

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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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.

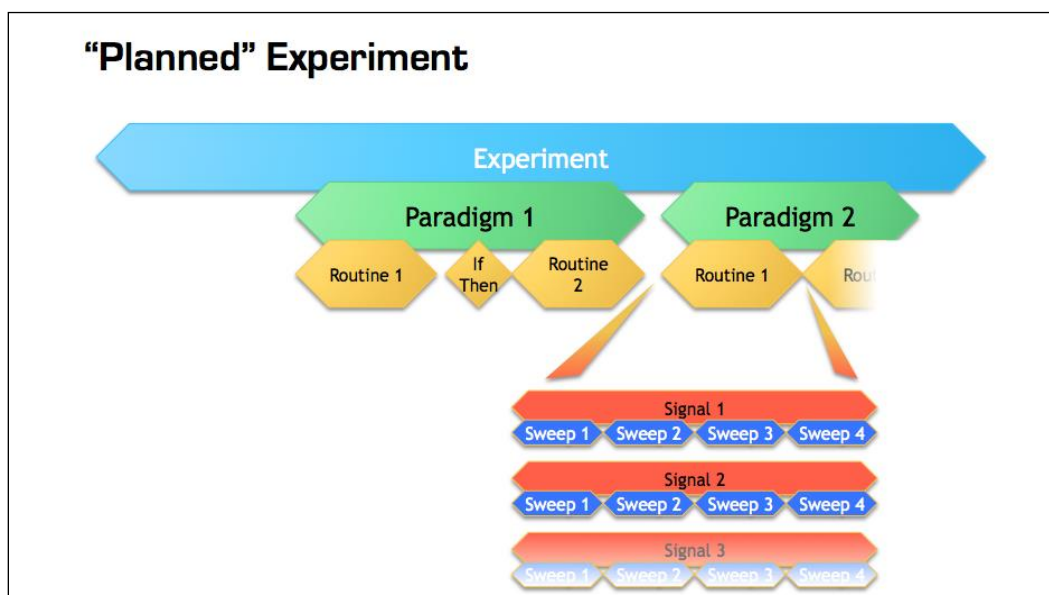


Figure 1-1. Data Structure - Planned Paradigms

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.

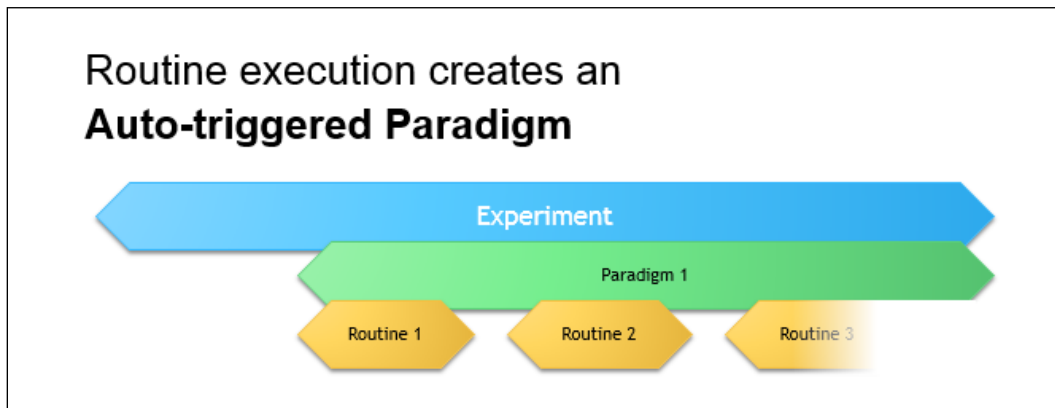


Figure 1-2. Data Structure - Auto-triggered Paradigms

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 Parameters:

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 of 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.

SutterPatch Metadata:**99

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 Configuration

Operating System:	Windows:	Version 10 (64-bit versions) Most language packs are compatible. (listed in OS: Control Panel > System)
	macOS:	Version 10.11 (El Capitan) or later. (listed in OS: Apple > About this Mac)
	Note:	Virtual machines and OS emulators, such as Parallels and VMWare Fusion, are not supported.
CPU (Central Processing Unit):	Dual-core i5	
RAM (Random Access Memory):	3 GB	
Hard Disk Free Space:	500 GB	
Display Resolution:	1024 x 768 (XGA)	
Computer Ports:	(1) USB 2.0 High Speed port	
	Note:	USB hubs are not supported.

Recommended Configuration

Operating System (for Windows):	Version 10 (64-bit)	
RAM (Random Access Memory):	8 GB	
Hard Disk Free Space:	SSD (Solid State Drive) 500 GB or greater	
	Note:	The drive should be configured as the primary system drive.
Display Resolution:	1920 x 1080 (Full HD)	
	Note:	High resolution (> 96-DPI) displays, such as Retina, 4K, 5K, Quad-HD and Ultra-HD, are not supported.

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

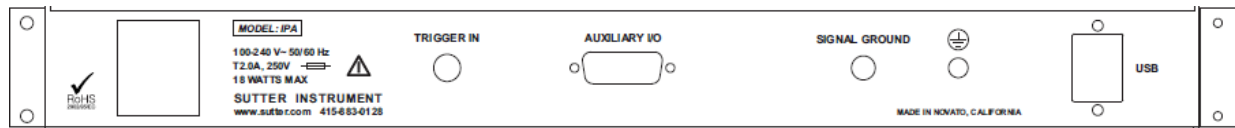


Figure 2-1. Rear of IPA Cabinet

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 HEAD-STAGE 1, and the higher serial-numbered headstage to HEADSTAGE 2, as each headstage is individually tuned to its channel.

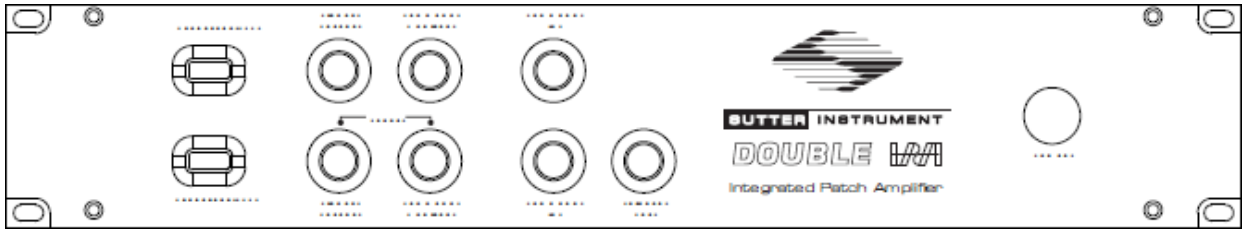


Figure 2-3. Front of Double IPA Cabinet

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:

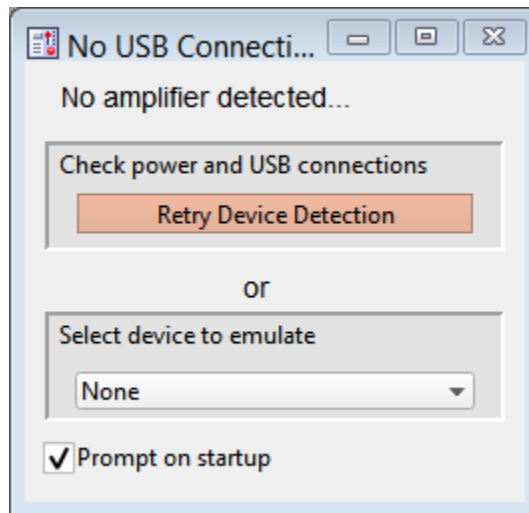


Figure 2-6. Emulation Modes

- a. Reconnect it, and then click 'Retry', or
 - b. Select 'Emulate IPA' or 'Emulate Double IPA'.
 - c. If amplifier hardware is not attached, and 'Prompt on startup' is disabled, the program will automatically start up in the last known emulation state.
6. The Dashboard panel is displayed.

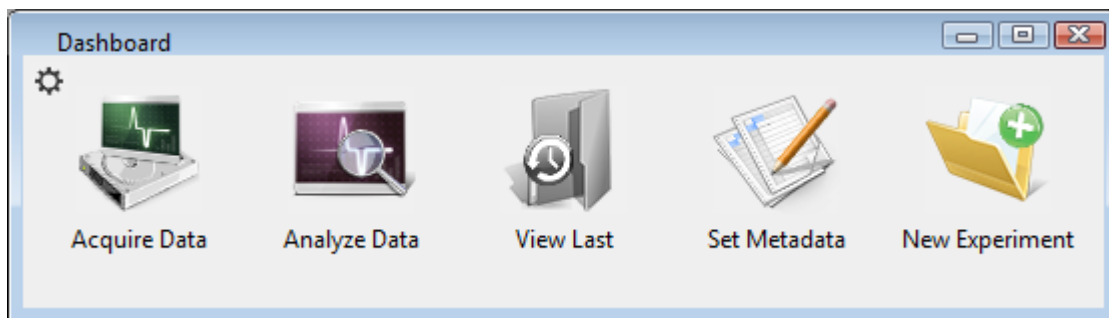


Figure 2-7. Dashboard.

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

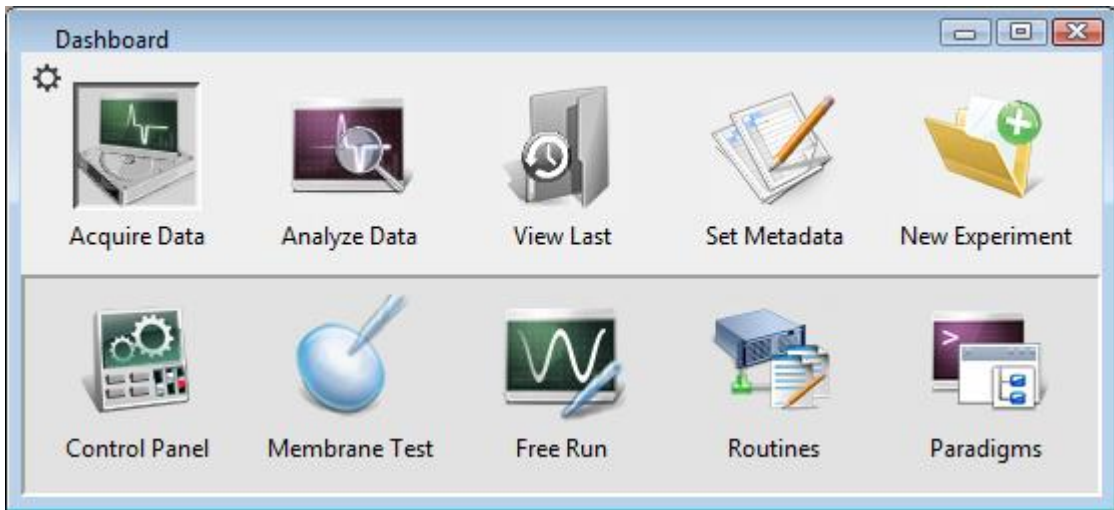


Figure 2-8. Acquisition Dashboard.

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

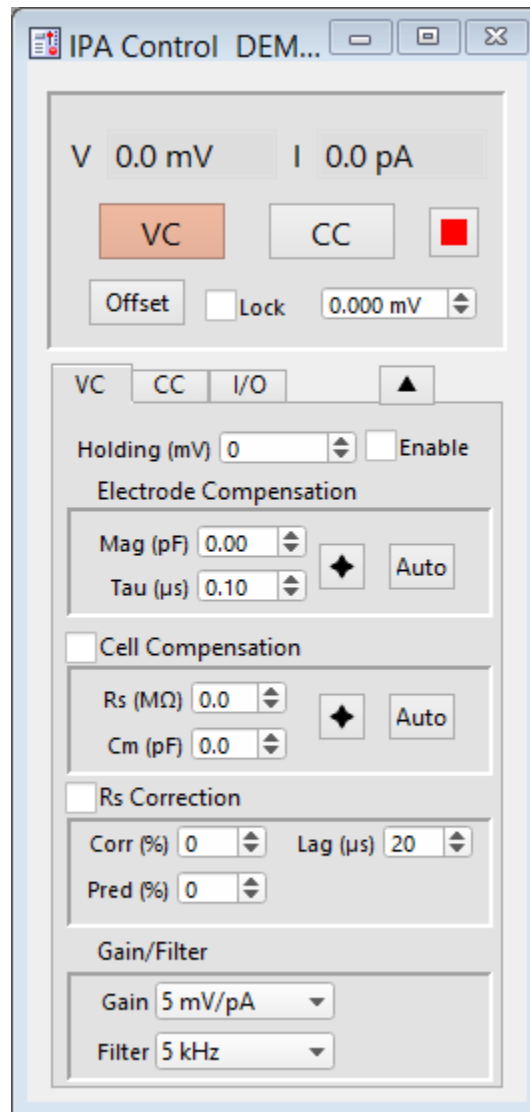


Figure 2-9. Amplifier Control Panel

- a. If “DEMO” 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.

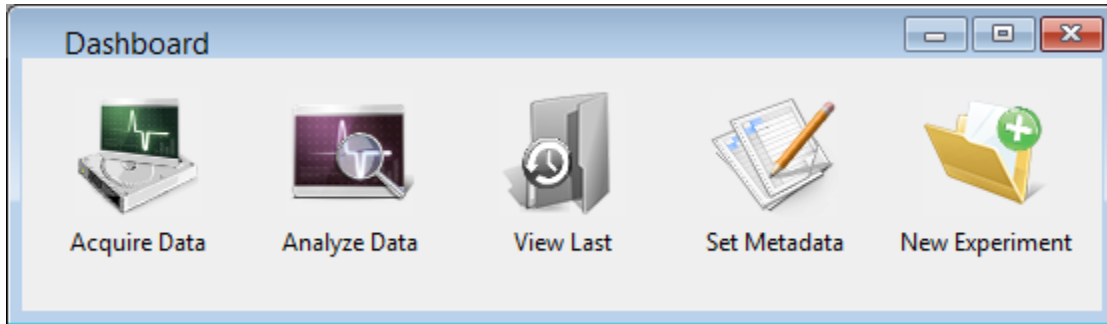


Figure 2-10. Dashboard

2. Click on the 'Membrane Test' icon.

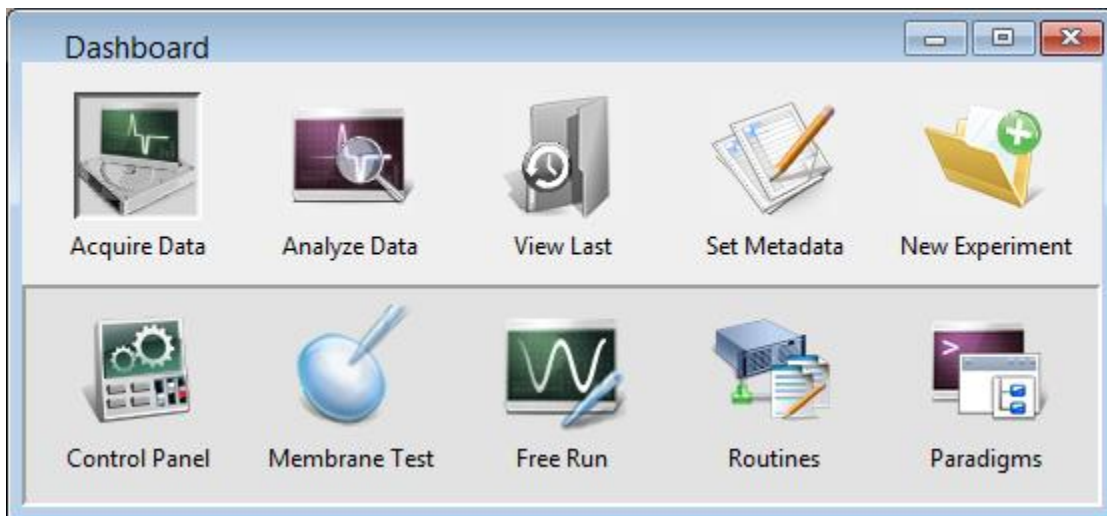


Figure 2-11. Dashboard – Acquisition

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: $\sim 10 \text{ M}\Omega$

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.

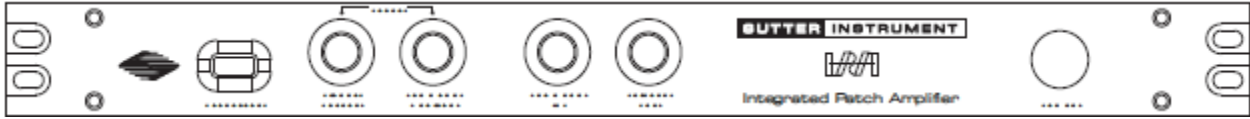


Figure 3-1. Front of IPA Cabinet

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.
<ul style="list-style-type: none"> VC mode: 	mV/pA	Variable gain (Amplifier Control Panel)
	Example:	Membrane Test
	Model Cell:	BATH position (10 MΩ)
	Amplitude	= 10 mV
	Gain	= 5 mV/pA
	Current response	= 10 mV/10 MΩ = 1 nA (V/R = I)
	Scaled output voltage	= I * Gain 1 nA * 5 mV/pA = 5 V
<ul style="list-style-type: none"> CC mode: 	mV/mV	Variable gain (Amplifier Control Panel)
	Example:	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
Example:	Voltage Clamp	
Command voltage:	10 mV	
Scaled output voltage:	$V * Gain$	
	$= 10 \text{ mV} * 10 \text{ mV/mV}$	
	$= 100 \text{ mV}$	
• CC mode:	0.5 mV/pA	Constant gain
Example:	Current Clamp	
Command current	$= 200 \text{ pA}$	
Scaled output voltage	$= I * Gain$	
	$= 200 \text{ pA} * 0.5 \text{ mV/pA}$	
	$= 100 \text{ mV}$	
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
Example:	Voltage Clamp	
External Command	$= 5 \text{ mV}$	
Stimulus signal	$= 20 \text{ mV}$	
Total stimulation	$= (V * Gain) + \text{StimOUT}$	
	$= (5 \text{ mV} * 10 \text{ mV/mV}) + 20 \text{ mV}$	
	$= 70 \text{ mV}$	
• CC mode:	2 pA/mV	Constant gain
Example:	Current Clamp	
External Command	$= 1 \text{ mV}$	
Stimulus signal	$= 5 \text{ pA}$	
Total stimulation :	$(V * Gain) + \text{StimOUT}$	
	$= (1 \text{ mV} * 2 \text{ pA/mV}) + 5 \text{ pA}$	
	$= 7 \text{ pA}$	

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.

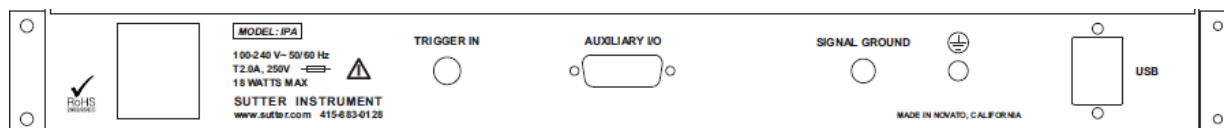


Figure 3-3. Rear of IPA Cabinet

[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, 20 kHz filter)

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”.

