPatch will need to be exited and restarted to complete the factory reset.

Warning! When you do a factory reset, you will lose ALL your configuration data (including Metadata, Scope, Preferences, etc.)

4.3.7 Shortcut Editor

Keyboard control of SutterPatch is available by configuring keyboard shortcuts.

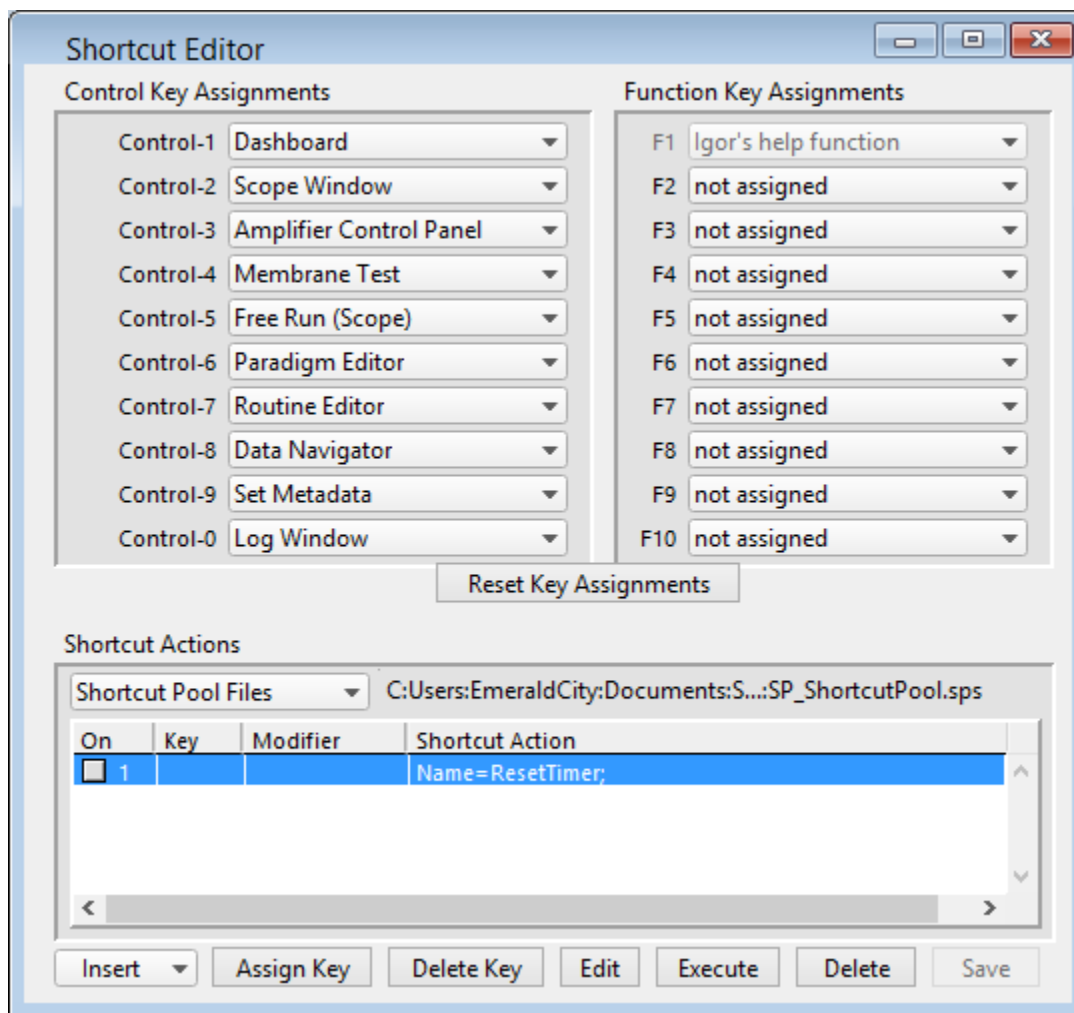


Figure 4-74. Shortcuts Editor

Note: All Control/Command, Function and Shortcut Action key combinations are automatically added to the SutterPatch / Shortcuts submenu.

Control Key Assignments / Command Key Assignments

A Control Key shortcut assignment can be changed by clicking on its drop-down list and selecting a new menu item.

- Windows 'Control' key: CTRL
- macOS 'Command' key: ⌘

Use a shortcut Control Key by holding down the ‘CTRL’ or ‘Command’ key while simultaneously clicking its assigned number.

A list of the Control Keys and their default SutterPatch menu items:

Control-1	Show Dashboard
Control-2	Show Scope Window (Scope window must be open.)
Control-3	Amplifier Control Panel
Control-4	Membrane Test
Control-5	Free Run (Scope)
Control-6	Paradigm Editor
Control-7	Routine Editor
Control-8	Data Navigator
Control-9	Set Metadata
Control-0	Log Window

Other SutterPatch menu items available for shortcut assignment:

Reset Acquisition
Equation Editor
Template Editor
Analysis Editor
Shortcut Editor
Camera Module
Set Preferences

Function Key Assignments

Computer keyboards usually include a set of Function keys for special actions. Configure a ‘Function Key’ shortcut assignment by clicking on its drop-down list and selecting a new menu or action item.