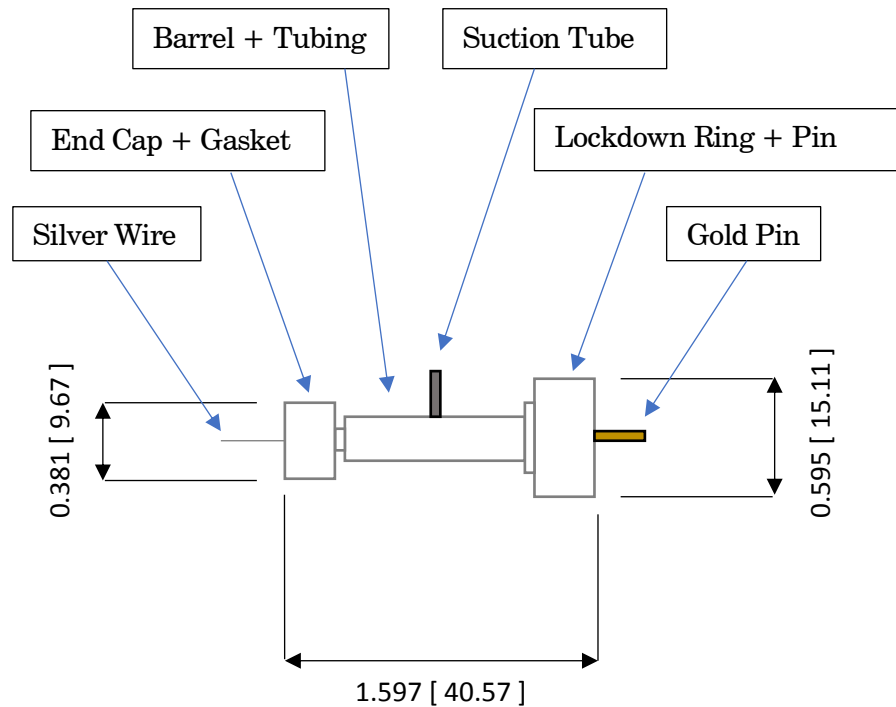


Figure 3-4. Electrode Holder

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 IPA Amplifier Control Panel

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.

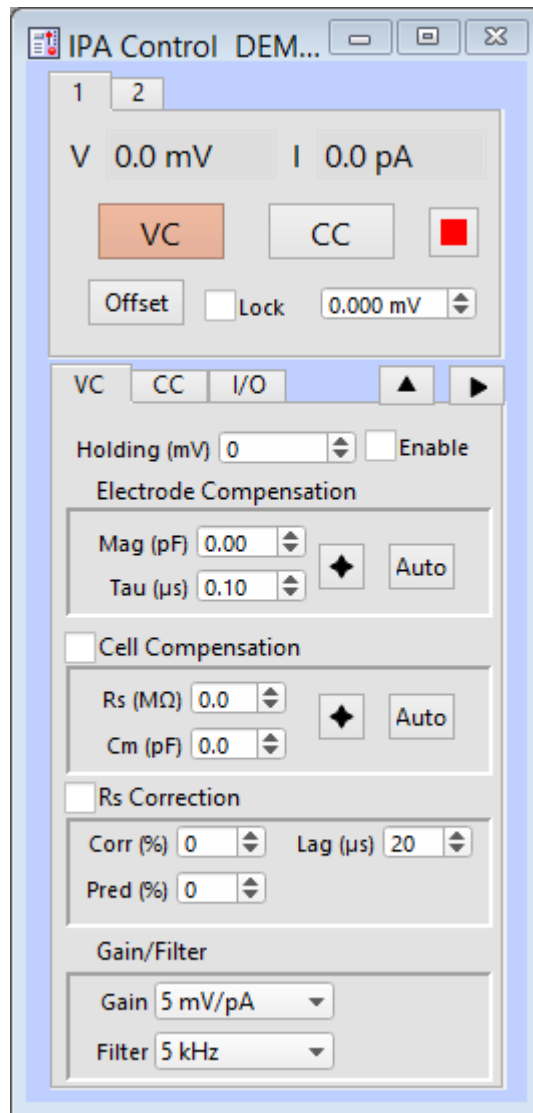


Figure 3-5. Amplifier Control Panels

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 from Current Clamp to Voltage Clamp mode. The button for the active mode is highlighted in red. A “gentle” switch is used to protect cells against transition spikes that could degrade the electrode seal and integrity of the recording. The amount of voltage to hold the current steady is automatically injected into the cell during the transition (< 100 ms), before stepping to the new voltage command level.
CC	CC button:	Switch the IPA amplifier from Voltage Clamp to Current Clamp mode. The button for the active mode is highlighted in red. A “gentle” switch is used to protect cells against transition spikes that could degrade the electrode seal and integrity of the recording. The amount of current to hold the voltage steady is automatically injected into the cell during the transition (< 100 ms), before stepping to the new current command 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 all attached Sutter IPA/DIPA amplifiers and their headstages to factory defaults.

Offset

Click on the Offset button to apply a potential to auto-zero the current signal. This value is an approximation that might need further adjustment, and is to the right of the Offset button.

[±250.000 mV] This value is independent of the holding potential.

The electrode offset should be initially adjusted in voltage-clamp mode, when the electrode is placed into the bath solution. This feature 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.

Note: In Demo mode, this value is auto-set to -0.200 mV.

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

- For fine adjustments, use the up / 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 then 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 contains the amplifier Voltage Clamp controls:

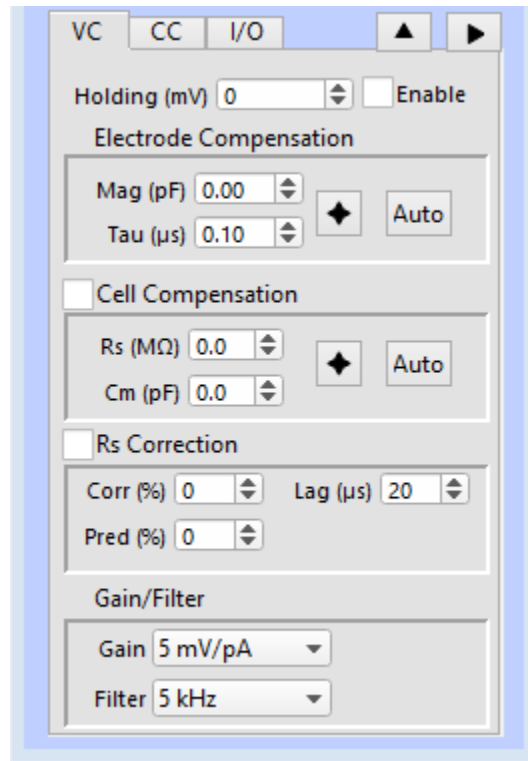


Figure 3-6. VC Control

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). This control is active during acquisition - changes are applied to the next sweep.

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

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

- For fine adjustments, use the up / down spinners to increase or decrease the setting by 1 mV.
- For moderate adjustments, increase the spinner increment to 10 mV by holding down the Shift key and then 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.

Note: The IPA Compensation and Correction controls do not affect demo data.

Electrode Compensation: Electrode capacitance compensation.
[These fields are shared with CC mode.]

Mag (pF): [0.00 – 25.00] Magnitude

Tau (μ s): [0.10 – 4.50]



Opens a 2-D slider 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 using the 2-D slider panel or 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 2-D slider 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 use the 2-D slider panel or iteratively adjust the ‘Rs’ and ‘Cm’ controls until the signal is adequately compensated (i.e., smooth transitions without transients.)

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

Note: Disable ‘Cell Compensation’ when measuring Series Resistance or Membrane Capacitance in the Membrane Test (Cell mode), so that

the calculations are based on uncompensated cell activity.

However, you can leave 'Cell Compensation' enabled when using Membrane Test Pulse Type 'Lock-in' frequencies up to 2 kHz. (At higher Lock-in frequencies, the Lock-in phase delay is affected.)

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

<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)

kHz

0.5

1

2

5

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

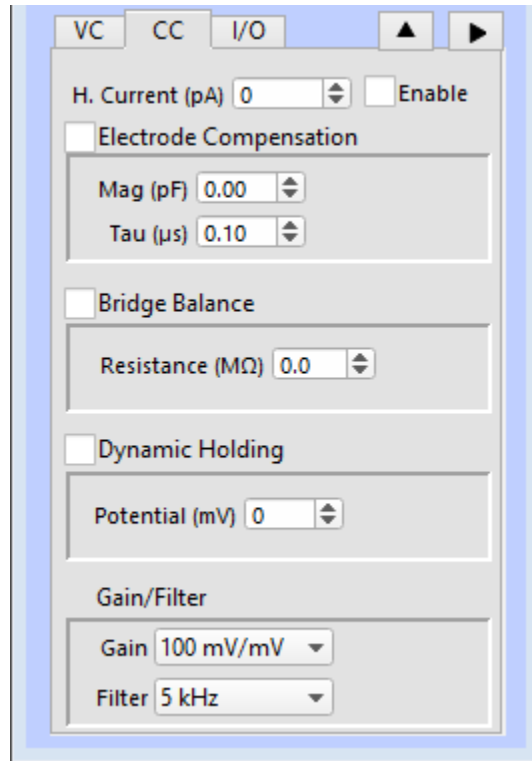


Figure 3-7. CC Control

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

H. Current (pA): [±1000]

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

Note: The IPA Compensation controls do not affect demo data.

Electrode Compensation Electrode capacitance compensation.
[These fields are shared with VC mode.]

Mag (pF): [0.00 – 25.00] Magnitude

Tau (µs): [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.

Tip: If you consistently lose cells when switching into CC mode, consider

adjusting the CC mode electrode compensation value in Set Preferences / Hardware / Stability Control.

Bridge Balance

Resistance (M Ω): [0 – 200.0]

Bridge Balance is an adjustment to remove the voltage effects of series resistance on current clamp recordings. It corrects distortion of the signal due to a voltage drop across the electrode during current flow (injection).

Note: If you are simply recording voltage (I=0), then Bridge Balance can be left disabled, i.e., completely ignored.

Instead of an 'Auto' bridge balance button, the series resistance value from the voltage clamp mode is carried over. Start in VC mode and use Auto 'Cell Compensation'. When you switch into CC mode, the VC mode Cell Compensation value is copied into the Bridge Balance 'Magnitude' field.

To manually determine the correction value, run the Membrane Test and zoom in on the initial rising phase. With Bridge Balance disabled, there is a DC shift visible at the beginning of the rising signal. Enable Bridge Balance and adjust until the DC shift disappears.

However, after adjustment, there may still be a small glitch at the beginning of the rise, due to electrode and headstage capacitance that doesn't entirely go away. In some cases, this can make it difficult to determine the exact value, but as long as the series resistance is significantly smaller than the cell resistance, the errors are very small.

Dynamic Holding

Potential (mV): [\pm 1000]

Maintain the membrane holding potential at a set target level without it drifting over time.

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 Dynamic Holding 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

<u>kHz</u>
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

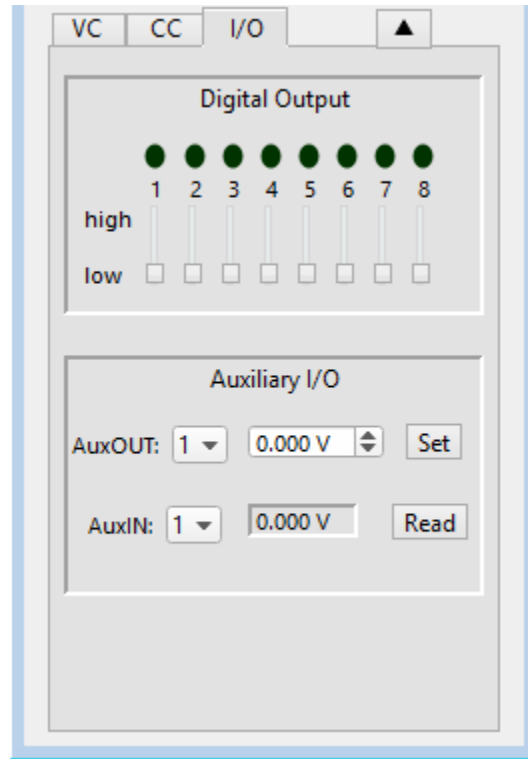


Figure 3-8. I/O Control

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.

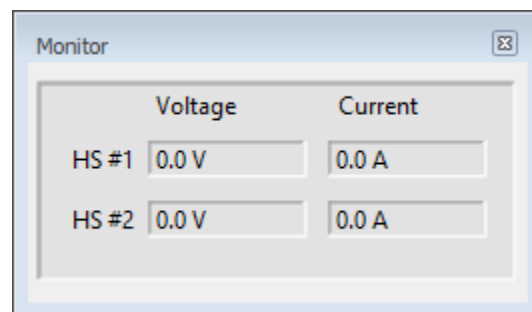


Figure 3-9. Headstage Monitor

3.8 Lock-In Adjustments

The “lock-in” system is used to detect very small changes in membrane capacitance measurements, such as for exocytosis measurements. Our system uses a digital implementation of phase-sensitive detection (PSD) to make its measurements.

- 1) To perform lock-in analysis, a reference waveform needs to be supplied to the headstage. A suitable sine wave needs to be generated via the Routine Editor / Waveform Editor / Sine / Sine Wave Cycles / For Lockin. (Also see Membrane Test.)
- 2) The lock-in response signal is processed via a virtual input channel. Other measurements, such as membrane conductance, can also be reported. Calculations are made using ‘conductance’ (1 / resistance) instead of ‘resistance’.

Setup in the Routine Editor / Input Channels / Virtual#:

Math Type	LockIn
Current Channel	Select a (source) input channel with a “Current” signal.
Trace Kind	Select the LockIn measurement to perform. The selected ‘Trace Kind’ is automatically set as the Virtual Channel label.
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.
Cycles to Average	[1 – 1000]
Cycles to Skip	[1 – 1000]
V-reversal	[±1000 mV] When using a calculated stimulus trace, enter the reversal potential for the ion under study, such as for (Na ⁺) sodium spikes or (K ⁺) potassium tail currents.

- 3) The lock-in phase detection can be manually tuned via the menu item SutterPatch / Hardware Control / Lock-In Adjustments. Phase adjustments are made to optimize the headstage signals, 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.
[$\pm 1.00 \mu\text{s}$]

Reset Reset to '0.00 s'

Attenuation Adjustment Apply a gain to the calculations.
[0.001 – 9.999]

Reset Reset to '1.000'

List Results Display results in the Command window.

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. Due to its sensitivity, run the electrode compensation on a pulse before running LockIn adjustments.

Start with the Phase Delay Adjustment, and then follow with the Attenuation Adjustment.

The sensitivity of the SutterPatch software lock-in results is as good as for a hardware lock-in amplifier.

When making absolute capacitance measurements, you can improve the consistency of the measurements by adjusting the lock-in phase adjustment to a known reference capacitance, such as with the model cell.

The SutterPatch lock-in calculations are based on the Lindau/Neher method of time-resolved capacitance measurements in single cells. (See the Algorithms appendix for the reference.)

Note: Demo mode is not designed to respond to lock-in phase and attenuation adjustments.

3.9 System Integration

The IPA systems can be integrated with other suitable laboratory equipment.

3.9.1 Using Peripheral Equipment

The IPA system can control peripheral equipment, such as:

- Cameras

- Light sources
- Pulse generators
- Solution changers
- Wavelength switchers

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.

3.9.2 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 has its own numbered tab [1 – 4] in the Amplifier Control Panel. Up to 16 analog input channels can be configured, using a mix of headstage, auxiliary and virtual input channels from either amplifier. However, Digital Outputs (8) 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.

3.9.3 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%.

3.9.4 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

<u>BNC</u>	<u>Channel</u>
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.

3.10 IPA Maintenance

This unit should require minimal maintenance when operated according to specifications.

3.10.1 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.

3.10.2 Cleaning

Routine cleaning of the IPA system is required to prevent excessive dust accumulations. Wipe all exterior surfaces with a dry, soft, cotton cloth.

3.10.3 Calibration

The IPA amplifiers do not support user calibration.

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.

Signal Parameters

Channel: Select an input channel to monitor:

Current1
 Voltage1
 AuxIN1 - 4

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

Duration: The duration of the acquired/displayed sweep.
 [100, 200 ms, 0.5 , 1 , 2 s]

Sampling rate: [5, 10, 20, 50 kHz]

4.1.3 Membrane Test Windows

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 (Membrane Test)

This Scope (Membrane Test) window operates similarly to the Scope (Acquisition) 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.

However, differences to the Scope window are that unsupported buttons and controls were removed (Persistence Display, Signal Layout, Sweeps Counter). Also, during an Experiment, the Autoscale button state persists between windows.

The IPA Membrane Test always opens in a “running” state.

Closing the Membrane Test Scope or Analysis window halts any acquisition.

Membrane Test Analysis

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

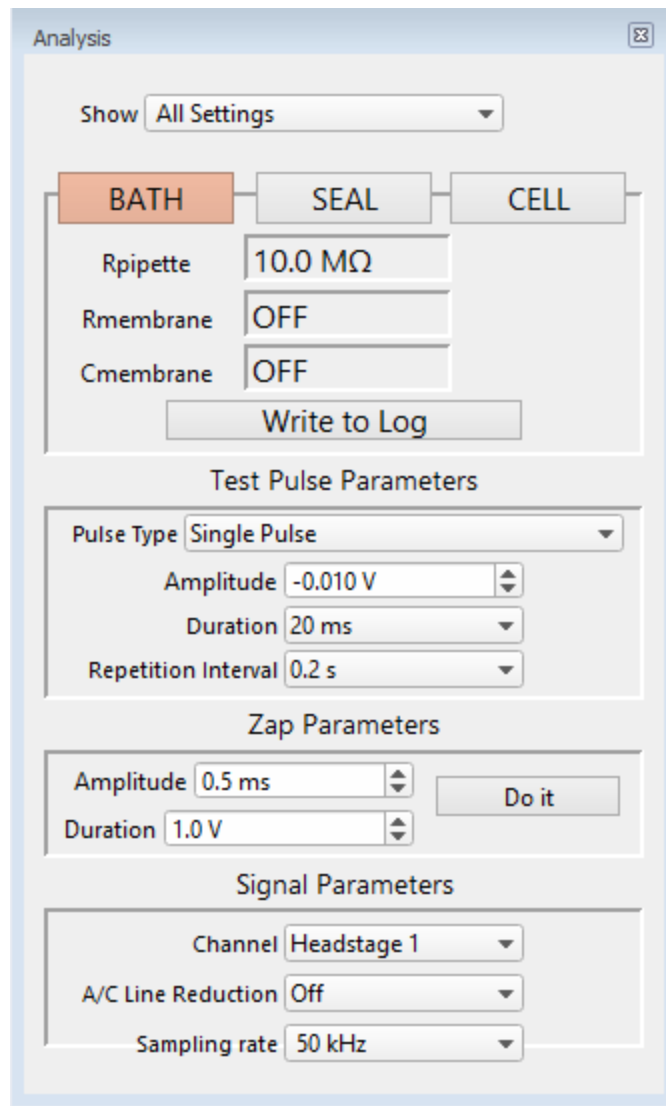


Figure 4-1. Membrane Test Monitor

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 M Ω . In slice patch experiments, pipette resistances well above 10 M Ω are not uncommon.

R_{pipette} (M Ω) Pipette Resistance meter

[Model cell = ~10 M Ω]

2) Seal When an on-cell patch is formed between the pipette and the cell, voltage transition spikes are visible. The seal resistance increases as the seal forms. The goal is to achieve a “gigaseal” with a resistance above 1 G Ω .

R_{seal} (M Ω) Seal Resistance meter

[Model cell > 10,000 M Ω]

3) Cell After breaking through the cell membrane and creating a whole-cell patch, membrane resistance and capacitance measures are calculated from the resulting capacitance spikes. These values can be periodically checked to monitor the health of the cell.

R_{series} (M Ω) Series Resistance meter

[Model cell = ~10 M Ω]

R_{membrane} (M Ω) Membrane Resistance meter

[Model cell = ~500 M Ω]

C_{membrane} (pF) Membrane Capacitance meter

[Model cell = ~28 pF]

Warning: In Cell mode, disable the Amplifier Control Panel ‘Cell Compensation’ and ‘Rs Correction’ to allow capacitance spikes to be generated for the calculation of the Series Resistance and Membrane Capacitance values.

Note: ‘Series Resistance’ and ‘Access Resistance’ are equivalent terms. It is helpful to monitor the Series Resistance, as if it increases by more than 5%, the electrode tip might be clogged.

Write to Log Click the ‘Write to Log’ button to copy the measurements to the Log window.