Function Keys:

F1	Igor Pro Help function	< reserved >
F2 – F12	not assigned	< available >

On some keyboards, you also need to press the ‘Fn’ key to use function keys.

On some Windows systems, F12 is reserved for debugging.

macOS reserves nearly all function keys for itself. In order to use function keys for an application, you must first check a checkbox in the Keyboard control panel. Even then, macOS will intercept some function keys.

Reset Key Assignments

Reset all Control and Function Key Assignments to their default assignments.

Shortcut Actions

Additional custom keyboard Actions can be created and their shortcuts managed.

Shortcut Pool Files

New Shortcut Pool	Create a default Shortcut Pool.
Load Shortcut Pool	Load the Shortcuts of a previously saved Shortcut Pool file into the Shortcut Pool.
Revert to Last Saved	Undo any unsaved changes to the Shortcut Pool.
Save Shortcut Pool	Save the Shortcut Pool using its existing file name and path.
Save Shortcut Pool As...	Save the Shortcut Pool to a new file, and switch to the new file. The default file name is the same as the original file name.
Save Shortcut Pool Copy...	Save the Shortcut Pool to a new file, but do not switch to the new file. The default file name has 'Copy of' prepended to it.
Merge Shortcut Pools	Insert the Shortcuts from a previously saved Shortcut Pool file into the loaded Shortcut Pool.

[Shortcut Pool file path]

Columns

On	Enable/disable the Shortcut Action.
Key	The assigned keyboard key.
Modifier	The keyboard “modifier key” used in a key combination.

Windows

- CTRL Only use with keys ‘0 – 9’.
- ALT Keys ‘0 – 2’ reserved by Igor for File / Recent Experiments.
- SHIFT
- FUNC
- CAPS LOCK Ignored.

macOS

- Command Only use with keys ‘0 – 9’.
- Option
- Shift
- Control Keys ‘0 – 2’ reserved by Igor for File / Recent Experiments.
- Caps Lock Ignored.

Shortcut Action An Action’s instructions and settings.

Click a field in the pool to highlight an Action and make it the active entry. Click-and-drag a field to reposition an Action in the pool.

Insert Adds an Action to the ‘Shortcut Action’ list and opens its Shortcut Actions Editor for setup.

Although these Actions operate similarly to Paradigm steps, an additional Label field can be used to name the Action in the SutterPatch Shortcuts menu.

Amplifier

Analysis

Camera

Execute

Export

Front Window

Hide Window

Paradigm Load & Run / Stop / Pause / Resume a Paradigm.

Reset Timer

Routine

Scope Operation

Set Axis

Set Checkbox

Set Variable ‘Label’ entry only displays in the Shortcut Action column.

Set Tag

Start Acquisition In open Scope acquisition window.

Stop Acquisition In open Scope acquisition window.

View Last

Write Log

Assign Key	<p>This button opens the Shortcut Key Input dialog (or double-click in a “Key” or “Modifier” field) to input the desired keyboard combination for a letter, number, or symbol.</p> <p>Note: Available keyboard letters, numbers, and symbols can vary from computer to computer, depending on the computer OS and Igor Pro’s key usage. (Reserved keys typically open another window type, or are non-responsive.)</p> <p>Tips: If the CAPS LOCK button is on when assigning a key, the key is case insensitive.</p> <p>Keyboards often have a Function (FN) button to allow special access to the Function keys.</p> <p>Although the F1 function key is reserved in Igor Pro, it can be assigned if used with a modifier key.</p>
Delete Key	Remove the Key entry for the selected Action.
Edit	Open the Shortcut Editor dialog (or double-click in a “Shortcut Action” field) to change the Action’s parameters.
Execute	Run the selected Action.
Delete	Remove the selected Action from the ‘Shortcut Action’ list.
Save	Saves any changes to the current Shortcut Pool file.

4.3.8 Sample Files

Sample settings files (subject to change) are included in the ... / Documents / SutterPatch / Parameters folder:

Equation Pool

SP_EquationPool.txt	For all amplifiers.
1. X3pi	3*pi
2. ElapsedTime	ParadigmTime - time
3. Temperature	aux[1]*1.23 – 273.15

Paradigm Pool

SP_ParadigmPool_IPA.spp	Paradigms for D/IPA amplifiers.
1. Amplifier_Setup	Set initial amplifier settings.

- | | |
|-------------------------------|--|
| 2. Start_one_Series | Start acquisition of one routine. |
| 3. Set_amplifier_and_start_IV | Set amplifier to a known state, then start a routine. |
| 4. Start_two_Series | Start acquisition of two subsequent routines. |
| 5. Start_ForEachSweep | Start acquisition of a routine, individually triggering each sweep. |
| 6. Interactive_acquisition_1 | Run an interactive acquisition stopping at a given analysis condition. |
| 7. Interactive_acquisition_2 | Run an interactive acquisition loop that selects between 2 routines, and manually stop via a Checkbox. |
| 8. Toggle_Persistence | Use a Checkbox to toggle Scope trace persistence while acquiring a routine. |
| 9. Switch_Headstages | Switch between multiple headstages. |
| 10. Tuning_with_Keys | Use the keyboard to increment or decrement a Routine's stimulus output by 10 mV. |
| 11. Tuning_with_Input | Use the paradigm "Input" control to increment or decrement a Routine's stimulus output. |

LockIn_IPA.spp

Paradigms for IPA lock-in tuning.