Valid measurements are logged to headstage “(HS#1)” and/or “(HS#2)”.

Test Pulse Parameters

Pulse Type

- Single Pulse [1, 2, 5, 10, 20, 50, 100, Other: ± 999.00 mV]

A single monopolar square pulse.

	<ul style="list-style-type: none"> • Double Pulse 	A symmetrical bipolar (biphasic) square pulse.
	<ul style="list-style-type: none"> • Triangle 	A train of 5 symmetrical bipolar triangular pulses. [dPatch only]
	<ul style="list-style-type: none"> • Sine 	A train of 5 sine wave pulses. [dPatch only]
	<ul style="list-style-type: none"> • 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]	Set long enough for the signal to reach its asymptote, or measurements can be incorrect.
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	[0.1 - 1.0 V]	Set the amplitude of the square wave zap pulse.
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.

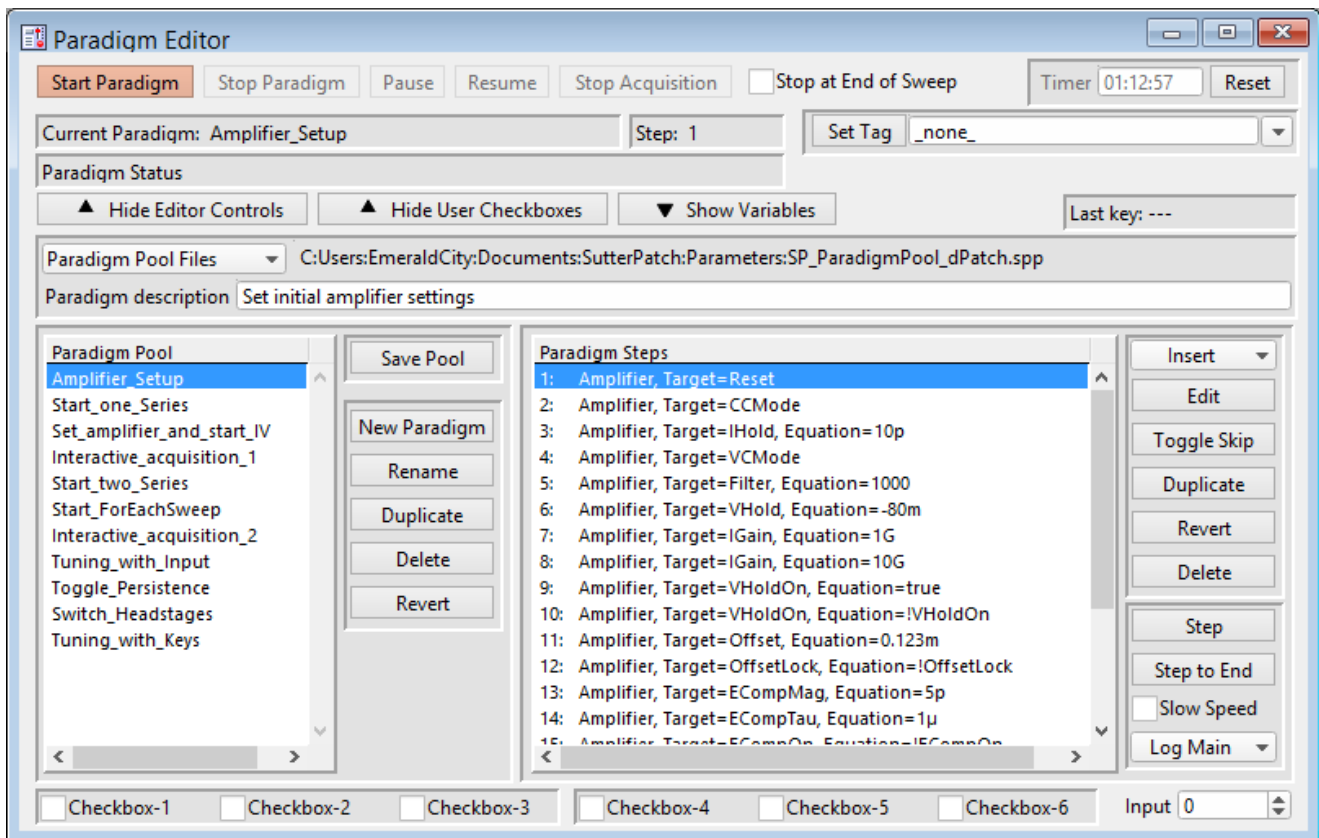


Figure 4-2. Paradigm Editor

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.
- To select multiple Paradigms, use a Shift-Click mouse drag, or individually Shift-Click the Paradigm names. Multiple Paradigms can thus be deleted, or saved to a new Paradigm Pool.

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 Inserts 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 to Log

Alert

Beep

Comment

Wait

Pause

Break

Chain

For Loop

Jump

Label

If

ElseIf

Else

(See details in Insertable Steps list below.)

Edit

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.

Also, if a highlighted Step's text is partially hidden, use the Edit button to view the entire entry.

Toggle Skip

Mark a step so it is not executed.

A semicolon is prepended to the Step number to "comment out" the instructions, and a Skip status is appended to the Step text.

Example: A 'Beep' command in Paradigm step #2:

```
; 2 Beep, Skip=true
```

The leading semicolon ";" prevents this step from being executed by the instruction queue, and the 'Skip' status

is displayed.

Duplicate	Insert a copy of the selected step.
Revert	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.
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.

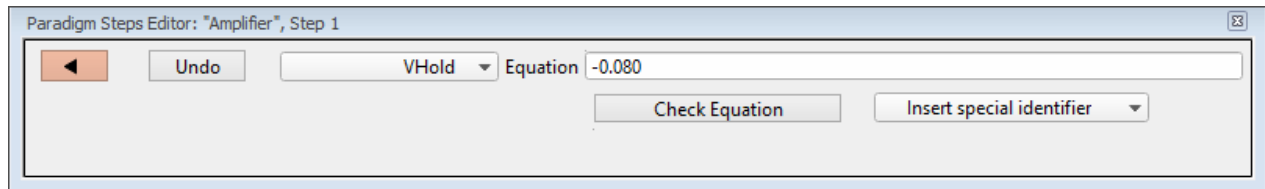


Figure 4-3. Step: Amplifier

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



Close the 'Paradigm Steps Editor'.

Undo

Remove 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.

VCMMode (amplifier voltage clamp)

Place the amplifier into Voltage-Clamp mode.

Hold	(IHold in CC-mode, VHold in VC-mode) [$\pm 0.000,000,020$ A (± 20 nA), or ± 1.000 V (± 1000 mV)] Set the active headstage holding level.
IHold	(amplifier holding current, A) [$\pm 0.000,000,020$ 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: <ul style="list-style-type: none"> • 0.5 mV/pA • 1 mV/pA • 2.5 mV/pA • 5 mV/pA • 10 mV/pA • 25 mV/pA <p>To help reduce signal saturation from too high a gain, a 90% threshold promotes the equation value to the next higher Gain setting.</p>

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)

LockInAdjustOn (set LockIn adjustments On)

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.

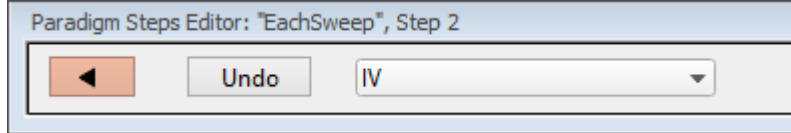


Figure 4-4. Step: Each Sweep

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



Close the ‘Paradigm Steps Editor’.

Undo

Remove any unsaved edits to this step.

[drop-down list]

Select a Routine name from the loaded Routine Pool.

For example, when tracking whole-cell Rs 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.

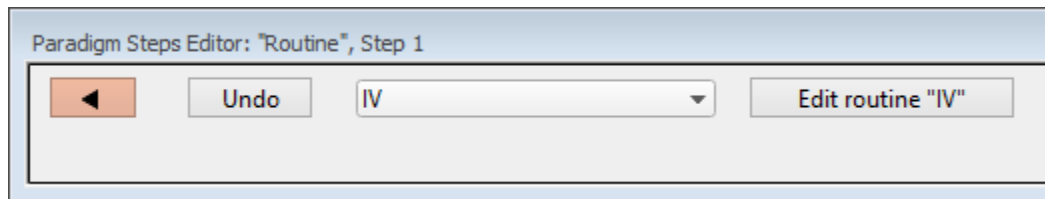


Figure 4-5. Step: Routine

Default Setting: *Routine, Target=untitled*



Close the 'Paradigm Steps Editor'.

Undo

Remove 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>]

Edit routine “xxx” Open the Routine into` the Routine Editor for “last minute” changes.

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.

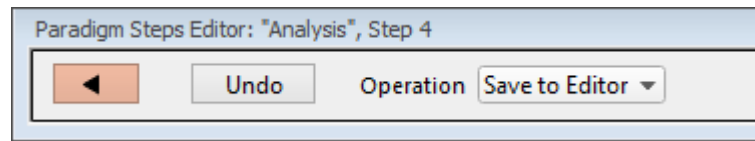


Figure 4-6. Step: Analysis

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



Close the 'Paradigm Steps Editor'.

Undo Remove 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.

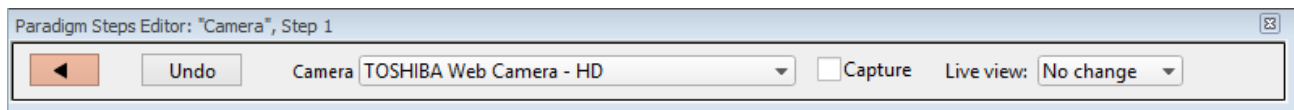


Figure 4-7. Step: Camera

Default Setting: *Camera, Camera=_Camera_Name, Capture =true*



Close the 'Paradigm Steps Editor'.

Undo Remove 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:

- No Change Keep last settings
- Stop Stop live view
- Start Start live view

Clear Key

Clear 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.

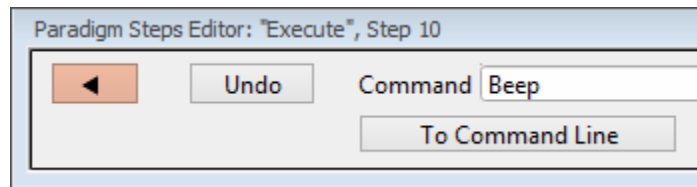


Figure 4-8. Step: Execute

Default Setting: *Execute, Command=Beep*



Close the 'Paradigm Steps Editor'.

Undo

Remove 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 an 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.

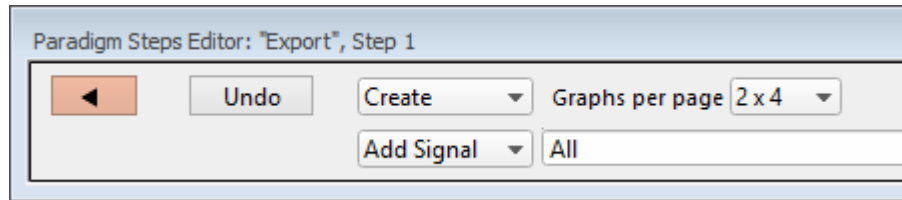



Figure 4-9. Step: Export

Default Setting: *Export, Signal=Layout*

	Close the 'Paradigm Steps Editor'.
Undo	Remove 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	Select all entries.
All Signals	Select all input signals.
< List of input signals >	

All Analyses Select 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.

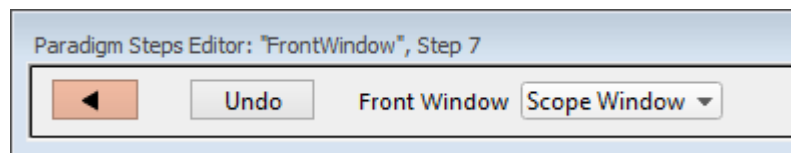


Figure 4-10. Step: Front Window

Default Setting: Front Window, Target=Scope Window



Close the 'Paradigm Steps Editor'.

Undo Remove 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.

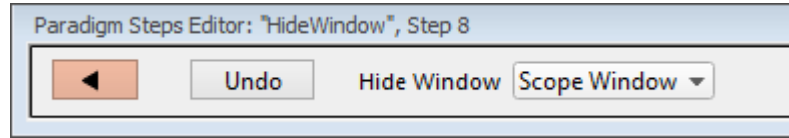


Figure 4-11. Step: Hide Window

Default Setting: HideWindow,Target=Scope Window



Close the 'Paradigm Steps Editor'.

Undo

Remove 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.

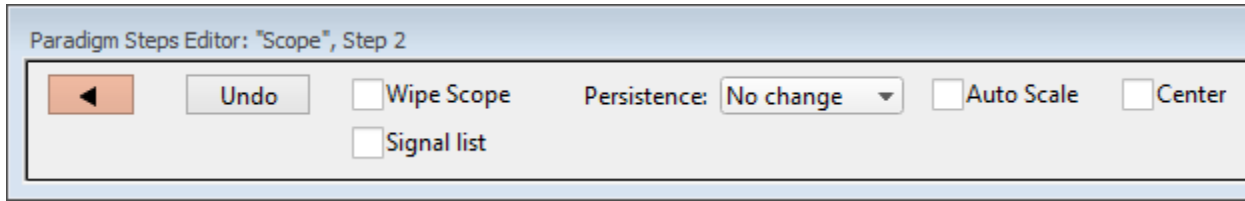


Figure 4-12. Step: Scope Operation

Default Setting: Scope, Wipe=false



Close the 'Paradigm Steps Editor'.

Undo

Remove any unsaved edits to this step.

Wipe Scope

Clear the Scope window of sweeps

Signal list:

Enable to display a list of input signals

Add Signal

Clear

Clear the signal list

All Signals

Select all signals

[list of all available input signals]

[list of selected input signals]

You can directly edit the list. User-defined signal labels can also be used.

Persistence:

No change

On

Off

Autoscale

A one-time Autoscale of the Y-axes of all selected signals to their incoming data, i.e., to their visible sweeps data limits, and resets the X-axes to the full sweep duration.

Center

Centers the active signal so the mean of the Y-axis data is vertically centered in the signal pane. Only the Y-axis offset is automatically adjusted, not the scaling; the X-axis is unaffected.

Set Axis

Modify the axis scaling of a signal.

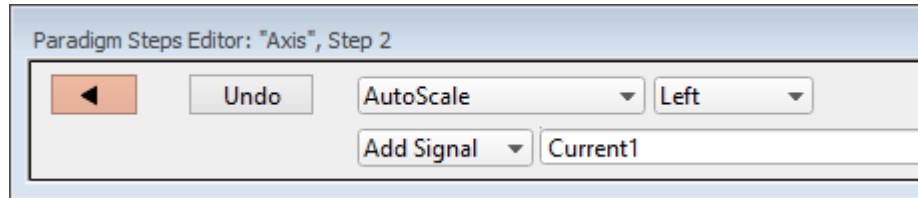


Figure 4-13. Step: Set Axis

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



Close the 'Paradigm Steps Editor'.

Undo Remove any unsaved edits to this step.

[drop-down list] Select the operation to perform.

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] Select the axis orientation.

Left	Apply to the Y-Axes on the signal list.
Bottom	Apply to the X-Axis for all signals.

Add Signal Manage the signal list.

Clear Clear the signal list.

All Signals Select all signals

[list of all available input signals]

[list of selected signals] Select an input signal to modify from the list, or 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”.

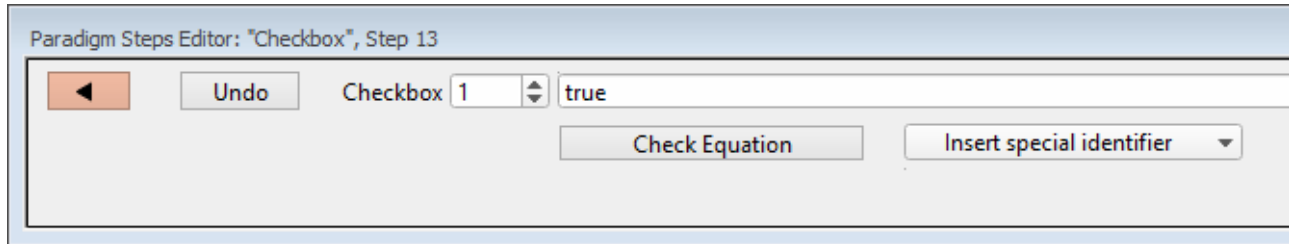


Figure 4-14. Step: Set Checkbox

Default Setting: *Checkbox, Count=1, Equation=true*



Close the ‘Paradigm Steps Editor’.

Undo

Removes any unsaved edits to this step.

Checkbox

Checkboxes [1 – 3] are local: they are cleared whenever a Paradigm is started.

Checkboxes [4 – 6] are global: they are not automatically cleared, so their status persists across all Paradigms in the Experiment.

[Equation field]

A free-form text field, evaluated to a value, and applied to the Checkbox.

[Errors are reported under this field.]

Check Equation

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

- 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.

Go to Preferences > Metadata to set the detail level.

Default Setting: *Metadata, Value=*

The “Set Metadata Paradigm Step Value” dialog opens.

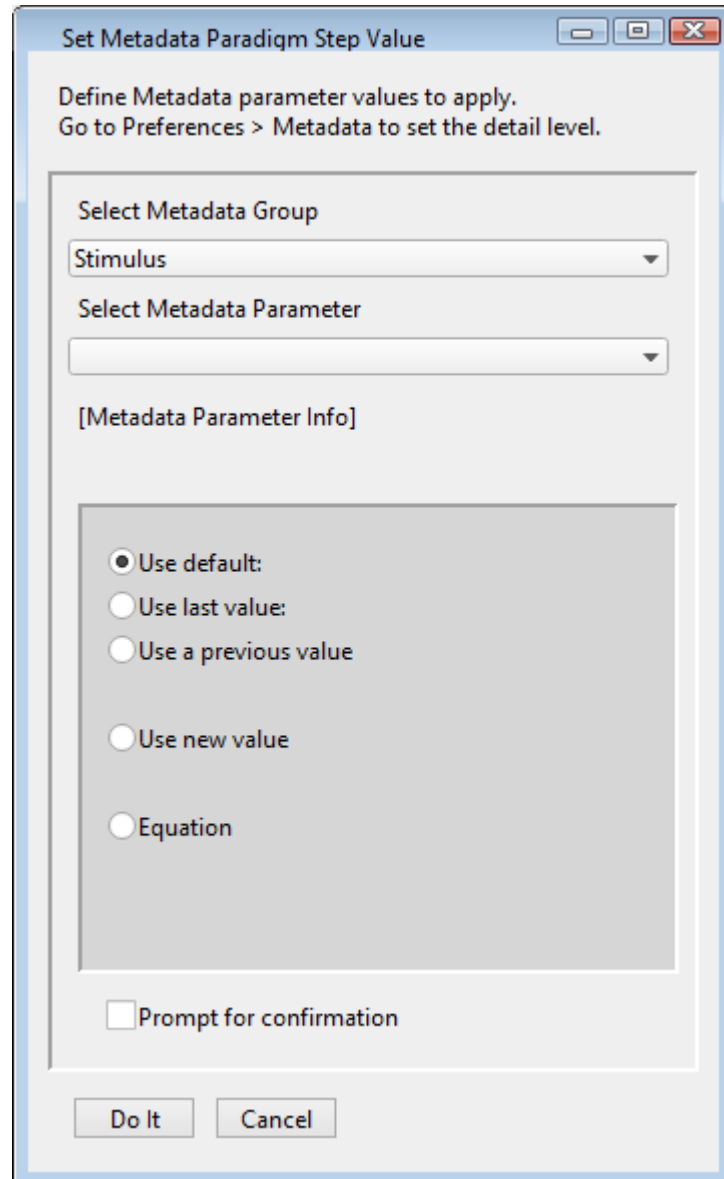


Figure 4-15. Step: Set Metadata

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.

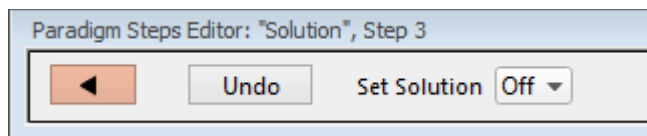


Figure 4-16. Step: Set Solution

Default Setting: *Solution, Target=Off*



Close the ‘Paradigm Steps Editor’.

Undo

Remove 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.

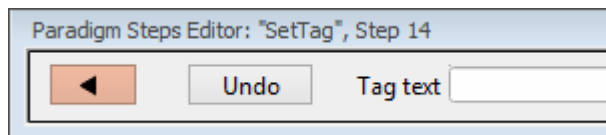


Figure 4-17. Step: Set Tag

Default Setting: *SetTag, Text=*



Close the ‘Paradigm Steps Editor’.

Undo

Remove 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.

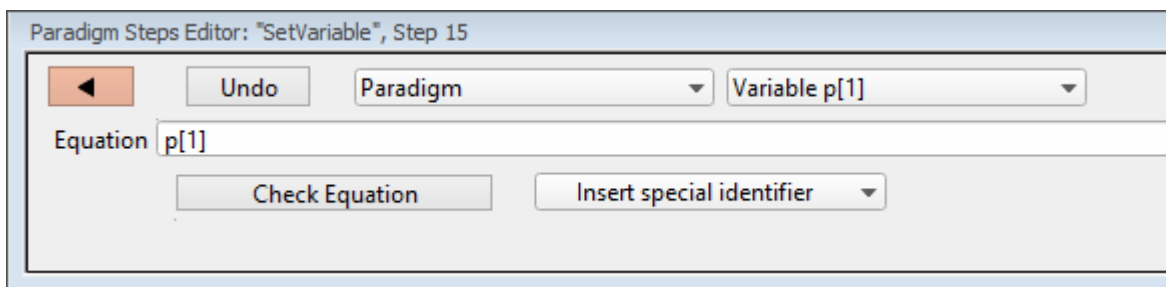


Figure 4-18. Step: Set Variable

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



Close the ‘Paradigm Steps Editor’.

Undo Remove 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 unused and 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.

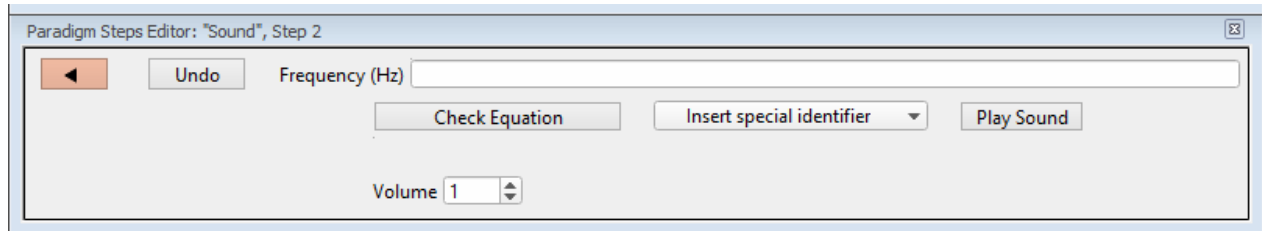


Figure 4-19. Step: Sound

Default Setting: *Sound, Equation=, Volume=1*



Close the 'Paradigm Steps Editor'.

Undo

Remove 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 last recording in a Scope (reanalysis) window.

Write to Log

Enter text to be written to the Log window.

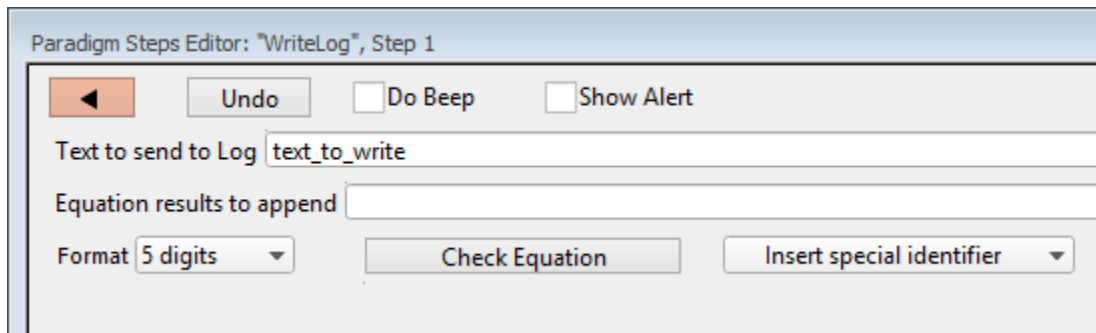


Figure 4-20. Step: Write to Log

Default Setting: *WriteLog, Alert=true, Text=text_to_write, Equation=, DoBeep*



Close the 'Paradigm Steps Editor'.

Undo	Remove 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 window.
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

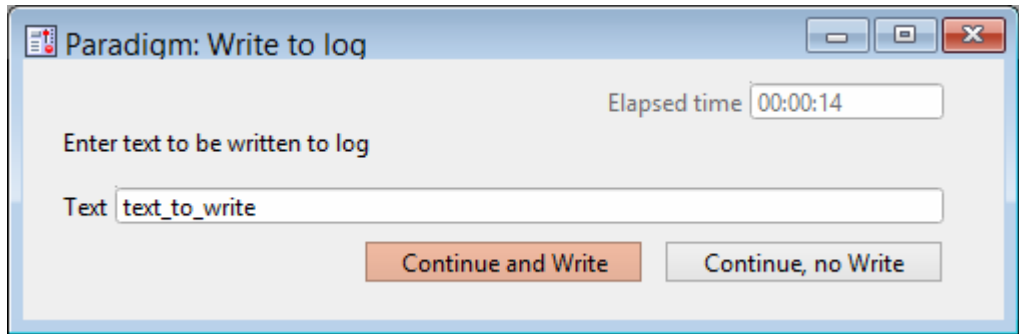


Figure 4-21. Write to Log Window

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

Alert

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

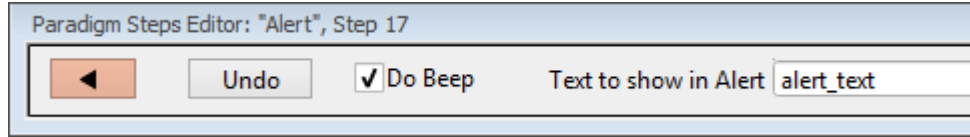



Figure 4-22. Step: Alert

Default Setting: *Alert, Text=alert_text, DoBeep=true*

-  Close the ‘Paradigm Steps Editor’.
- Undo Remove 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.

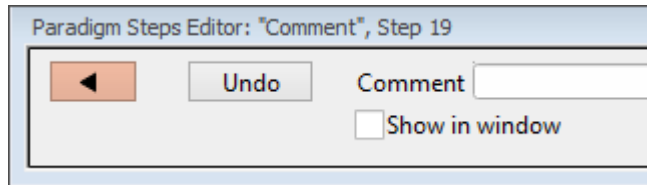



Figure 4-23. Step: Comment

Default Setting: *Comment, Text=*

-  Close the ‘Paradigm Steps Editor’.

Undo	Remove 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.

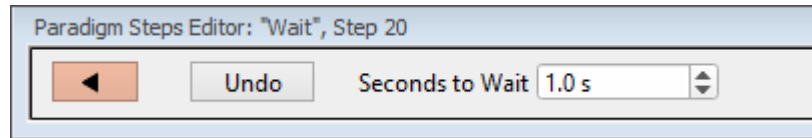


Figure 4-24. Step: Wait

Default Setting: *Wait, Time=1*



Close the 'Paradigm Steps Editor'.

Undo Remove 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.

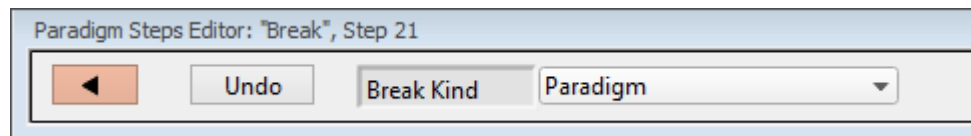


Figure 4-25. Step: Break

Default Setting: *Break, Kind=Paradigm*



Close the 'Paradigm Steps Editor'.

Undo

Remove any unsaved edits to this step.

Break Kind: Paradigm
ForLoop

Chain

Use to link step execution to another Paradigm.

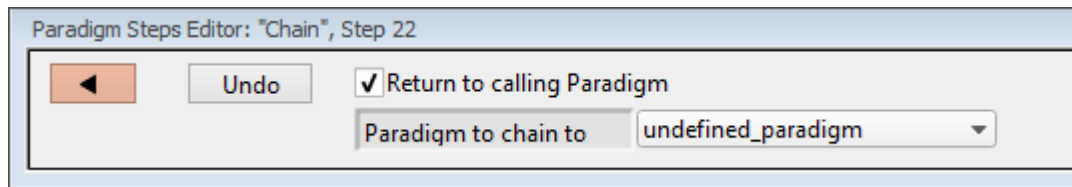


Figure 4-26. Step: Chain

Default Setting: *Chain, Target=undefined_Paradigm, Return=true*



Close the 'Paradigm Steps Editor'.

Undo

Remove 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.

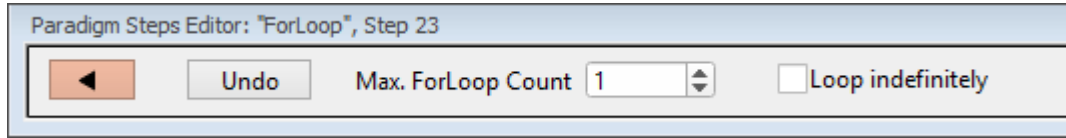


Figure 4-27. Step: For Loop

Default Setting: *ForLoop, Max=1*
ForEnd



Close the ‘Paradigm Steps Editor’.

Undo

Remove 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.

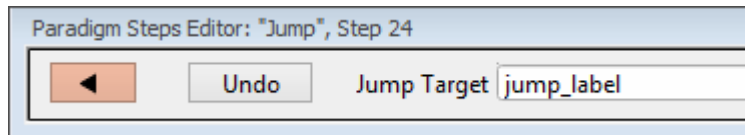


Figure 4-28. Step: Jump

Default Setting: *Jump, Target=jump_label*



Close the ‘Paradigm Steps Editor’.

Undo

Remove 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.

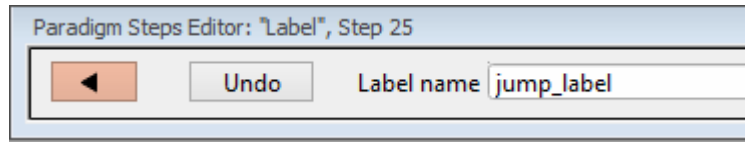


Figure 4-29. Step: Label

Default Setting: *Label, Target=jump_label*



Close the 'Paradigm Steps Editor'.

Undo Remove 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.

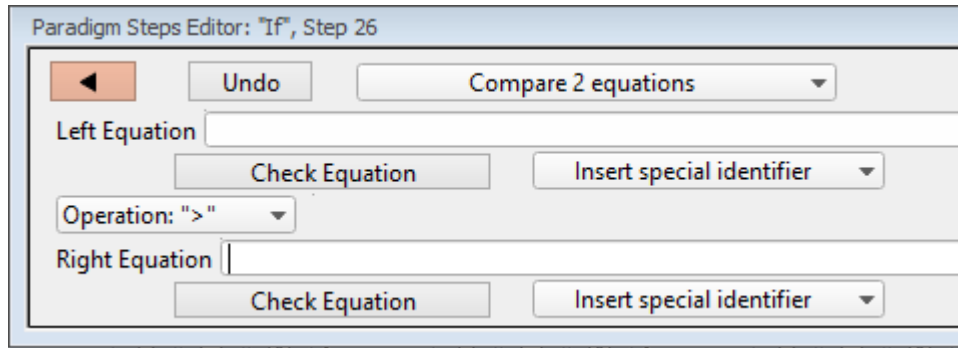


Figure 4-30. Step: If

Default Setting: *If, Left=, Operation=>, Right= EndIf*



Close the 'Paradigm Steps Editor'.

Undo Remove 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. 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
	Note: Be careful when comparing two floating-point numbers for equality, as minor variations in resolution can affect the outcome.
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 keyboard. The "Last key" field is cleared at the start of a Paradgim.
Key to check for	Enter a text key, or insert a "special" key.
Insert special key	Use a "non-text" key.
○ 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 are cleared at the start of a Paradigm.

[4 – 6]

Experiment-level “global” checkboxes persist across Paradigms for the entire Experiment.

- 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.

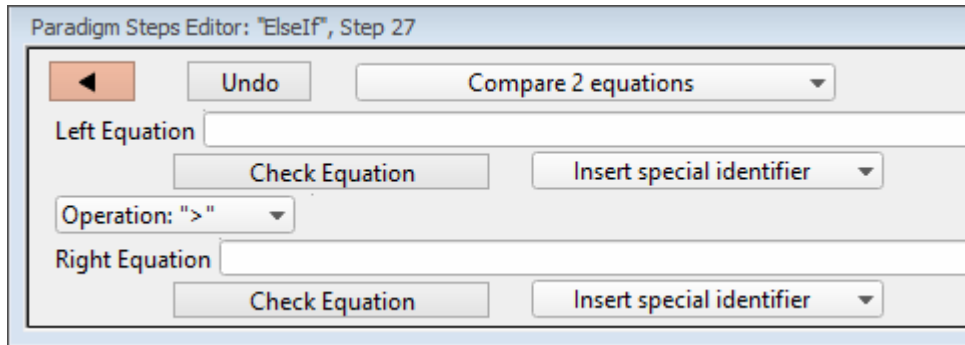



Figure 4-31. Step: Else If

Default Setting: *ElseIf, Left=, Operation=>, Right=*

 Close the 'Paradigm Steps Editor'.

Undo Remove 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

Note: Be careful when comparing two

		floating-point numbers for equality, as minor variations in resolution can affect the outcome.
	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.</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 bottom of the Paradigm Editor window, and are cleared at the start of a Paradigm.</p>
	Checkbox	
	[1 – 3]	Paradigm-level "local" checkboxes ..
	[4 – 6]	Experiment-level "global" checkboxes persist across Paradigms for the entire Experiment.
•	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.

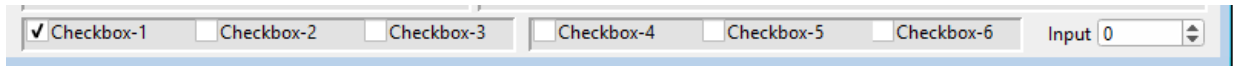


Figure 4-32. Checkboxes

[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.

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.

```
ForEachSweep
  EachSweep, Target=IV
  If, Left=sweep, Operation='=', Right=LastSweep- 1
  Alert, Text=LAST SWEEP, DoBeep=true
  EndIf
ForEachEnd
```

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]

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

IHold (amplifier holding current, A)

[± 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.

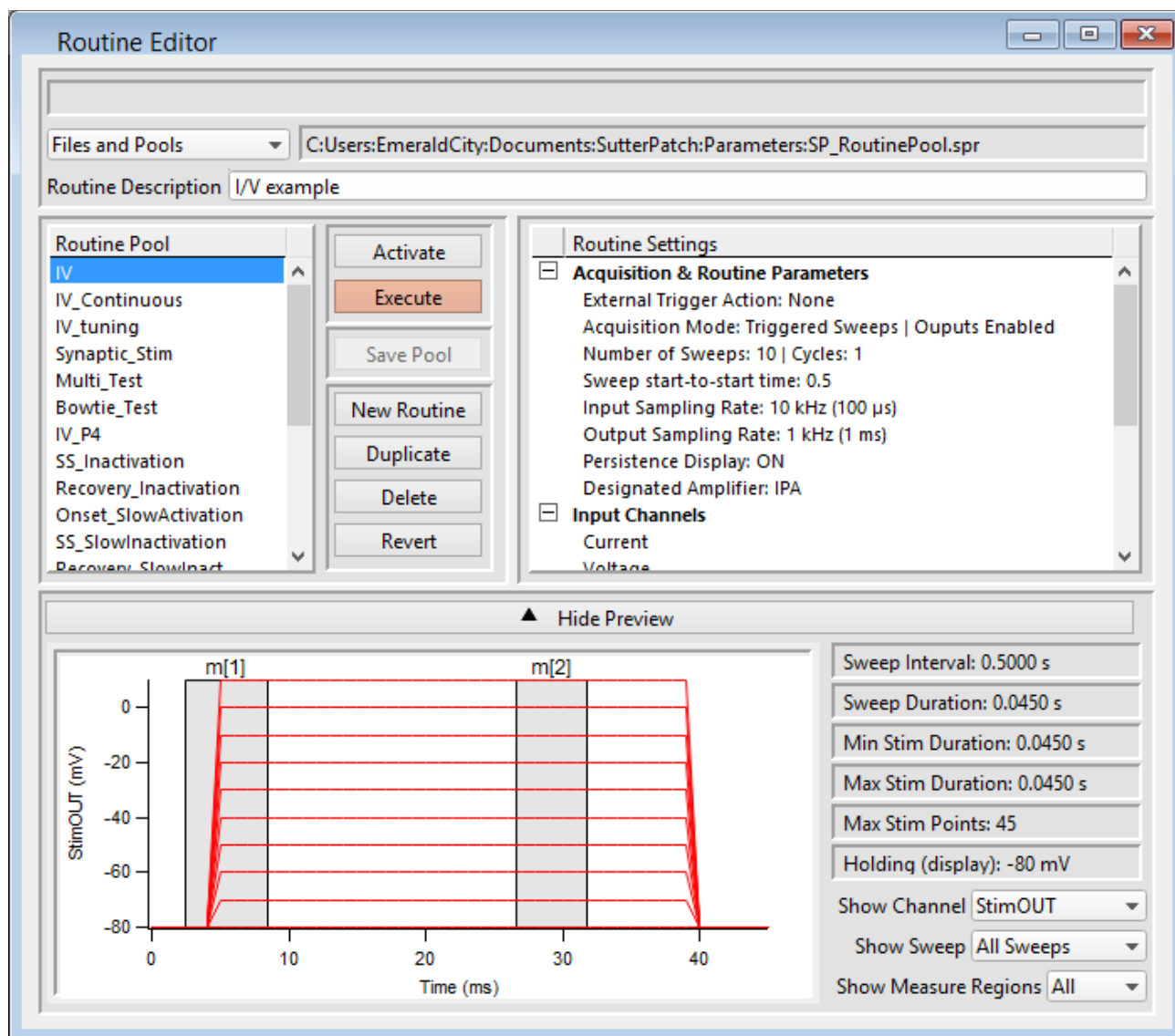


Figure 4-34. Routine Editor

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" (PGF Sequences) 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 History	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 dialog:

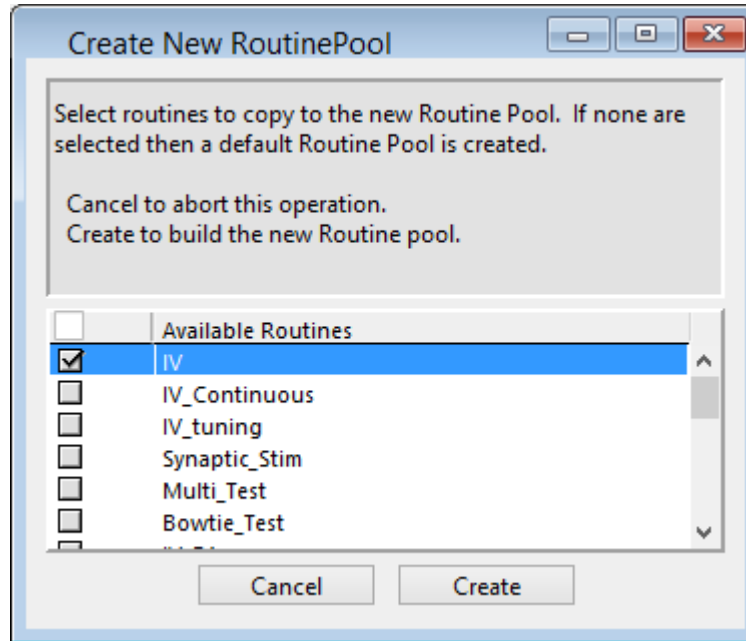


Figure 4-35. New Routine Pool

Create a new Routine Pool populated with a “blank” Routine, or select Routines from the loaded pool to populate the new 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.