1. LockIn_Adjust_500Hz
2. LockIn _Adjust_1kHz
3. LockIn_DoAdjust

Routine Pools

SP_RoutinePool_DIPA.spr

Routines for two-headstage DIPA amplifiers.

SP_RoutinePool_IPA.spr

Routines for one-headstage IPA amplifiers.

- | | |
|--------------------------|------------------------------------|
| 1. IV | I/V for voltage-clamp mode. |
| 2. IV_Continuous | I/V with continuous acquisition. |
| 3. IV_tuning | I/V for sample "tuning" paradigms. |
| 4. Synaptic_Stim | Synaptic stimulation. |
| 5. Multi_Test | Multi-channel waveform. |
| 6. Bowtie_Test | Multi-channel waveform. |
| 7. IV_P4 | I/V with 4 leak pulses. |
| 8. SS_Inactivation | Steady-state inactivation. |
| 9. Recovery_Inactivation | Recovery from inactivation. |

10. Onset_SlowActivation	Onset Slow activation.
11. SS_SlowInactivation	Steady-state slow activation.
12. Recovery_SlowInact	Recovery from slow inactivation.
13. Test_Pulse	Test pulse.
14. ContinuousNoOut	Acquisition without any output waveform.
15. Amplitude_Equations	Equations for waveform output.
16. Template_SpontAct	Template wave from a recorded signal.
17. TemplateTest	Template wave for waveform output.
18. TemplateAndVirtual	Template wave and recording virtual signals.
19. IV_VC_CC	IV for voltage- and current-clamp modes. [For two-headstage DIPA amplifiers.]
20. A_T_InactRec_P4	Inactivation with leak subtraction.

LockIn_IPA.spr Routines for IPA lock-in tuning.

1. phase delay
2. LockIn _500Hz
3. LockIn _1kHz

Solution Pool

SP_SolutionPool.spo For all amplifiers.

1. undefined

Template Pool

SP_TemplatePool.spt For all amplifiers.

1. minis
2. R1_S1_10minSpontAct
3. Template1_Sweep5

Sample data (subject to change) are included in the ... / Documents / SutterPatch / Example folder:

Experiments

ActionPotentials.pxp	Action potentials.
LargeAPs.pxp	Large action potentials.
MiniExample.pxp	Spontaneous miniature synaptic potentials.

4.3.9 Startup

The SutterPatch application startup sequence:

1. Click on the Igor Pro 8 icon to launch SutterPatch.
2. A “splash” screen displays file opening information, and a blank Experiment window is created.
3. Next, a splash screen displays SutterPatch launch options:
 - Igor Only Run Igor Pro (without launching SutterPatch).
 - Open Launch SutterPatch from a saved Experiment.
 - Start Launch SutterPatch for a new Experiment.
4. After clicking ‘Start’, the splash screen displays a progress bar while compiling the code.
5. Then a ‘Save experiment as’ dialog displays a standard file saving dialog to name the Experiment file.
6. If a Sutter amplifier is not attached or not communicating with the computer, the ‘No USB Connection’ pane allows you to try re-establishing the USB connection(s), or to select a hardware-emulation demonstration mode (IPA, Double IPA, dPatch).
 In “demo” mode, all functions are available, except that the input and output channels use simulated data, and the Amplifier Control Panel and acquisition Scope window are labeled with “Demo”.
7. The Dashboard panel is displayed. Additional SutterPatch windows might open, if they were displayed in the prior experiment.

5. PROGRAMMING

5.1 Data Format

SutterPatch data are written in a 64-bit double-precision binary floating-point format. This supports a decimal precision of 17 significant digits.

The data are stored within an Igor Experiment (*.pxp) file.

For large data sets, an optional HDF5 file format will be available for streaming data acquisition without resaving the experiment at the end of a recording.

5.2 Data Structure

SutterPatch recorded data are stored as multidimensional data waves, and are listed per signal in the Data Browser. Select a data wave in the Data Browser and right-click to ‘Edit’ the Signal data in a spreadsheet-style table. The two-dimensional data wave is displayed with one row per sample point and one column per trace, with the number of

data table columns increasing with the number of sweeps.

Warning! The raw data can be directly edited in the Data Browser – this is not recommended, as it permanently alters the data.

Note: While SutterPatch does not read the older Igor one-dimensional wave data-format, graph data for each axis can be separately exported to it. See the Analysis Editor / Files menu.

5.3 Data Paths

The Data Browser path uses a “root” of ‘SutterPatch’. The Data Browser right-click ‘Copy Full Path’ command copies a Signal’s data wave path to the clipboard.