To select multiple Routines, use a Shift-Click mouse drag, or individually Shift-Click the Routine names. Multiple Routines can thus be deleted, or saved to a new Routine Pool.

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	Open or refresh the Scope window with the latest Routine settings, but do not start acquisition. This button is renamed to "In Progress" during a recording.
Execute	Open or refresh the Scope window and immediately start recording. The latest Routine settings are applied to the Scope window. This button is renamed to "Convert" if a routine designed for a different amplifier type is selected.
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.

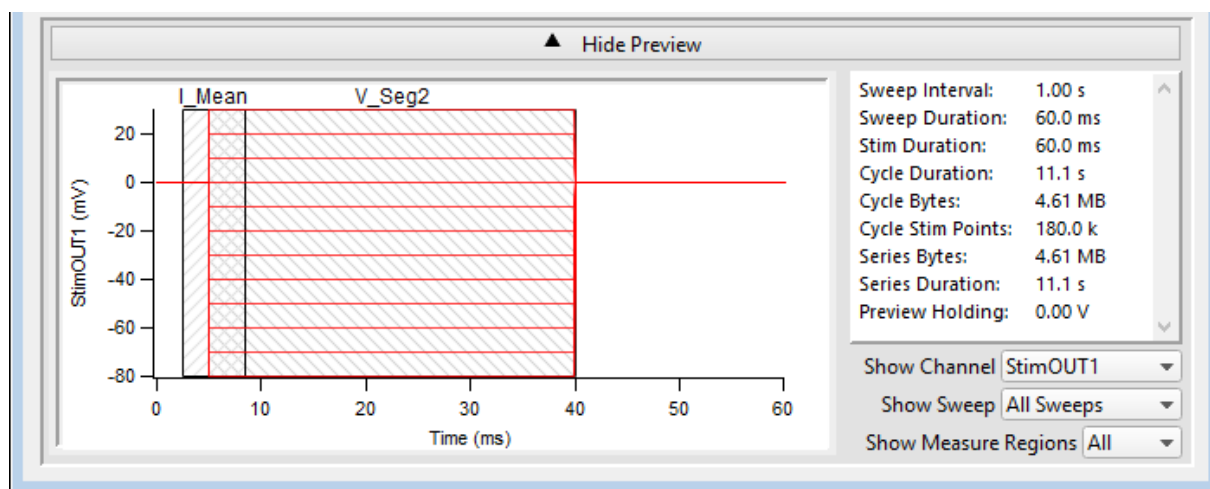


Figure 4-36. Waveform Preview Pane

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

Measurement cursors and regions can be manually dragged in the Preview pane, but only if Real Time Measurements & Graphs is the active section and the cursors' Region Timing is configured relative to 'Sweep Time'; cursors configured relative to 'Segment Time' are not draggable. If a measurement region is draggable, clicking on it will highlight it in black.

When you click-drag a measurement region to reposition it, its Region Timing 'Start/End Time' settings both update to maintain a fixed duration. To change the region's duration, click-drag the region's right-edge cursor; its Region Timing 'End Time' setting is updated, while its 'Start Time' is unchanged.

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).



Copy and paste the waveform preview into a Layout window. If no Layout window is open, a new one is created.



Copy the waveform preview to the system clipboard.

Show / Hide Preview Expand or collapse the Preview pane.

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.
Sweep Duration:	The amount of time in a sweep during which signal recording occurs with the active Routine.
Stim Duration:	The maximum amount of time during which output stimulation occurs in a sweep. Set in Output Channels & Waveform / Waveform Editor / Duration.
Cycle Duration:	The amount of time for a cycle. Set in Acquisition & Routine Parameters.
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 channels to preview.
Show Sweep:	Select how to display autoscaled sweeps traces in the preview pane. <ul style="list-style-type: none"> • Time Course Display all traces in continuous linear time. • All Sweeps Display all traces overlaid from time zero. (not available for “Show Channel: All Channels”) • Sweep # Display a trace from a single sweep.
Show Measure Regions:	A list of measurement regions to preview.

- 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.

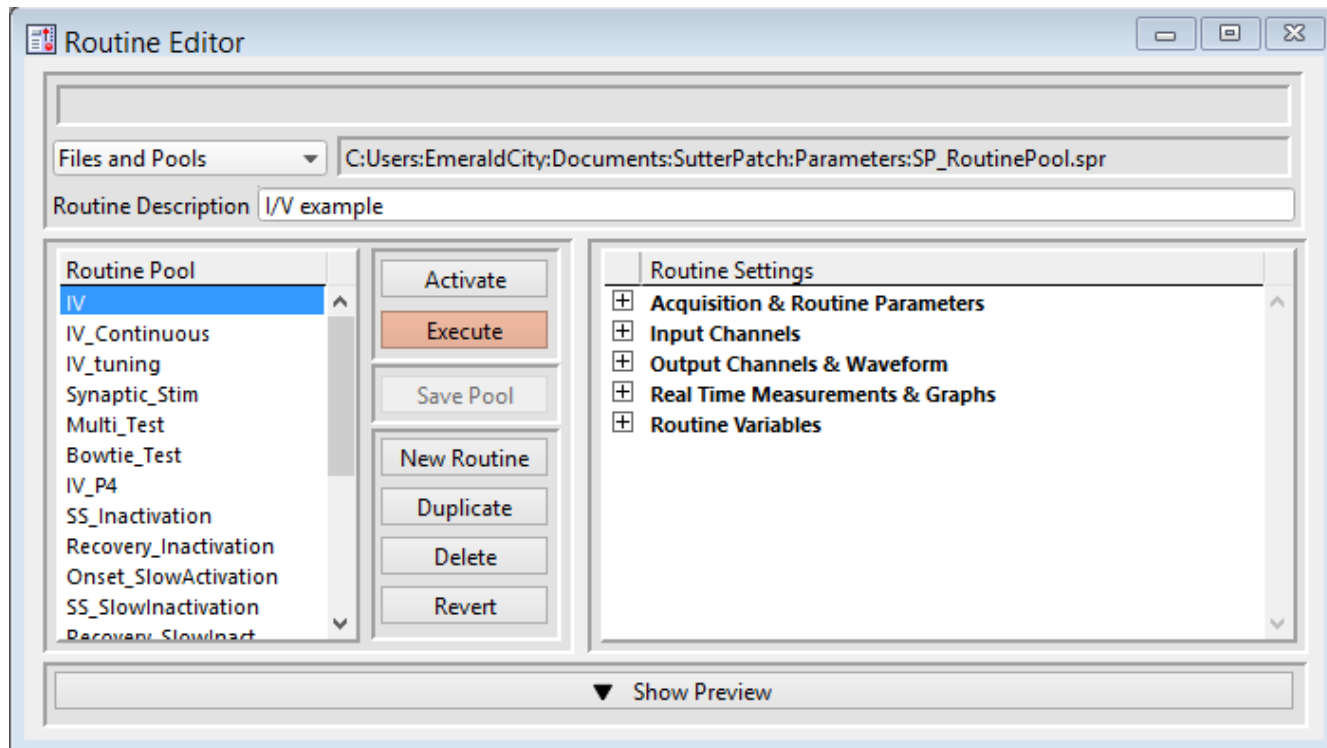


Figure 4-37. Routine Settings

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.

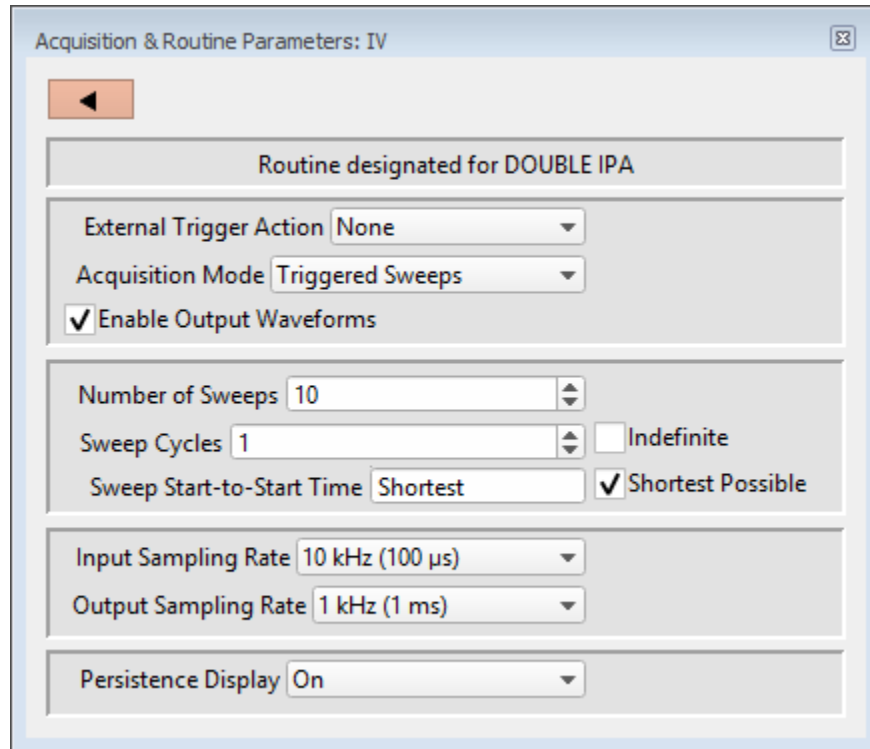


Figure 4-38. Acquisition & Routine Parameters

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 signal from other laboratory equipment to trigger the recording of each sweep in a Series.

Connect the external trigger signal to the '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 allow for system delays, there is a short gap (~100 ms) between sweeps. The resolution of the Sweep Start-to-Start time is 1 ms.

- **Continuous Sweeps:** Uninterrupted data without time gaps between sweeps are recorded when the ‘Sweep Start-to-Start Time’ is set equal to the ‘Sweep Duration’.

Data are displayed in sweeps, not in a continuous “rolling” display.

This option does not support:

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

Warning! High data throughput has the potential to overload system resources and interfere with data processing.

Note: The IPA demo mode display of continuous sweeps includes artificial gaps between them. Recording with hardware attached does not have any gaps

Enable Output Waveforms

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

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 recorded by a single Series.

Indefinite [for ‘Triggered Sweeps’]

The number of Sweep Cycles is undefined - sweeps continue to record until acquisition is stopped.

Indefinite Post Stimulation [for ‘Continuous Sweeps’]

After the designated number of Sweep Cycles is reached, the Output Waveform is disabled while Sweeps continue to record until acquisition is stopped.

The largest file size that SutterPatch can record is 2.5 G samples.

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 [for 'Triggered Sweeps']

The longest waveform duration in the Series + overhead processing time (100 ms).

Sweep Duration [for 'Continuous Sweeps']

The sweep duration is the longest waveform duration in the Series.

[Outputs enabled] As set in Output Channels & Waveform / Waveform Editor.

[Outputs disabled] As set in Input Channels / Edit Signals / Waveform Editor.

Note: In demo mode, the sweep start-to-start time can vary during acquisition, especially 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 and default 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 for more complex waveforms.

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.

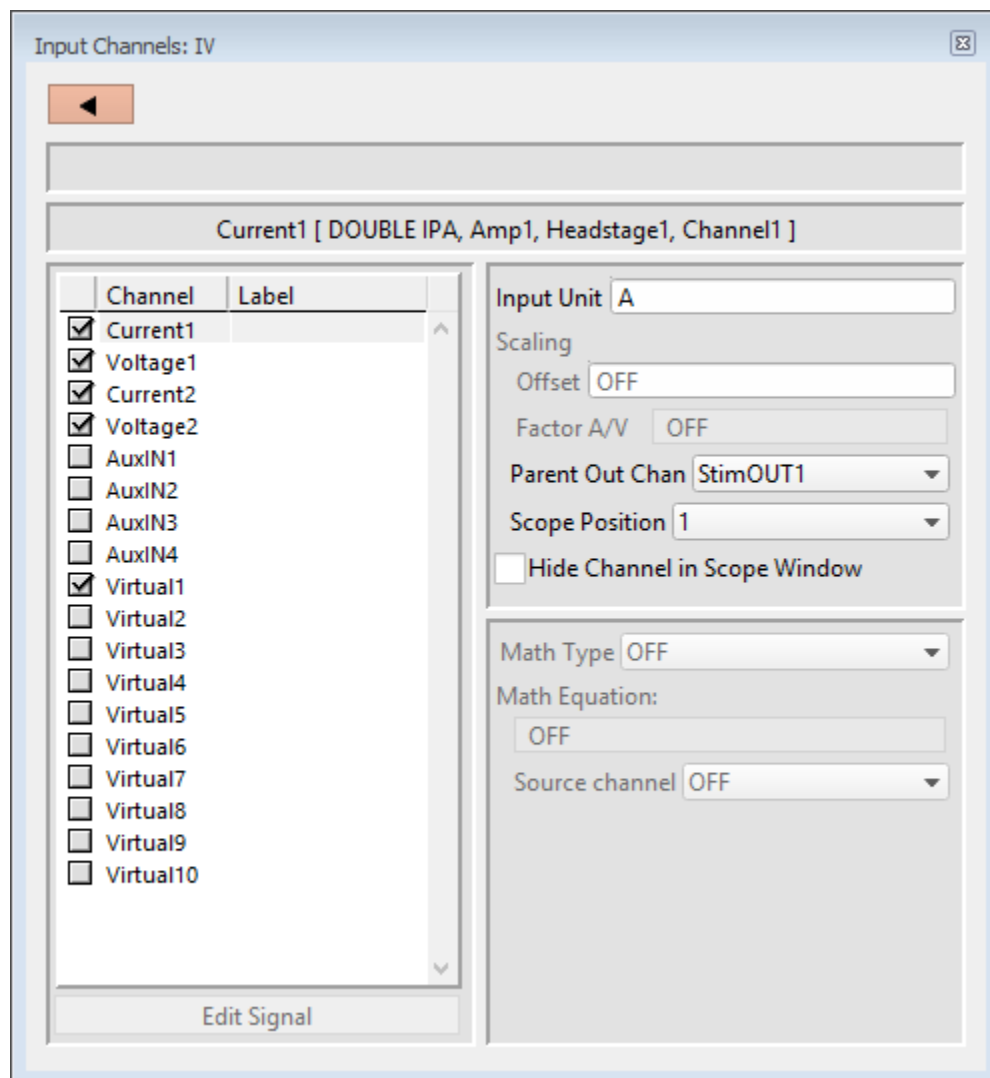


Figure 4-39. Input Channels

- [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.

When a virtual input channel is enabled, its default 'Math Type' label is automatically generated for it.

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 which input channel. The output channel timing is also used for measurements with ‘Cursors Relative to Segments’.

Auxiliary Input channels allow you to change the ‘Parent Out Chan’.

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

Virtual input channels allow you to perform a variety of mathematical transformations on input signals in real time. To enable a virtual signal, highlight a signal name. When 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 fixed value from all data points in an input trace. This is useful for adjusting for an offset, or resetting a baseline.

Source Channel Select an input channel to process.

Baseline From Select how to calculate the subtraction value.

- Value Subtract a fixed value.

Value Spinner adjusts in 1 pA or 1 mV increments.

- Trace Subtract the average of the entire input trace.

- Sweep Time Subtract the average of the data between the Start Time and End Time.

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.

Start Ratio Set the starting time of the data to be averaged, as a ratio relative to the starting time of the Segment duration.

Start Time [derived value]

End Ratio Set the ending time of the data to be averaged, as a ratio relative to the ending time of the Segment duration.

End Time [derived value]

- **BesselFilter** A frequency-domain filter with excellent response characteristics for preserving the shape of a biological signal.

- | | |
|--|---|
| Source Channel | Select an input channel to filter. |
| Filter Bandwidth | Select a frequency range. |
| <ul style="list-style-type: none"> • LowPass | Allow signal frequencies less than the cutoff frequency, and block all higher frequencies, such as high frequency noise. |
| <ul style="list-style-type: none"> • HighPass | Allow signal frequencies greater than the cutoff frequency, and block all lower frequencies. |
| Filter Order | [1, 2, 4, 8] |
| | Number of “poles” in the filter. A higher number provides a sharper (more accurate) response, but consumes more processing time and system resources. |
| Cutoff Frequency (Hz) | [100 to $< \frac{1}{2}$ the sampling rate] |
| | Restrict frequencies from this boundary point onwards. |
- Differentiate Apply differentiation to an input signal. The instantaneous rate of change in the signal is displayed.

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

- Equation Specify an equation to process an input signal.

Source Channel	Select an input channel to process.
----------------	-------------------------------------

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'th paradigm variable
- s[series-count, sweep-count, trace-count, routine name]

Access 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

Access the input trace (data wave) in Scope position “n” for the last sweep of the current Series. This numbering can differ from the Scope Position "n", if signals are re-arranged or hidden.

Tip: You can duplicate a trace by using this.

Undo All changes in the equation editing session are discarded.

(See the Equation Editor for more details.)

- Integrate Display the integral of the data signal. This is equivalent to the signed area under a curve.

Source Channel Select an input channel to integrate.

- Leak Remove leakage current from the data signal. This is the small passive current when the cell is in a resting state.

This feature is only enabled when the Routine includes an output channel with P/N Leak Pulse enabled.

Source Channel Select an input channel to process.

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 included in the leak current of the main signal.

- LineFreq Remove AC line frequency noise (hum) from the data signal.

Source Channel Select an input channel to process.

Line Frequency	50 Hz	Most of rest of world.
	60 Hz	Canada, (Carribbean), Central America, (Japan), Mexico, (South America), South Korea, Taiwan, USA.

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.

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

Calculations are made using ‘conductance’ (1 / resistance) instead of ‘resistance’.

Current Channel Select a (source) input channel with a “current: signal.

Trace Kind Select the LockIn measurement to display.

The selected ‘Trace Kind’ is automatically set as the Virtual Channel label.

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.

Cycles to Average [1 – 1000]

Cycles to Skip [1 – 1000]

V-reversal [±1000 mV]

When using a calculated stimulus trace, enter the reversal potential for the ion under study, such as for (Na⁺) sodium spikes or (K⁺) potassium tail currents.

Note: See the SutterPatch Algorithms Appendix for the math used in the LockIn computation.

- 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 sliding “window”.

Note: For best performance, only odd values are used.

- 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 average.

Average Type

Cumulative Average all processed sweeps together

Run Average Average the last “N” sweeps

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

Select a sweep to be 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.

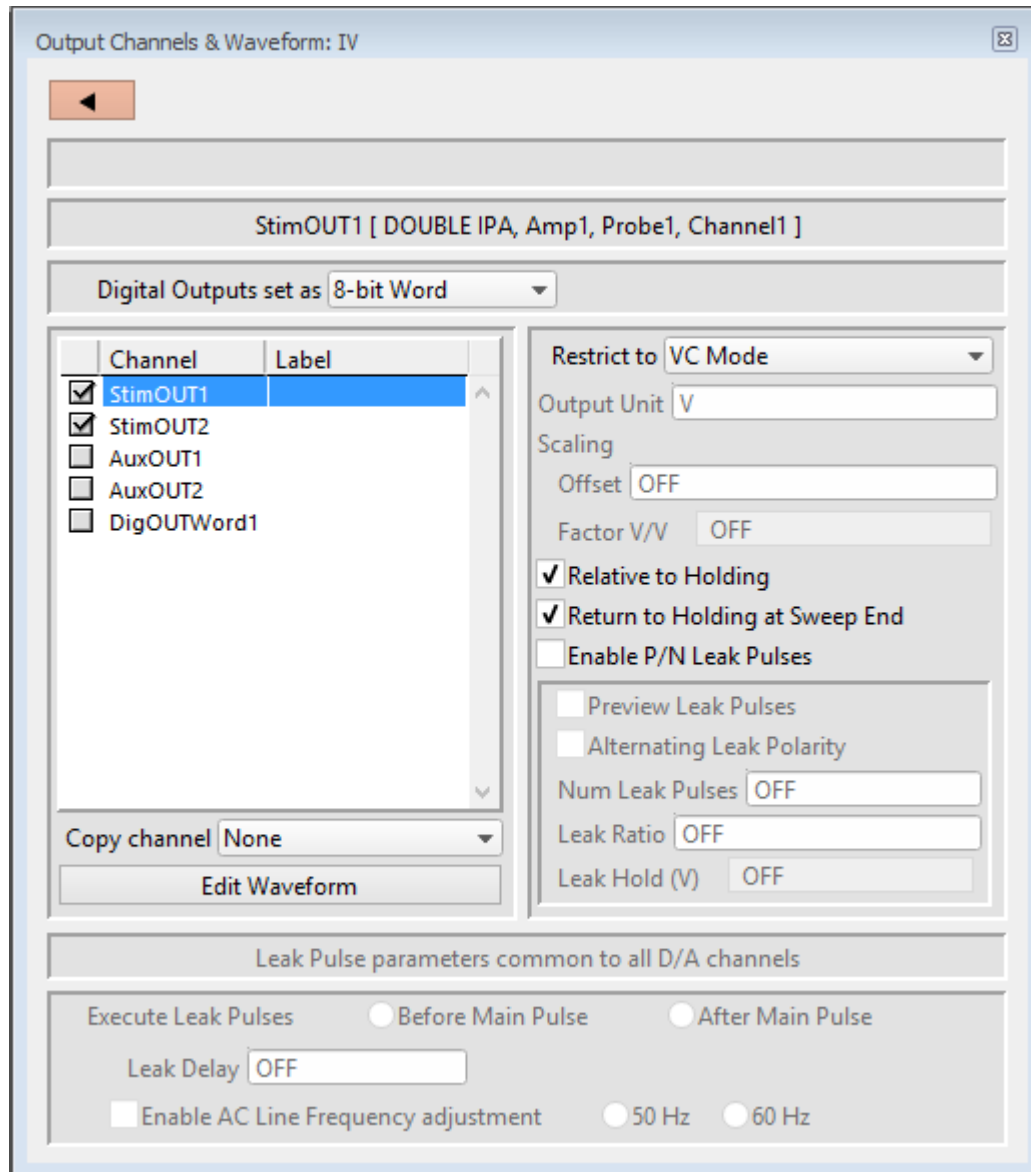


Figure 4-40. Output Channels & Waveform

[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's waveform to another channel, for 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 the '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 headstage 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.

For the Auxiliary channels, the command waveform is summed with the Amplifier Control Panel I/O Auxiliary Output settings.

For digital channels, the command waveform is relative to the Amplifier Control Panel I/O Digital Output settings.

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 Amplifier Control Panel holding level or I/O Auxiliary and Digital Output levels while the system is not acquiring data, i.e., the time between sweeps after a sweep acquisition ends, but before 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 level (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.

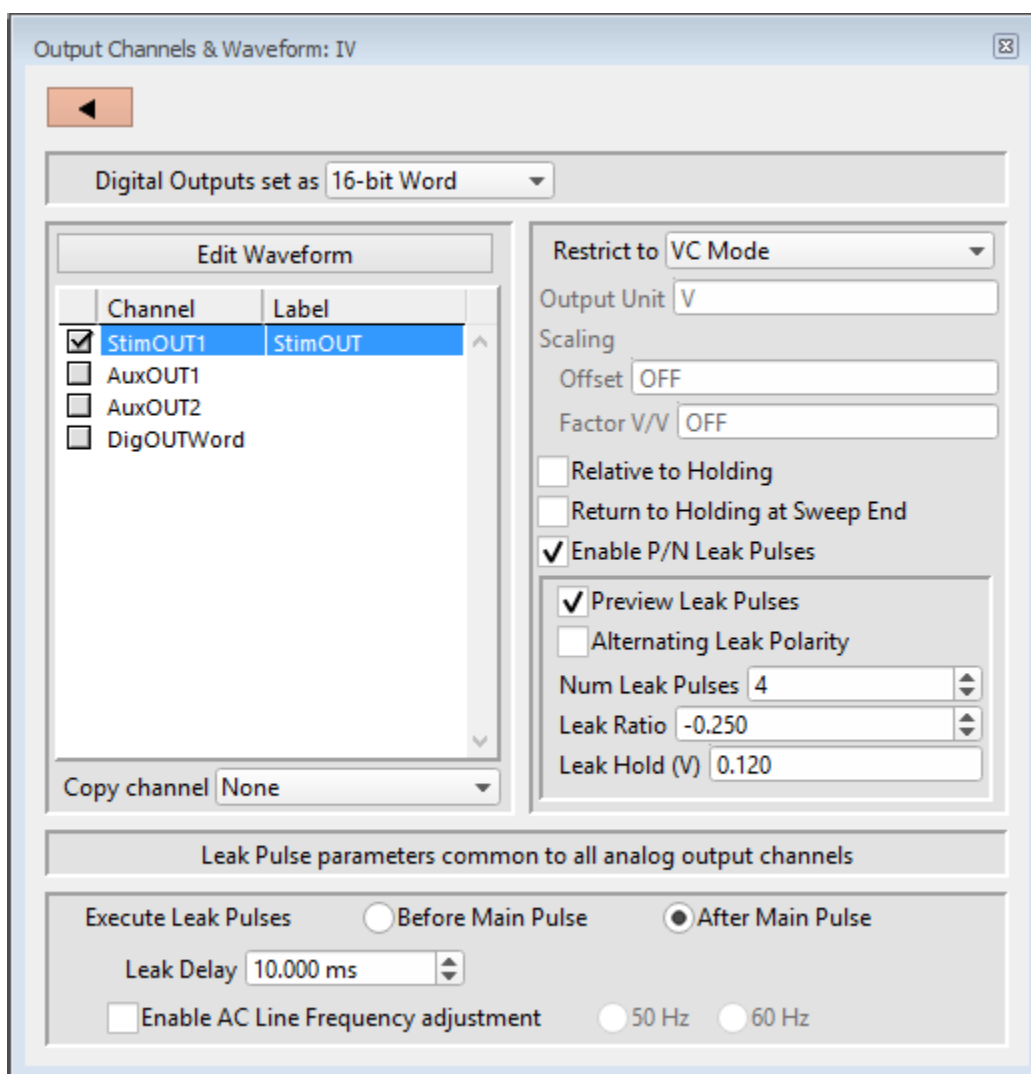


Figure 4-41. Output Channels & Waveform with Leak

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.

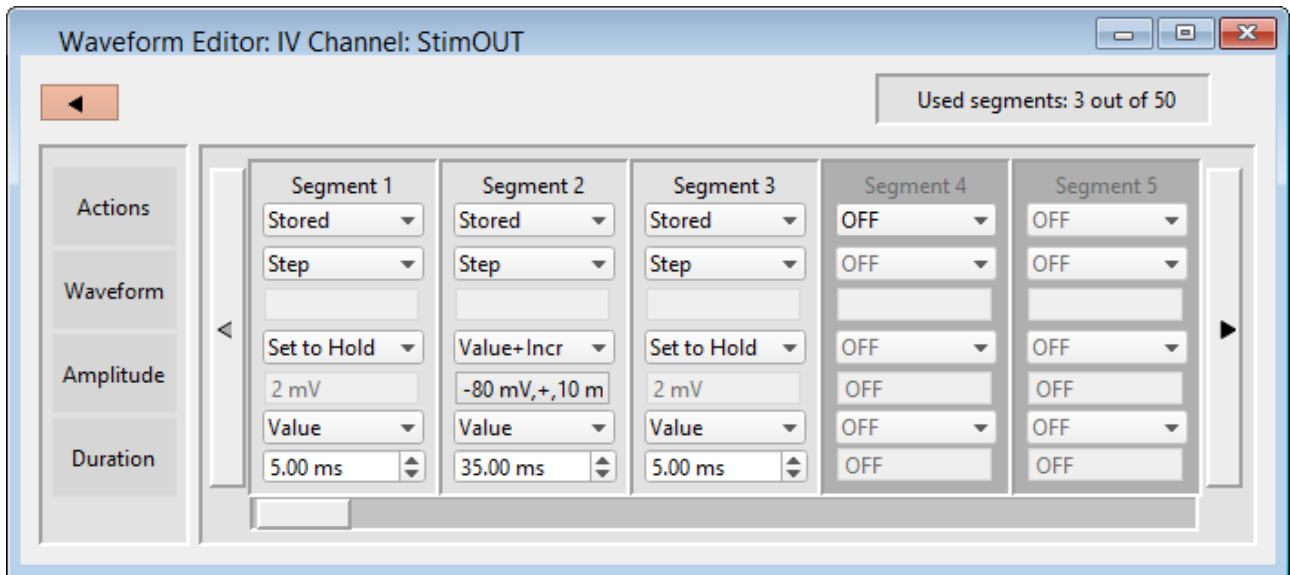


Figure 4-42. Waveform Editor

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	<p>Insert a default Segment into the current position, and increment the position of the following Segments, i.e. move them to the right.</p>
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	<p>Select the waveform shape.</p>
Step	<p>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.</p> <p>The first segment typically consists of a Step waveform set to the holding level amplitude (Set to Hold).</p>
Ramp	<p>A straight line connects the last point of the preceding segment (or holding level) to the last point of the</p>

current segment.

Sine

The waveform is a sinusoidal wave.

Sine Wave Cycles	Multiple	One or more cycles
	Single	One cycle, where the Cycle Duration is equal to the Segment Duration.
	For LockIn	For capacitance measurements in a virtual input channel. For multiple Sine Lock-In segments in a Routine waveform, use the same Cycle Amplitudes and Cycle Durations for each Segment.

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 successive pulse.

[± 0.75 V, ± 20.000 nA]

Step1 Amplitude Amplitude of first pulse.
Value [± 0.75 V, ± 20.000 nA]
Var_r[1 – 16]

Step1 Width Duration of first pulse (ms).
Value []
Var_r[1 – 16]

Step2 Amplitude Amplitude of second pulse.
Value [± 0.75 V, ± 20.000 nA]
Var_r[1 – 16]

Step2 Width Duration of second pulse (ms).
Value []
Var_r[1 – 16]

Segment Duration Squarewave 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 an arbitrary waveform to a segment.

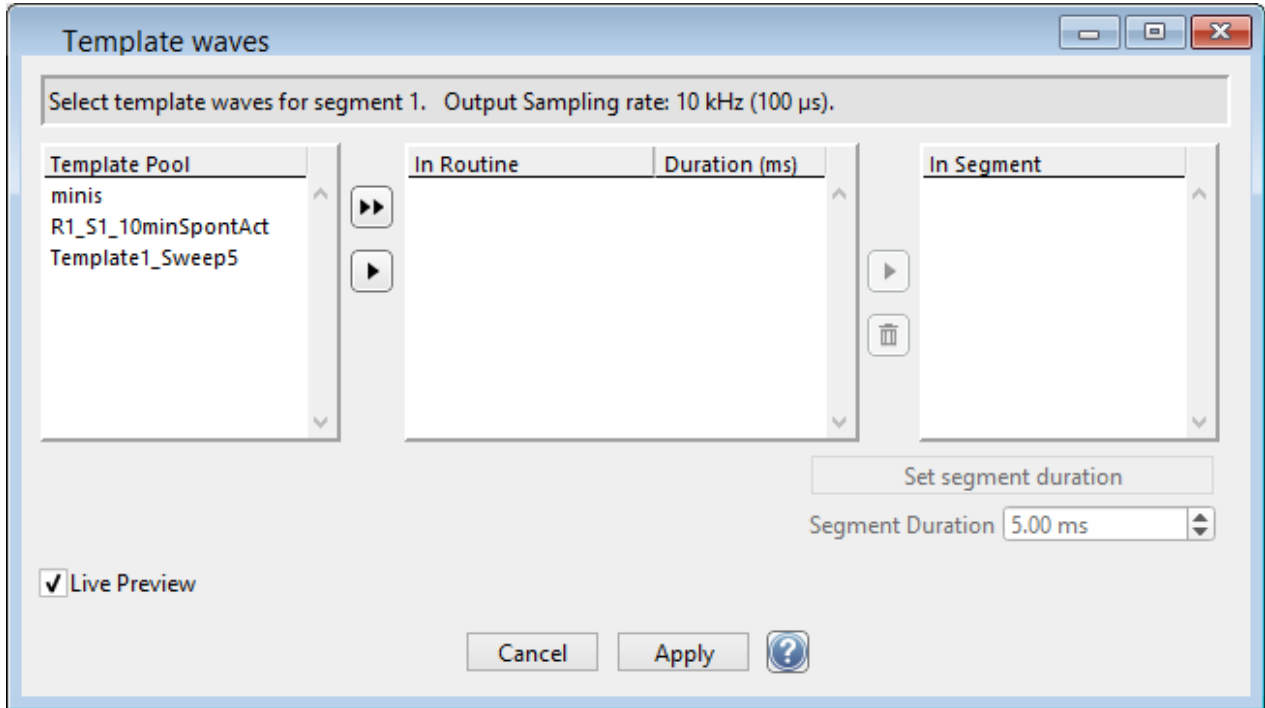


Figure 4-43. Template Waves

[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 data are 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.

Triangle	The waveform generates a train of triangular pulses.
Base Amplitude Increment	Increment the baseline amplitude for each successive pulse.
Peak Amplitude	Amplitude of the triangle pulse.
Ramp1 Width	Duration of the initial phase.
Ramp2 Width	Duration of the secondary phase.
Segment Duration	Duration of the triangle train.

Amplitude (*analog*) Set the waveform amplitude for a Segment.

For the Chirp, Sine, Squarewave and Triangle waveforms, this is used as a baseline offset.

Set to Hold Use the Amplifier Control Panel holding level for the Segment amplitude.

Tip: Record an initial baseline Segment to help interpret your data. Use in Segment 1.

For voltage-clamp experiments, records the leak current along with the actual holding voltage.

For current-clamp experiments, records 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.
	<ul style="list-style-type: none"> ▪ 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]
	When typing in a value, if no unit type is entered, the unit type defaults to seconds (s). If you enter a number followed by an ‘m’ or ‘ms’, the unit type is milliseconds (ms).
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.

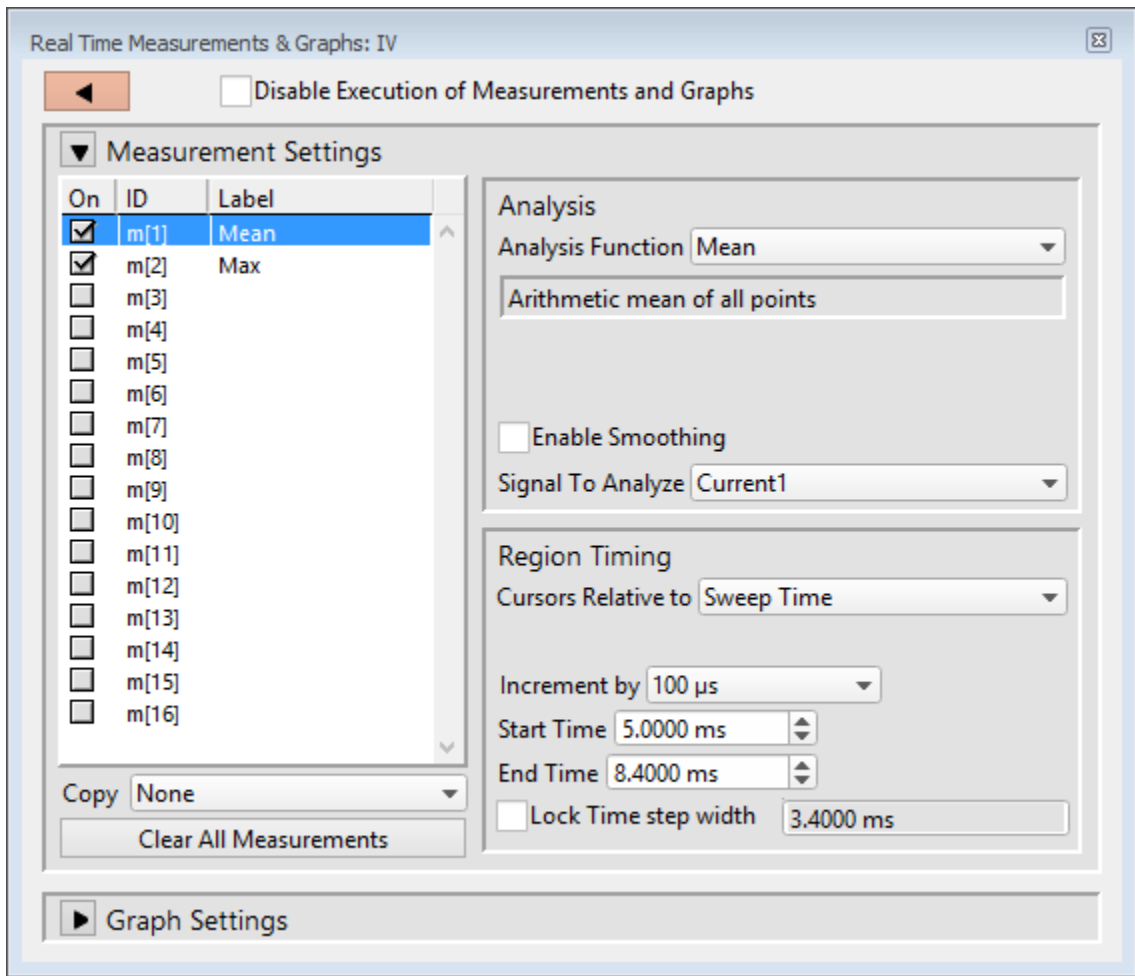


Figure 4-44. Real Time Measurement Settings

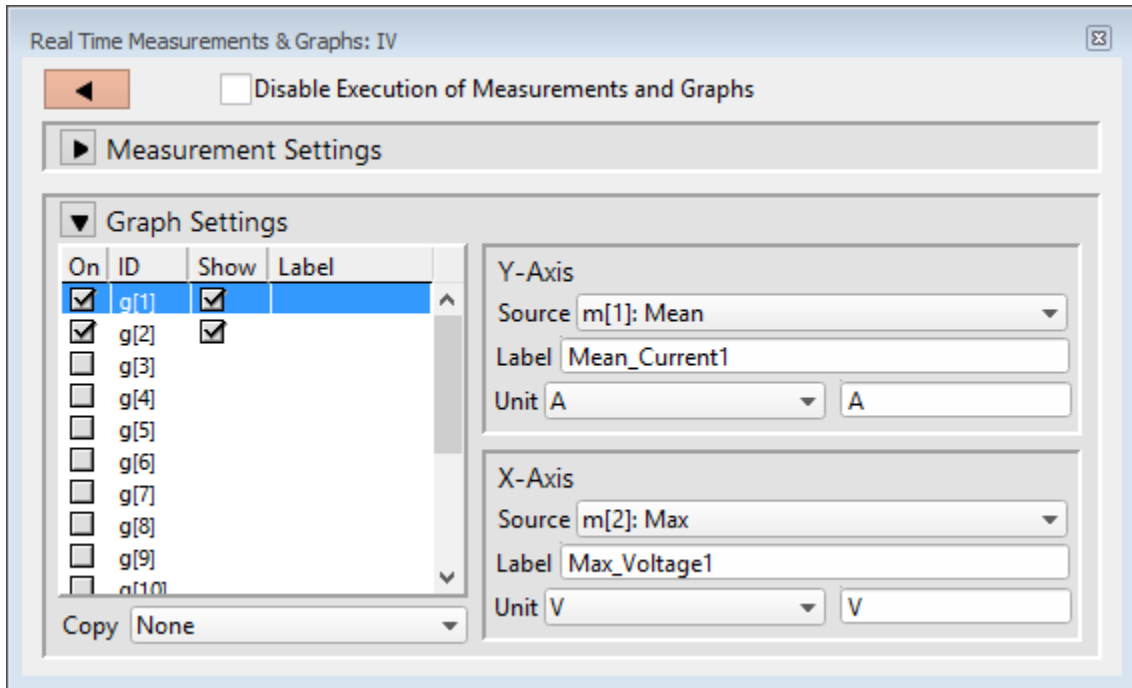


Figure 4-45. Real Time Graph Settings

Disable Execution of Measurements and Graphs

Block all measurements and analyses with one click.