Note: This path references Igor’s internal data folders, and not the computer OS file system.

The object’s path name can be used in user functions and executable commands. However, when referencing an active Scope window, the path name to the data wave can be substituted by “t[#]”, where ‘#’ refers to the signal position number in the Scope window.

5.4 User Functions

SutterPatch functionality can be extended through the use of user-defined Functions.

To create a user Function:

1. Open the menu for Windows / Procedure Windows / Procedure Window.
2. Enter your user code into the Procedure window, following its ‘#pragma’ and ‘#include’ lines.

Example:

```
#pragma TextEncoding = "Windows-1252"
#pragma rtGlobals=3      // Use modern global access method..
#include "SP_Globals", optional
```

```
Function SayHello()
```

```
    DoAlert 0, "Hello World!"
```

```
End
```

Note: The Function name must include trailing open/close parentheses “()”

3. Click on the Compile button at the bottom of the window.
4. Enter the Function name (including parentheses) into the Command window and press ‘Enter’, or use it in a Paradigm ‘Execute’ step.

For more information on creating your own functions, see the Igor Help topics on Programming / User-Defined Functions, and Procedure Windows.

Warning! User-defined functions only exist during the Experiment. They are not stored when the Experiment is closed. If you plan to re-use them in other Experiments, save them to a separate file, such as with a word processor.

Also, while user-defined functions are stored internally by Igor, there is no visible list, so you will need to maintain such a list manually.

6. MAINTENANCE

6.1 Inspection

Periodically inspect all cables and connections to make sure that all cables are sound and that all connections are firm and evenly seated.

Warning! Turn off the IPA power before plugging/unplugging headstages.

6.2 Cleaning

Routine cleaning of the IPA system is required to prevent excessive dust accumulations. Wipe all exterior surfaces with a dry, soft, cotton cloth.

7. TROUBLESHOOTING

7.1 Technical Support

Technical support is provided to customers at no charge.

Support hours: 8:00 AM - 5:00 PM PST (Pacific Standard Time).

Telephone: (+1) 415.883.0128

Fax: (+1) 415.883.0572

E-mail: info@sutter.com

Address: Sutter Instrument Company
One Digital Drive
Novato, CA 94949

When contacting us for technical support, please provide your SutterPatch version and “build” numbers to help us troubleshoot your situation. These numbers are found in the Start splash screen during program loading, or in the Log Window Startup events.

For issues regarding Igor Pro features (all non-SutterPatch menu items), please contact Wavemetrics, Inc. for technical support.

7.2 Manual

The IPA manual is installed as a PDF file along with the SutterPatch software. The latest version of the manual can be downloaded from our web site:

<https://www.sutter.com/AMPLIFIERS/SutterPatch.html>.

You can navigate through the PDF document using Table of Contents links, accessed via the Bookmarks tab on the left side of the PDF screen.

7.3 Help

Online help is available via the main Help menu, under ‘Igor Help Browser’ or ‘Help Topics’. The SutterPatch Help Topics / Help File names start with “SP_”. The online Help includes the same information as found in the PDF manual.

Most items in SutterPatch also include a short description as a tool tip. Hover the mouse over an item to see the tool tip.

7.3.1 Error Messages and Notifications

Some SutterPatch error messages or notifications will flash to get your attention, and automatically close after 5 seconds, and then write to the “History” window. To review such messages, see the Command window (Windows / Command Window).

7.4 Startup Issues

7.4.1 Installation Fails

Problem: The SutterPatch installation on Windows fails due to language pack incompatibilities.

Solution: Support for foreign language packs has been added. If language versions still cause problems, please contact Sutter Technical Support.

7.4.2 Application Not Loading

Problem: The SutterPatch application does not load – the startup sequence only loads Igor Pro.

Solution: If available, execute the Igor Pro menu command ‘Macros / Autocompile’.

7.4.3 Startup Errors

- Problem:** When starting up SutterPatch after installing a software update, program errors display.
- Solution:** Closing and re-opening SutterPatch sometimes clears up these errors.

7.5 Acquisition Issues

7.5.1 Acquisition Windows Lock Up

- Problem:** The Scope window, Routine Editor or Paradigm Editor lock up during acquisition.
- Solution:** Use the menu command SutterPatch / Reset Acquisition to halt acquisition.
- A combination of SutterPatch-related and computer-related issues can contribute to your system's performance. For suggestions to improve it, see the Troubleshooting item Sluggish Acquisition below.

7.5.2 Signal Flat

- Problem:** A Scope input signal is completely flat during acquisition, i.e., zero amplitude.
- Solution:** The corresponding headstage might not be attached to its port. The headstage HDMI connectors do not lock on - make sure they do not disconnect from their port.
- Power off the dPatch system and reconnect the headstage.

7.5.3 Signal Saturated

- Problem:** A Scope input signal is completely saturated during acquisition.
- Solution:** The corresponding headstage is not attached to its port. Power off the IPA system and reconnect the headstage cable.
- If the headstage is attached, the Gain setting might be set too high. Reduce the output gain on the Amplifier Control Panel.
- Note:** The headstage HDMI connectors do not lock on - make sure they do not disconnect from their port.

7.5.4 Headstage Noise

- Problem:** The noise levels of the instrument suddenly and erratically increase.
- Solution:** If the headstages are touched, the noise level will greatly increase. Make sure you are grounded or working in a Faraday cage.

7.5.5 Paradigm Sound Reduced

Problem: The paradigm ‘Sound’ step volume is attenuated at lower frequencies.

Solution: Upgrade the computer speaker, such as with add-on speakers.

7.5.6 Offset Zero Delay

Problem: The Offset button in the IPA Control Panel has a short delay before it responds.

Solution: This can occur after running the Membrane Test due to internal processing.

7.5.7 Metadata Written

Problem: Unchecking ‘Write’ in a metadata prompt still writes Experiment and Routine parameters.

Solution: A known issue. Contact Technical Support, or check for fixes in the ‘SutterPatch Release Notes’ PDF file, from the SutterPatch Download tab
<https://www.sutter.com/AMPLIFIERS/SutterPatch.html>

7.5.8 Post-Acquisition Delay

Problem: Every time acquisition completes, there is a delay with the program operations.

Solution: The entire *.pxp Experiment file is resaved when a recording stops. Create new Experiments more often, so that file sizes are smaller and more manageable.

Alternatively, users can save data in an unpacked (*.uxp) format. However users will need to ensure that the unpacked data folder is kept with the *.uxp program file.

7.5.9 Sluggish Acquisition

Problem: Data acquisition is sluggish.

Solution: The computer’s available resources need to be increased to handle the system load.

A combination of SutterPatch-related and computer-related issues can contribute to your system’s performance. Here are some suggestions to improve it:

Close: Data Browser window – in Igor Pro 8, it consumes a lot of CPU time.

Disable: Computer screen saver, Power Save or Sleep modes.

Disable:	Scope window persistence display.
Disable:	Virtual input channels.
Reduce:	Routine / Acquisition & Routine Parameters / Output sampling rate.
Close:	Background software.
Remove:	Software for certain license protection USB keys (dongles).
Optimize:	Hard disk (defragment).
Upgrade:	Computer graphics card.
Increase:	Computer RAM, cache size or CPU speed.

7.5.10 USB Errors

Problem:	A USB communications error occurs.
Solution:	Right-click in the Control Panel and select Reset USB. If the red USB button does not turn green, then try to isolate the problem. <ul style="list-style-type: none"> a) Unplug and re-plug both ends of the USB cable from the amplifier to the computer. b) Try another USB cable. c) Try another USB port d) Remove any USB hubs.

7.6 Analysis Issues

7.6.1 Analysis Not Deleted

Problem:	An analysis cannot be deleted in the Analysis Editor.
Solution:	The analysis is still in use, i.e. displayed in another window, such as a graph window - close the window to allow the analysis to be deleted.

7.6.2 Signal Axes Overlay

Problem:	The X-axes and units are overlaid in the Scope window.
Solution:	There is not enough room for the X-axes and units due to the number of signals

displayed. Switch to a tiled signal layout, or reduce the number of visible signals by right-clicking a signal and selecting 'Hide Signal'.

7.6.3 Graphs & Layouts Not Visible

- Problem:** Cannot see SutterPatch Experiment graphs or layouts on non-SutterPatch computers.
- Solution:** Use the Igor menu command Windows / Graph or Windows / Layout to see the object. Right-click it to modify with Igor options.

7.7 General Issues

7.7.1 Buttons Unresponsive

- Problem:** When using a slower computer in emulation (demo) acquisition mode, acquisition-related actions might be difficult, such as clicking the Stop button.
- Solution:** You may need to click the button more than once or hold it down longer than usual.
- A combination of SutterPatch-related and computer-related issues can contribute to your system's performance. For suggestions to improve it, see the Troubleshooting item Sluggish Acquisition above.

7.7.2 Slow Sweeps

- Problem:** When displaying a large number of sweeps, the display slows down.
- Solution:** Disable Persistence display in the Scope window.

7.7.3 Window Maximizing

- Problem:** Maximizing a window only maximizes the title bar.
- Solution:** Certain fixed-size windows and panels will not maximize (Dashboard, Paradigm Editor, Set Metadata, Set Preferences, Log, Action Potential Analysis, Event Detection.) This is a reported Igor issue.

7.7.4 Command Window Frozen

- Problem:** The Command Window is blank and/or unresponsive.
- Solution:** Use Ctrl-J, or click on the Amplifier Control Panel, and the Command window is redrawn as an active window. This is a reported Igor issue.

7.7.5 File Operations Crash

- Problem: In Windows 10, file opening or saving crashes SutterPatch.
- Solution: Remove the Dell Backup and Recovery utility v1.8, or upgrade it to a newer version.

7.7.6 Strange Characters

- Problem: Extra or strange characters appear in various fields when using Igor Pro 7.0.1.1.
- Solution: Download and install the latest version of SutterPatch using the Full Installer available at <https://www.sutter.com/AMPLIFIERS/SutterPatch.html>.