Measurement Settings

On	Enable an analysis to run.
ID	Measurement regions are identified with an ID number: m[#] [1 – 16]
Label	These measurement labels display in the Waveform Preview and Scope windows, and can be used in equations. A Label name is automatically generated from the Analysis Function; double-click to manually edit.
Copy	Copy to the selected Measurement another Measurement's settings.

Clear All Measurements

All measurements and their settings are cleared.

Analysis

Analysis Function 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.)

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

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.	
	• Positive	Positive direction threshold crossing.
	• Negative	Negative direction threshold crossing.
	• Largest Change	Use the polarity direction of the largest change for Rise and Decay analyses.

Enable Smoothing

[2 – 200]

Set the number of Gaussian smoothing operations per measurement.

Smoothen noisy data to reduce the effects of high-frequency noise on measurements by averaging the data sample points with an unweighted sliding average.

Note: Smoothing is not applied to the analyses 'Segment duration' and 'Segment amplitude'. These are fixed values not subject to modification.

Signal to Analyze

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.

Region Timing

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

Note: Cursor Start times cannot be greater than their End times.

- Full Trace Encompasses the entire trace.
- Start Time Set the left cursor relative to the Trace start time (s).
- End Time Set the right cursor relative to the Trace start time (s).
- Sweep Time Set relative to the start time of a sweep (time zero).
- Increment by: []

When 'Lock Time step width' is enabled, the Start/End Time spinners increment by this amount.

The list of available values depends upon the input filter bandwidth.
- 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).
- Lock Time step width Fix the width of the measurement region.

The measurement width is maintained at a constant value when the cursor 'Start/End Time' is updated.

[] The width of the cursors in seconds.

The minimum width size is 2 sample points.
- Segment Time Set relative to the start time of a Segment. Uses the segment timing from the input signal's "Parent Output Channel".
- Out Channel [Output Channel list] [Segment #]
- Reset Ratios [0.0000] / [1.0000]

Reset the Start/End Ratios to span the entire segment.

Increment by:	[]	When 'Lock Time step width' is enabled, the Start/End Ratio 'Time' spinners increment by this amount.
		The list of available values depends upon the input filter bandwidth
Start Ratio	[0 = beginning of Segment]	Set the left cursor as a ratio of the Segment duration
Time	[0.0000 s]	Cursor start-time field.
End Ratio	[1 = end of Segment]	Set the right cursor as a ratio of the Segment duration.
Time	[1.0000 s]	Cursor end-time read-only field.
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.	
Lock Time step width	Fix the relative width of the measurement region.	
	The measurement width is maintained at a constant ratio value when Start Ratio 'Time' is updated.	
	[]	The width of the cursors in seconds. The minimum width size is 2 sample points.

Graphs

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 to manually enter a graph label for the Analysis.
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.

Y-Axis

Select a measurement ID to plot against the graph Y-Axis, or select a Y-Equation for a customized Y-axis plot.

Source Select a Measurement, or enter an equation
(see the Equation Editor for more details.)

Label Enter a customized name for the graph Y-Axis.

Equation Click to edit with the Equation Editor
(see the Equation Editor for more details.)

For example, to monitor cell compensation, use the Special ID 'RsComp'. Then track cell changes sweep-by-sweep by running a planned Paradigm with a Routine step inside a 'For Each' step.

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.

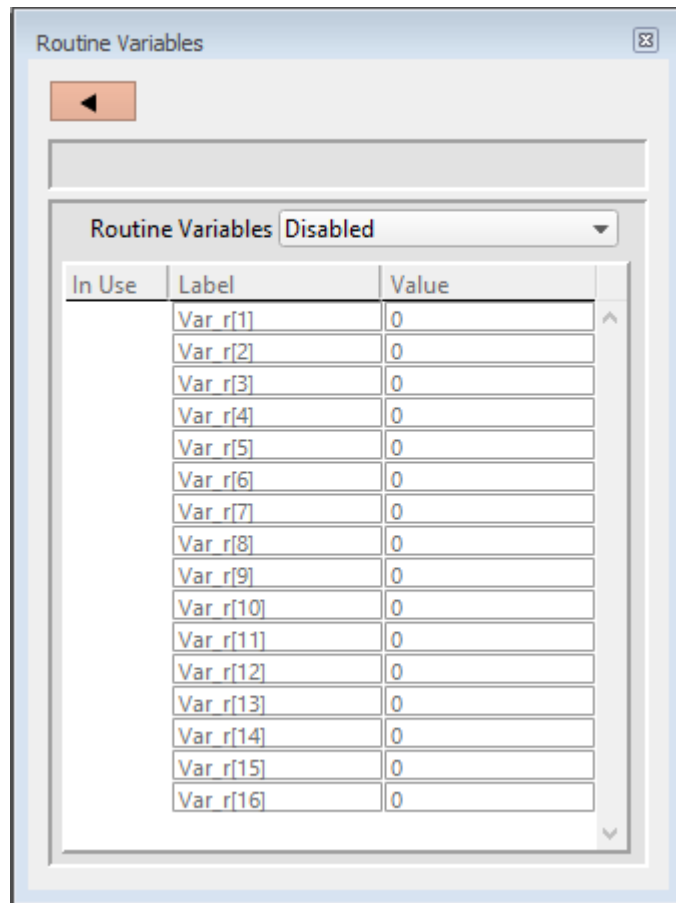


Figure 4-46. Routine Variables

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 (Acquisition)

This dialog can be used to make real-time changes, even during data acquisition, to the online measurements and graphs. Edits instantly override the loaded Routine settings for fast response.

When the Measurements dialog is closed, if parameter changes were made, an 'Update Measurements' dialog displays to save or discard those changes in the Routine.

Otherwise, this Scope (Acquisition) version of the dialog is very similar to the Routine Editor version,

To access this dialog, click on the Scope (Acquisition) window button 'Measurements / Edit Measurements'.

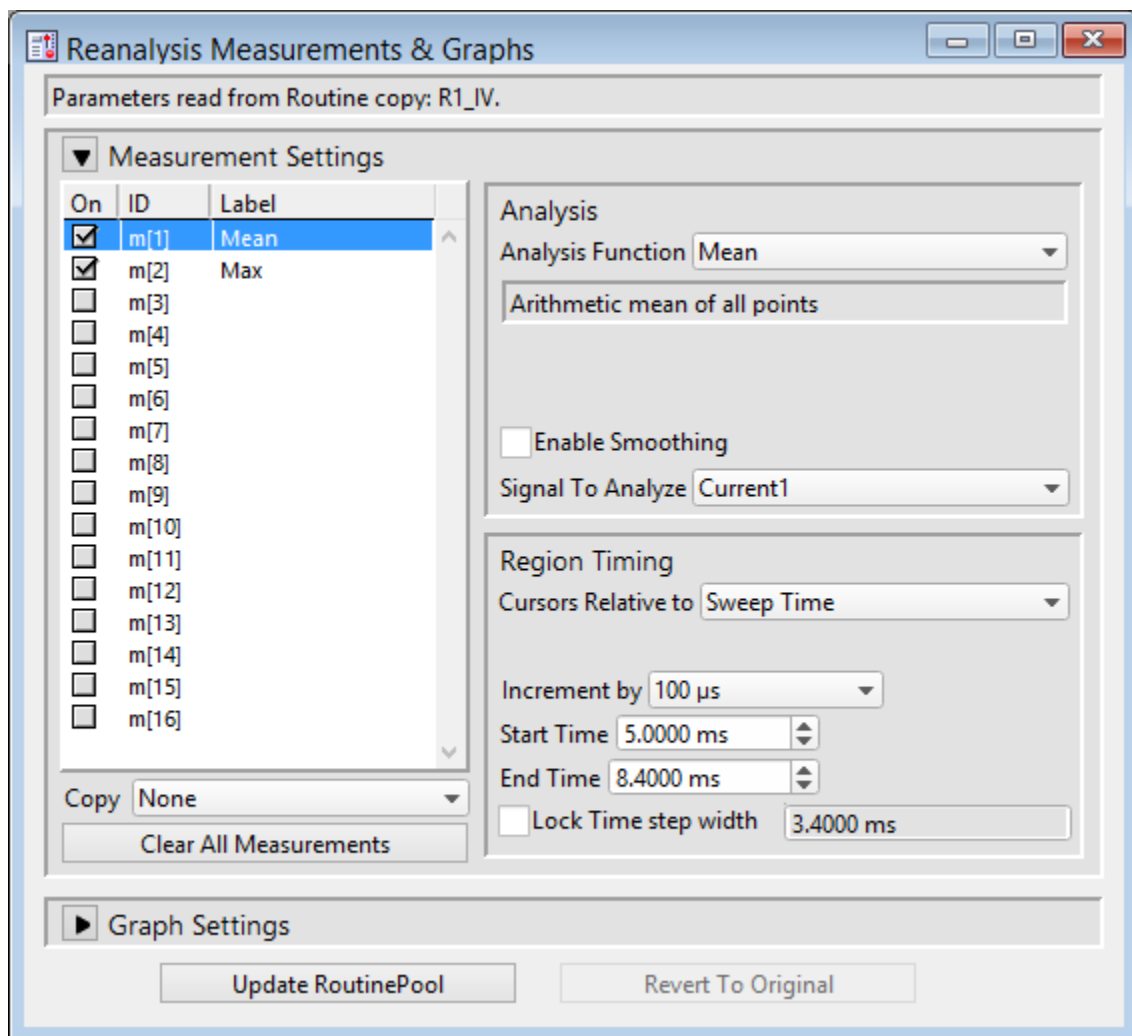


Figure 4-47. Reanalysis Measurement Settings (Acquisition)

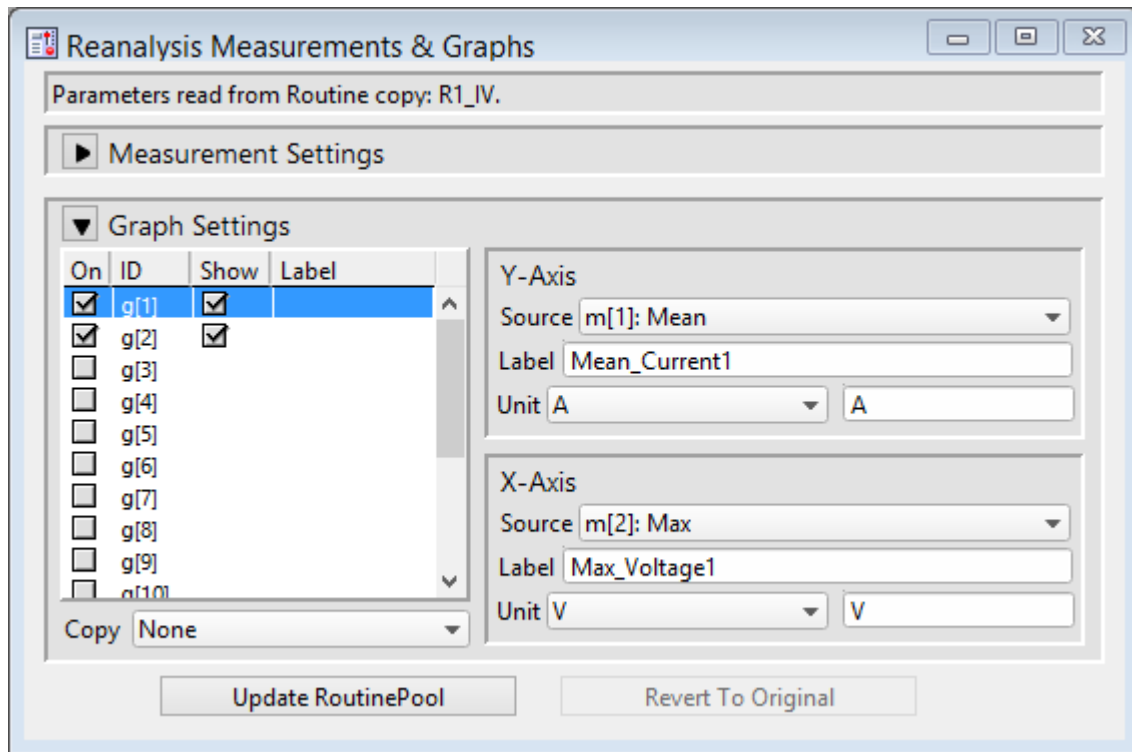


Figure 4-48. Reanalysis Graph Settings

Otherwise, this Scope version of the Real Time Measurements dialog is very similar to the Routine Editor version.

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.

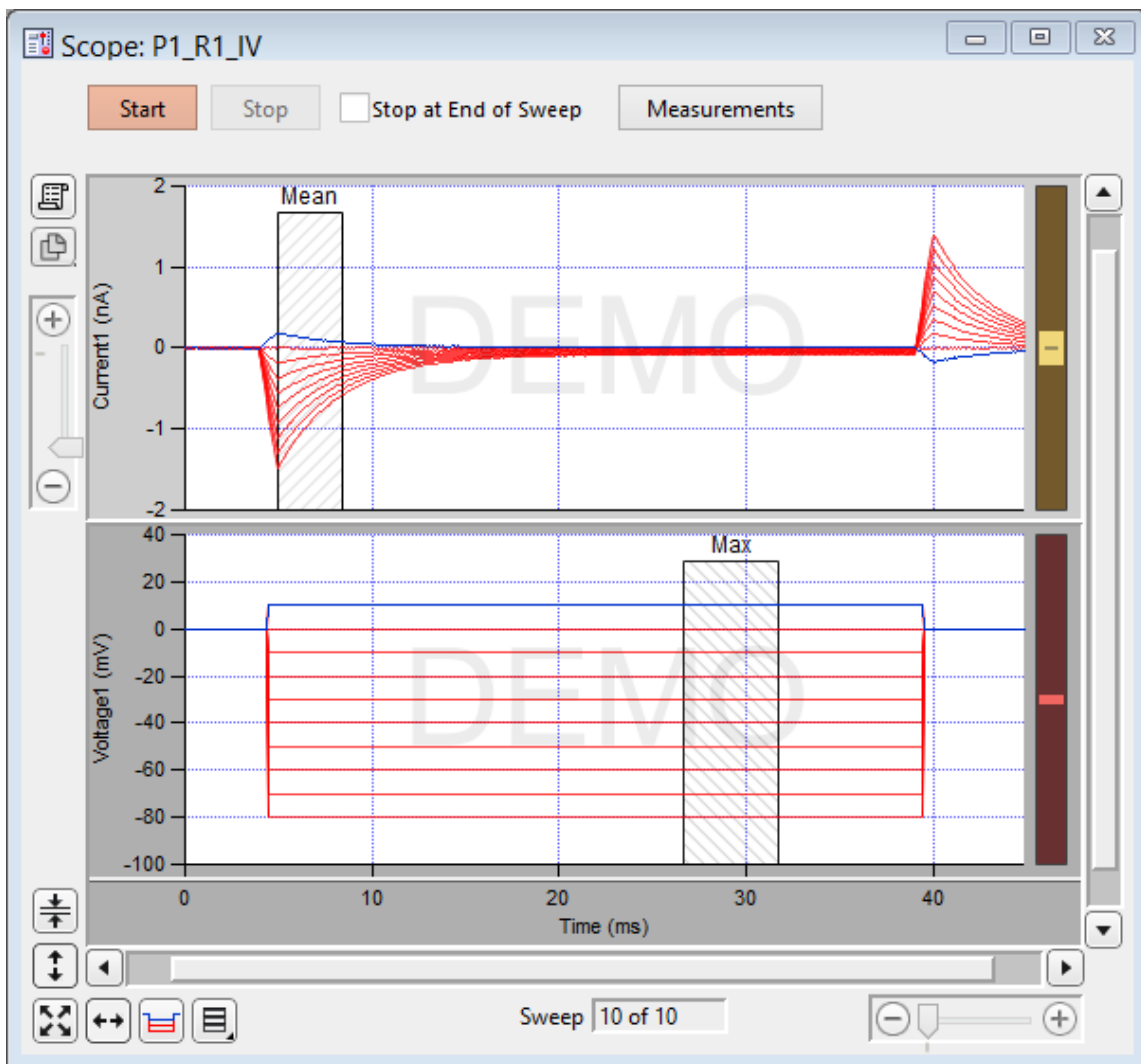


Figure 4-49 Scope (Acquisition) Window

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

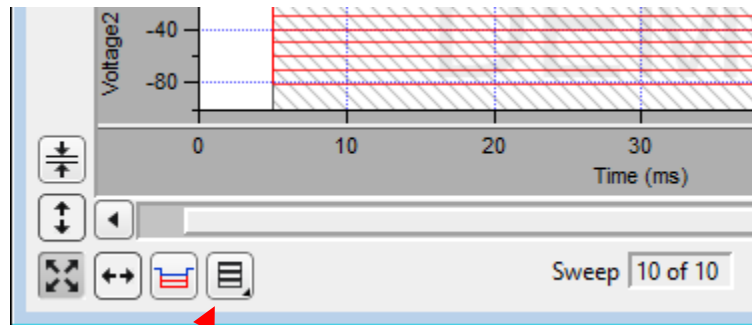



Figure 4-50. Signal Layout

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
- [m x n] Tiled configuration of signals with 'm' rows and 'n' 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

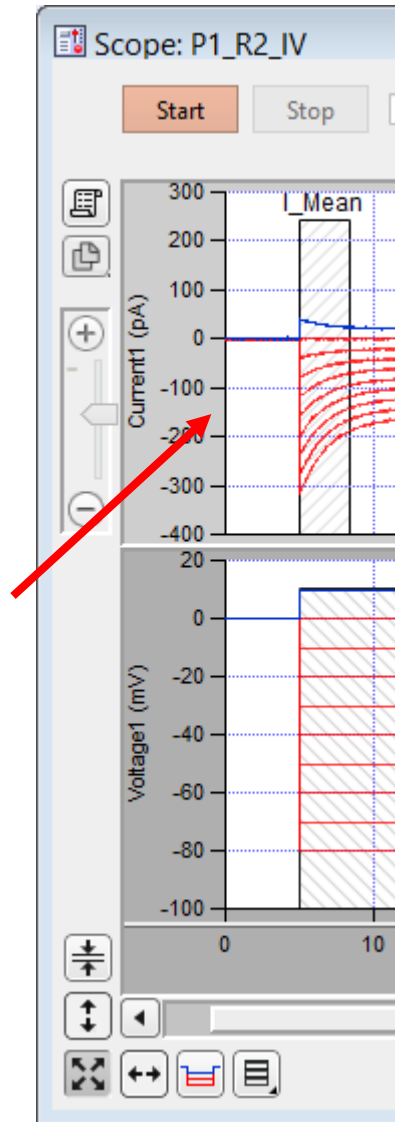


Figure 4-51. Axis Magnification

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 except 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