7.7.7 Wrong Default Settings

- Problem: Program preferences are non-standard or corrupted.
- Solution: Reset the SutterPatch preferences to their defaults via the SutterPatch / Set Preferences / General / Preferences Defaults button.

7.7.8 Weird Behavior

- Problem: There is weird or buggy behavior with the SutterPatch program.
- Solution: Reset the SutterPatch program settings to their factory defaults via the SutterPatch / Set Preferences menu command, by performing a Factory Reset.

7.7.9 Igor Pro Features

- Problem: There are a tremendous number of standard features in Igor Pro that can be used in conjunction with the SutterPatch application.
- Solution: Please refer to the Igor Help browser, or to Wavemetrics, Inc., regarding issues with Igor Pro features.

APPENDIX A: LIMITED WARRANTY

- Sutter Instrument Company, a division of Sutter Instrument Corporation, limits the warranty on this instrument to repair and replacement of defective components for two years from date of shipment, provided the instrument has been operated in accordance with the instructions outlined in this manual.
- Abuse, misuse, or unauthorized repairs will void this warranty.
- Warranty work will be performed only at the factory.
- The cost of shipment both ways is paid for by Sutter Instrument Company during the first three months this warranty is in effect, after which the cost is the responsibility of the customer.
- The limited warranty is as stated above and no implied or inferred liability for direct or consequential damages is intended.
- An extended warranty for up to three additional years can be purchased at the time of ordering, or until the original warranty expires. For pricing and other information, please contact Sutter Instrument Company.

APPENDIX B: SOFTWARE LICENSE

SutterPatch Software Licensing Agreement

IMPORTANT NOTICE

PLEASE READ THIS CONTRACT CAREFULLY. BY USING ALL OR ANY PORTION OF THIS PROPRIETARY SOFTWARE YOU ACCEPT ALL THE TERMS AND CONDITIONS OF THIS AGREEMENT. YOU AGREE THAT THIS AGREEMENT IS ENFORCEABLE LIKE ANY WRITTEN NEGOTIATED AGREEMENT SIGNED BY YOU. IF YOU DO NOT AGREE, DO NOT USE THIS SOFTWARE.

1. DEFINITIONS.

Section 1.0. Defined Terms. Terms defined in this Article 1 shall have the meanings given below. Defined terms may be used in the singular or plural.

Section 1.1. “Agreement” means this Software License Agreement, which includes this Agreement’s terms and conditions, Schedules, Exhibits, Addenda, and Amendments, if any, which are incorporated in, and form an integral part of, this Agreement.

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10. GENERAL PROVISIONS.

Section 10.1. Licensee warrants that Licensee has the requisite authority to enter into this Agreement.

Section 10.2. This Agreement constitutes the entire understanding and agreement between Licensor and Licensee regarding its subject matter, and supersedes all previous oral and written communications, agreements, memoranda, representations, or understandings between Licensor and Licensee regarding this Agreement. No other rights or licenses are granted to Licensee, except as expressly provided herein.

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Section 10.4. This Agreement is not transferable or assignable by Licensee under any circumstances, without the prior written consent of Licensor. This agreement shall be binding upon, and is made for the benefit of, each party, its successors, and permitted assignees (if any). For the purposes of this Agreement, any change in control of Licensee shall constitute an assignment or transfer of this Agreement. As used in this section, a change in control is defined as (i) any change in ownership of more than fifty percent (50%) of the voting interest in Licensee, whether by merger, purchase, foreclosure of a security interest or other transaction, or (ii) a sale of all or substantially all of the assets of Licensee.

Section 10.5. The relationship established by this Agreement between Licensee and Licensor shall be that of licensee and licensor. Nothing contained in this Agreement shall be construed as creating a relationship of agency, joint venture or partnership between Licensee and Licensor, so that neither party shall have any right whatsoever to incur any liabilities or obligations on behalf of the other party.

Section 10.6. The laws of California shall govern this Agreement. Any action or proceeding brought by either party against the other arising out of, or related to, this Agreement shall be brought only in a state or federal court of competent jurisdiction located in California and the parties hereby consent to the personal jurisdiction of said courts.

Section 10.7. In the event that any provision of this Agreement is found invalid or unenforceable pursuant to a judicial decree or decision, the remainder of this Agreement shall remain valid and enforceable according to its terms.

Section 10.8. The headings provided in this Agreement are for convenience and reference purposes only. In the event of a conflict between the terms and conditions listed in Articles 1 through 10, and the attached Schedules, the terms and conditions shall govern.

Section 10.9. A waiver of a breach, violation, or default under this Agreement shall not be a waiver of any subsequent breach, violation or default. Failure of either party to enforce compliance with any term or condition of this Agreement shall not constitute a waiver of such term or condition.

Section 10.10. All notices and communications shall be in writing and shall be deemed to have been duly given when delivered or three (3) Business Days after mailing by certified mail, return receipt requested, postage prepaid, addressed to the parties at their respective addresses or at such other addresses as the parties may designate by written notice in accordance with this section.

Section 10.11. Any amendments or addenda to this Agreement, may be executed in counterparts, each of which will be considered an original, but all counterparts together will constitute one agreement. A facsimile of a signed copy of this Agreement, or an electronic or other digital signature imprinted on this Agreement, may be relied upon as an original.

APPENDIX C: ACCESSORIES

- | | |
|-------------------------|---|
| • Ground Point GP-17 | Grounding point hardware |
| • Patch Panel | Rack-mountable analog and digital I/O BNC panel |
| • Quartz Pipette Holder | Quartz pipette holder |
| • Rack Pack | Rack mounting hardware |

APPENDIX D: FUSE REPLACEMENT

In the event that the instrument fails to power up when it is switched on, the power-line fuses should be checked to determine whether they have blown. Two fuses are located in the fuse holder in the power cord module on the rear of the amplifier.

To replace a fuse:

1. Unplug the power cord from the power entry module, revealing the fuse holder below.
2. Remove the fuse holder.

3. If a fuse is blown, it is recommended to replace both fuses.
4. Insert appropriately-rated replacement fuses (see below).
5. Replace the fuse holder in the power entry module and reconnect the power cord.

Mains Power Source	Fuses (Type: Time Delay/Time Lag, 5mm x 20mm, glass tube)	
	Fuse Rating	Manufacturer Examples
100 – 240 VAC	T2.0A, 250V	Bussmann: GMC-2-R, S506-2A, Littelfuse: 239.002.P

Table D-1. IPA Fuses

APPENDIX E. TECHNICAL SPECIFICATIONS



General Specifications

IPA & Double IPA Amplifiers	
Dimensions (in.) (includes handles & connectors)	IPA: 18.8 (W) x 11.8 (D) x 1.8 (H) DIPA: 18.8 (W) x 11.8 (D) x 3.5 (H)
Dimensions (cm) (includes handles & connectors)	IPA: 48.25 (W) x 30.0 (D) x 4.5 (H) DIPA: 48.25 (W) x 30.0 (D) x 9.0 (H)
Weight (lb) (with headstages)	IPA: 9.05 DIPA: 8.1
Weight (kg) (with headstages)	IPA: 4.1 DIPA: 3.7
Case	IPA: steel DIPA: aluminum
Communications	USB 2.0 (High Speed)
BNC Channels	4 auxiliary analog inputs (impedance: 1 M Ω) 2 auxiliary analog outputs (impedance: 16 Ω) 1 digital input trigger 1 digital output trigger 8 auxiliary digital outputs
Rack use	IPA: 19" rack-mount (1U) DIPA: 19" rack-mount (2U)
Benchtop use	Rubber feet
Signal Ground	4 mm Banana socket
Earth Ground	4 mm Banana socket
Safety	CE marking (Conformité Européenne)
Auxiliary I/O	DB-15 female connector


Port		
Auxiliary I/O Pinout	Pin	Definition
	1	Digital Output 1
	2	Digital Output 2
	3	Digital Output 3
	4	Digital Output 4
	5	Digital Output 5
	6	Digital Output 6
	7	Digital Output 7
	8	Digital Output 8
	9	Auxiliary Analog Input 1
	10	Auxiliary Analog Input 2
	11	Auxiliary Analog Input 3
	12	Auxiliary Analog Input 4
	13	Auxiliary Analog Output 1
	14	Auxiliary Analog Output 2
	15	Signal Ground
Configurations		Voltage-clamp Current-clamp
Whole Cell Capacitance Compensation		0 – 100 pF
Current-Clamp Rise Time (with 20 kHz low-pass filter & 100 MΩ load)		17.5 μs
Output Gain		0 – 25x

Table E-1. IPA & DIP Amplifier Specifications

IPA Headstage - Physical	
Construction	Anodized aluminum case
Dimensions (in.)	4.000 (L) x 1.375 (W) x 0.825 (H)
Dimensions (cm)	10.160 (L) x 3.493 (W) x 2.096 (H)
Cable Length (feet)	6
Cable Length (m)	1.83

Weight (lb) w/o cable w/cable	0.21 0.294
Weight (kg) w/o cable w/cable	0.095 0.133
Ground Socket (mm)	1
Feedback Resistor	500 M Ω

Table E-2. IPA Headstage - Physical

IPA Headstage - Noise (measured with 8-pole Bessel filter)	
Bandwidth	Open-Circuit Noise (RMS)
0.1 – 1 kHz	< 0.25 pA
0.1 – 5 kHz	< 0.75 pA
0.1 – 10 kHz	< 1.40 pA

Table E-3. IPA Headstage Noise

IPA & DIPA Data Acquisition	
Analog I/O Channel Type	Full Differential
Analog I/O Channel Amplitude (voltage)	± 10 V
Analog I/O Channel Amplitude (current)	± 20 nA
Analog I/O Channel Resolution	16-bit
Headstage Input Sample Rate	0.1 - 50 kHz
Headstage Input Filter Bandwidth	0.5 - 20 kHz
Headstage Output Sample Rate	0.1 - 10 kHz
Auxiliary Input Sample Rate	0.1 - 50 kHz

Auxiliary Output Sample Rate	0.1 - 10 kHz
Digital Input Sample Rate	0.1 – 50 kHz
Digital Output Sample Rate	0.1 – 10 kHz
Digital Input Trigger Level	+2.3 V
Digital Output Trigger Level	+3.3 V, 100 μ s

Table E-4. IPA Data Acquisition

IPA Expansion Panel	
Dimensions (in)	18.8 x 2 x 3.5
Dimensions (cm)	48 x 5 x 9
Weight (lbs)	3.5
Weight (kg)	1.6
Digital Output BNCs	8
Auxiliary Analog Input BNCs	4
Auxiliary Analog Output BNCs	2

Table E-5. IPA Expansion Panel

IPA Electrical	
Power consumption	18 Watts maximum
Mains fuse	250V 1A Slow Blow (5 mm x 20 mm) T2.0
Cables	Shielded grounded power line cord
Line Voltage	100 VAC – 240 VAC

Table E-6. IPA Electrical

IPA System Components

Carefully remove all components from the shipping container. The following should be included:

- (1) IPA Amplifier (IPA/E-1)
- (1) IPA Headstage (IPA-HS)
- (1) Polycarbonate Pipette Holder EH-P170 (standard)

- or (1) Quartz Pipette Holder EH-Q170 (optional)
- (1) Model Cell MCELL
- (1) Auxiliary I/O Adapter Cable
- (1) Rack-Mount Kit RACK-PK
- (1) Power Cord
- (1) USB 2.0 Cable
- (1) Quick Start Guide (with Igor Pro 8 Serial #)
- (1) USB Flash Drive (with SutterPatch and Igor Pro 8 software)

Double IPA System Components

Carefully remove all components from the shipping container. The following should be included:

- (1) Double IPA Amplifier IPA/E-2
- (2) IPA Headstages IPA-HS
- (1) Auxiliary I/O Adapter Cable
- (2) Polycarbonate Pipette Holders EH-P170 (standard)
- or Quartz Pipette Holder(s) EH-Q170 (optional)
- (1) Model Cell MCELL
- (1) Power Cord
- (1) USB 2 Cable
- (1) Rack Mount Kit RACK-PK
- (1) Quick Start Guide (with Igor Pro 8 Serial #)
- (1) USB Flash Drive (with SutterPatch and Igor Pro 8 software)

Pipette Holder Parts

- End Cap
 - Silicone Gaskets (O-rings, 6 ea.)
- | <u>Gasket ID</u> | <u>Color</u> |
|------------------|--------------|
| 1.1 mm | Clear |
| 1.2 mm | Green |
| 1.5 mm | Orange-Red |
| 1.75 mm | Blue |
- Silver Wire
 - Body/Barrel (standard: polycarbonate)
(optional: quartz)

- Wire Seal (tubing)
- Gold Pin
- Pin Cap
- Lockdown Ring

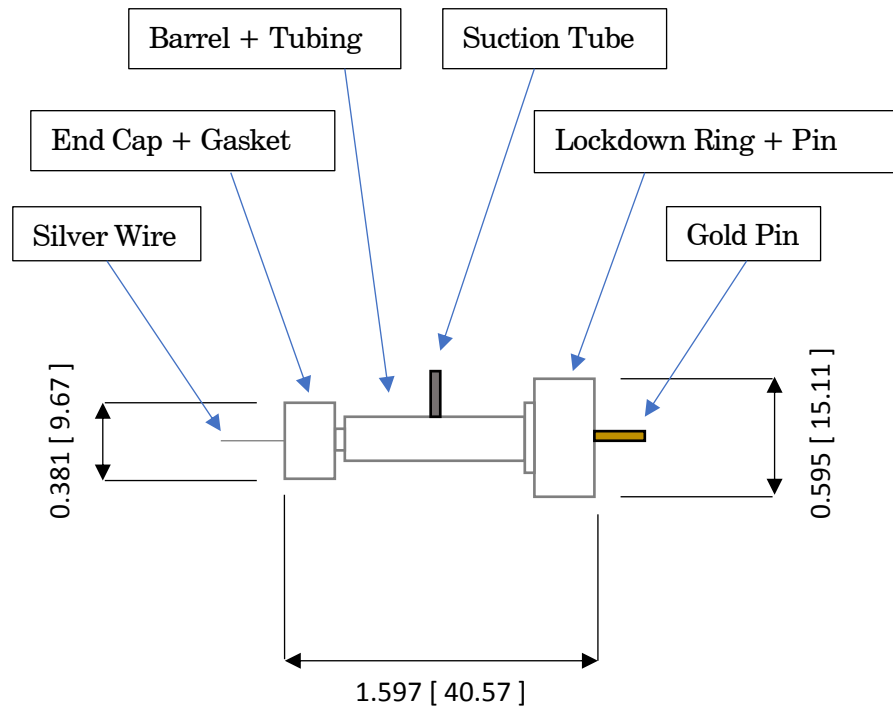


Figure E-1. Electrode Holder

Figure dimensions are in “inches [mm]”.

Model Cell Parts

- Model Cell
- Connector pins with crimp
- Ground wire