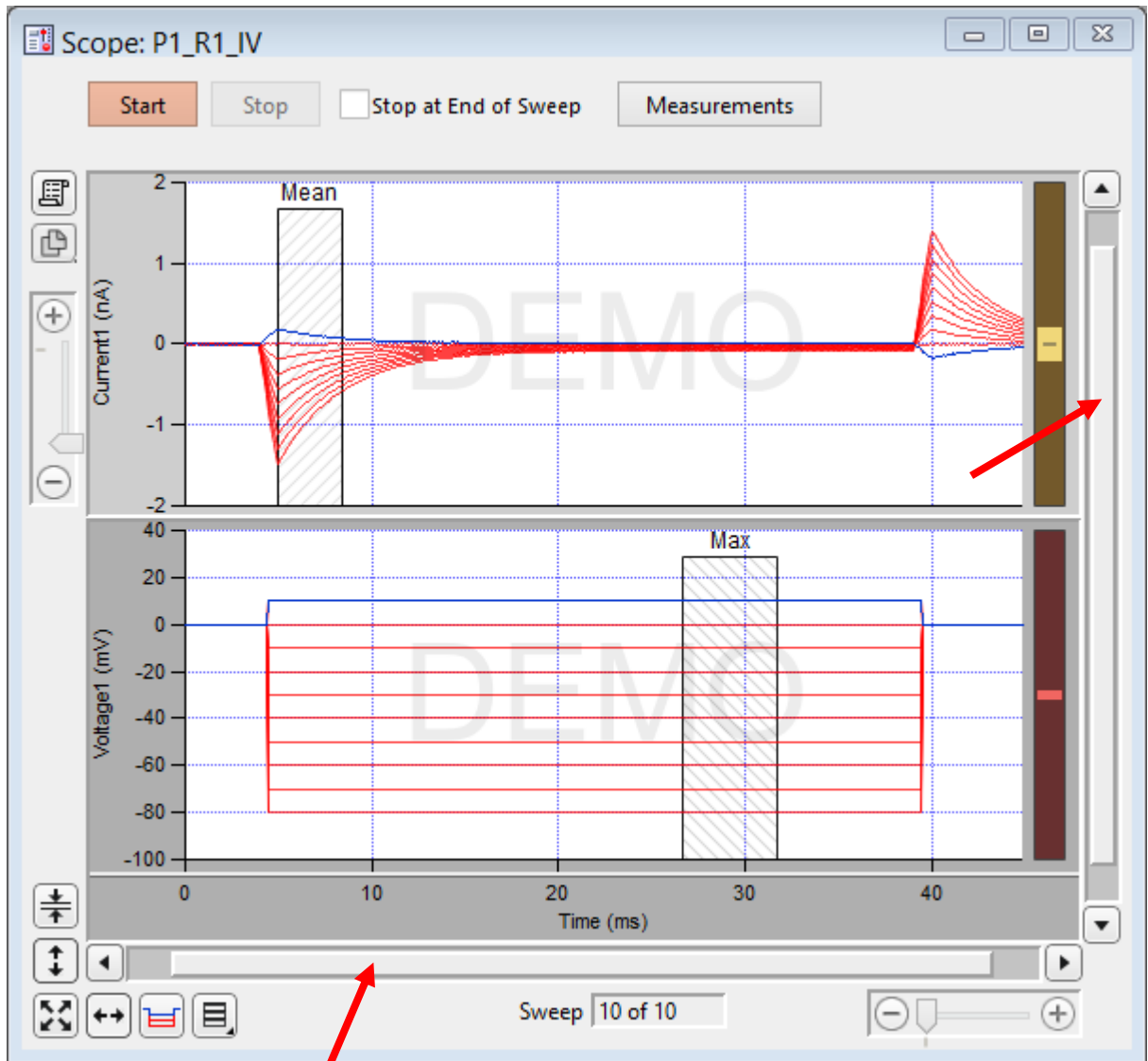


Figure 4-52. Axis Scroll Bars

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:

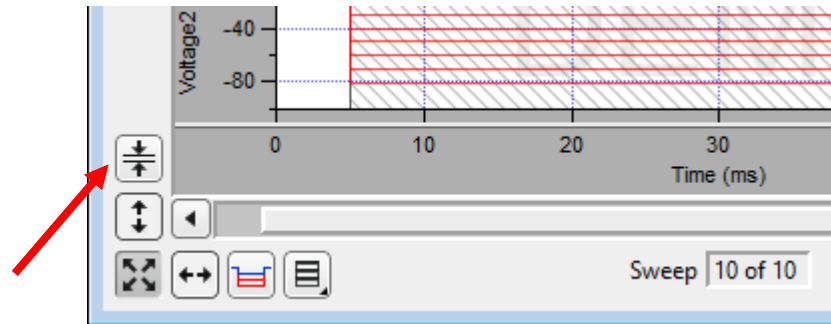


Figure 4-53. Center Button

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

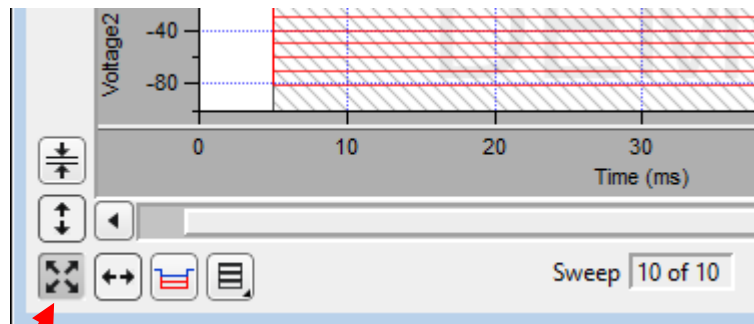


Figure 4-54. Autoscale Axes



Click to instantly autoscale 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, right-click the button in Windows, or Control-click in macOS - the continuous Autoscale button remains depressed in this state.



Click to autoscale the Y-axis of the selected signal.



Click to full-scale all X-axes.

for

- Amplitude Meters

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.

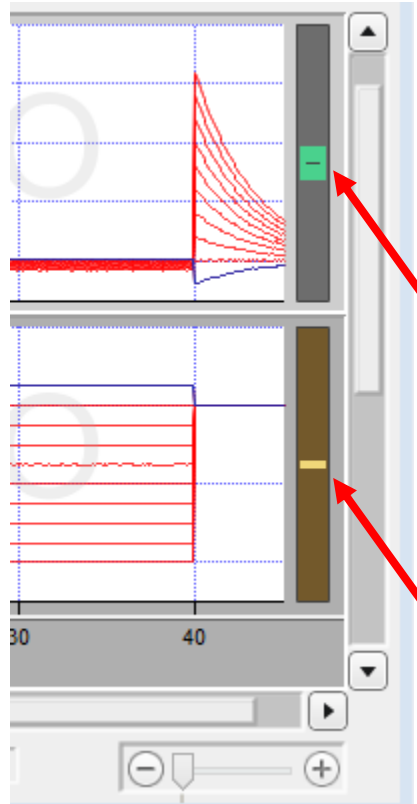


Figure 4-55. Amplitude Meters

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 style="text-align: center;">Start</p>	<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 style="text-align: center;">Stop</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>
<p><input type="checkbox"/> Stop at End of Sweep</p>	<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 style="text-align: center;">Measurements</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>

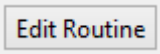



	Open the Routine Editor dialog, for configuration, management and activation of Routines. Routine configuration includes: Data acquisition parameters Input channels Output channels Stimulus waveforms Analysis measurements
Layout 	Copy and paste all visible Scope signals and analyses into a Layout window. If no Layout window is open, a new one is created.
Copy to Clipboard 	Copy the Scope window to the system clipboard.
Persistence Display 	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.
Sweep #:	The active sweep number vs. the total 'Number of Sweeps'; if multiple cycles are set, the active sweep cycle number is inserted between these.

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

Note: IPA amplifiers record both stimulus and response signals via physical analog channels, therefore there are no timing delays between the recorded signals.

4.1.8 Solution Editor

Control perfusion systems with “named” solution control settings.

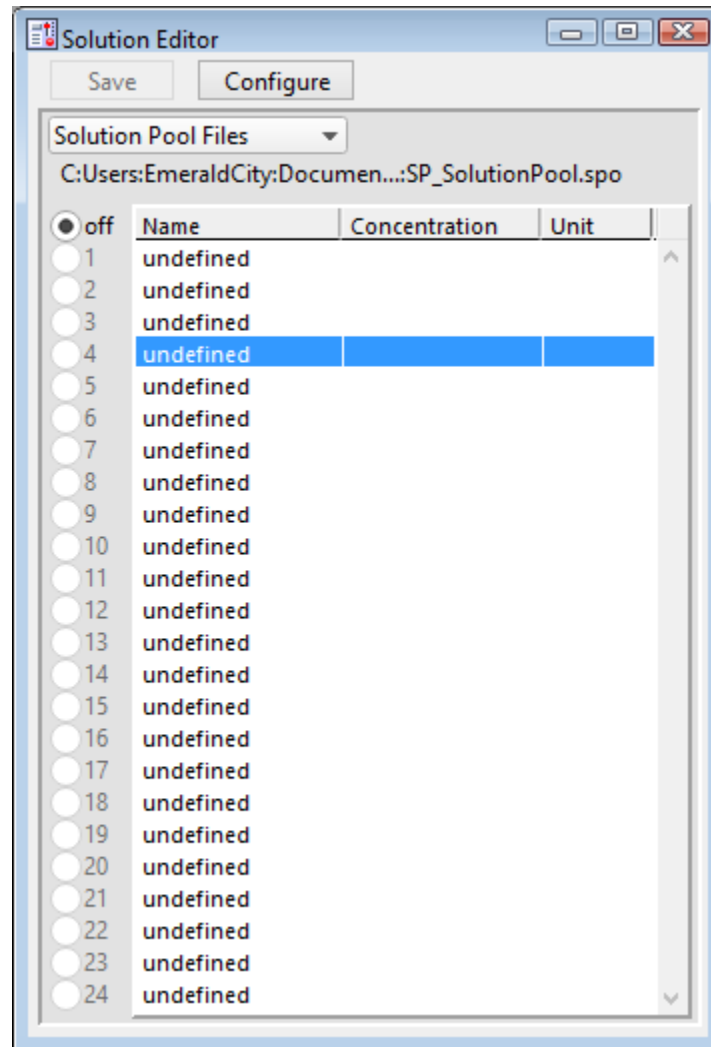


Figure 4-56. Solution Editor

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 “high”

4.1.9 Template Editor

Manipulate and manage templates for use in command waveforms.

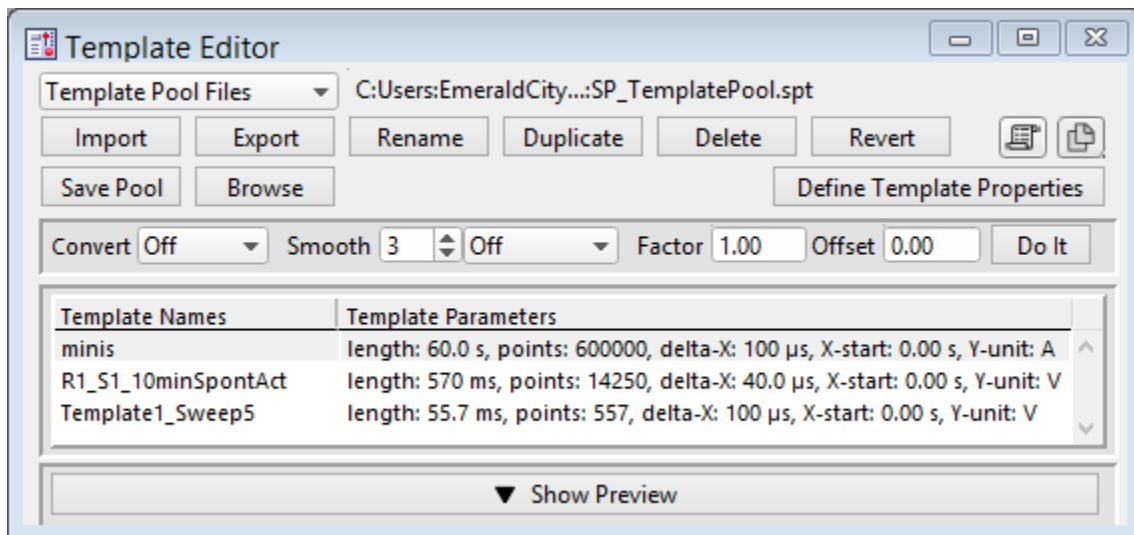


Figure 4-57. Template Editor

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	Select a template file (*.ibw). 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.
--------	--

An extracted template is composed of a single sweep:

- Scope window (Acquisition): last sweep
- Scope window (Analysis): selected sweep
- Preview pane: last or selected sweep

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.

Export	Export the selected template to a 1-D Igor wave file (*.ibw). To export a portion of a sweep, first extract (import) a portion of a sweep.
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.
 To Layout	Copy/append the template preview to the Layout window.
 To Clipboard	Copy the template to the system clipboard.
Save Pool	Save the template pool using its existing file name.
Browse	Create a template from the Experiment data in the Data Browser.
Define Template Properties	Update a data wave's X- and Y-axis parameters to be compatible with SutterPatch templates.
Enter X-increment	The data point time interval is changed, which also adjusts the length of the trace.
Enter X-start	The X-axis starting time for the data.
Enter Y-unit	The Y-axis base unit (enclose in double quotes.)
Convert	The data is interpolated to match the new sampling rate. While the number of samples is updated, the length of the trace is unchanged.
Smooth	Apply smoothing to the template. <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	Adjust the template scaling factor. Values are displayed with SI unit prefixes.
Offset	Adjust the template offset. Values are displayed with SI unit prefixes.
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/Hide Preview Display/Hide a preview pane with the selected template.

The preview pane X- and Y-axes can be controlled in two ways:

- 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 Data 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).

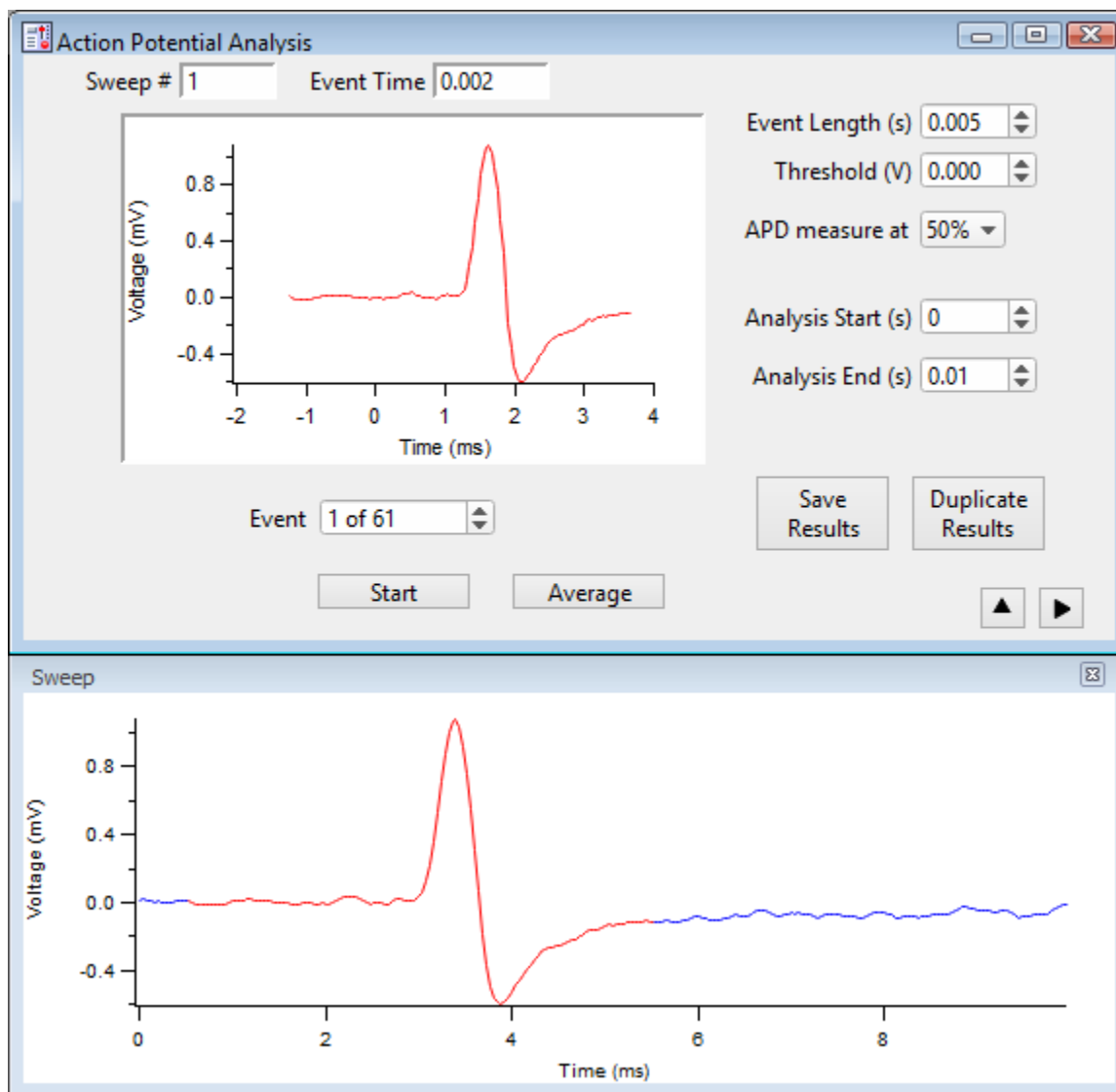


Figure 4-58. Action Potential Analysis

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)	[±0.100]	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.

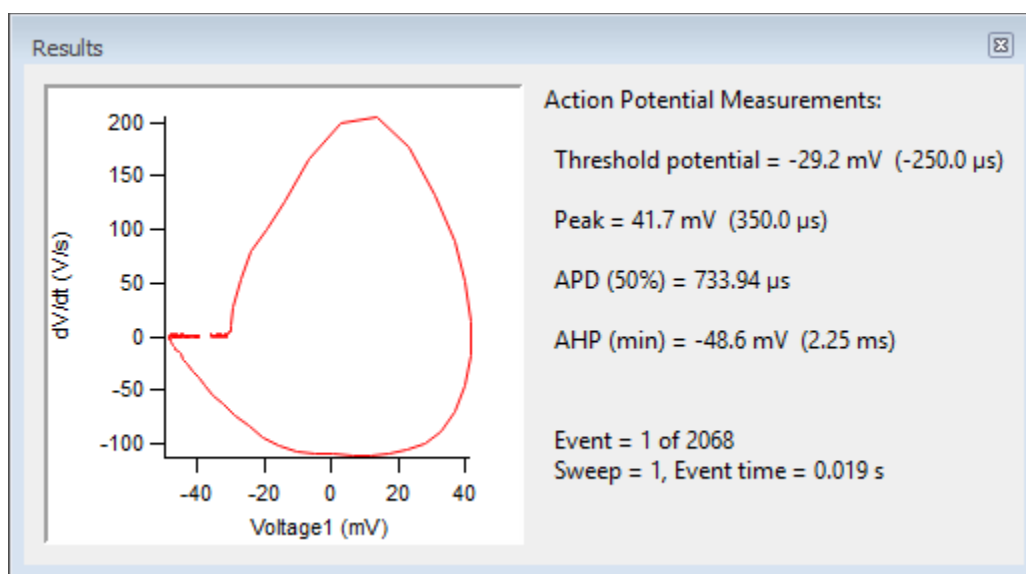


Figure 4-59. Action Potential Measurements

[Phase plot] dV/dt (V/s) vs. mV

[Y-axis vs. X-axis]

Threshold potential = [mV (μ s)]

Event starting amplitude (time from Threshold setting).

The actual biological start of an event, the reported Threshold potential, is calculated to be when the signal slope is $> 1 \text{ mV}/100 \mu\text{s}$ (10 V/s), or when 25% of the maximum slope is reached, whichever is smaller. As this timepoint is reported relative to the ‘Threshold’ detection timepoint, it is a negative number.

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.

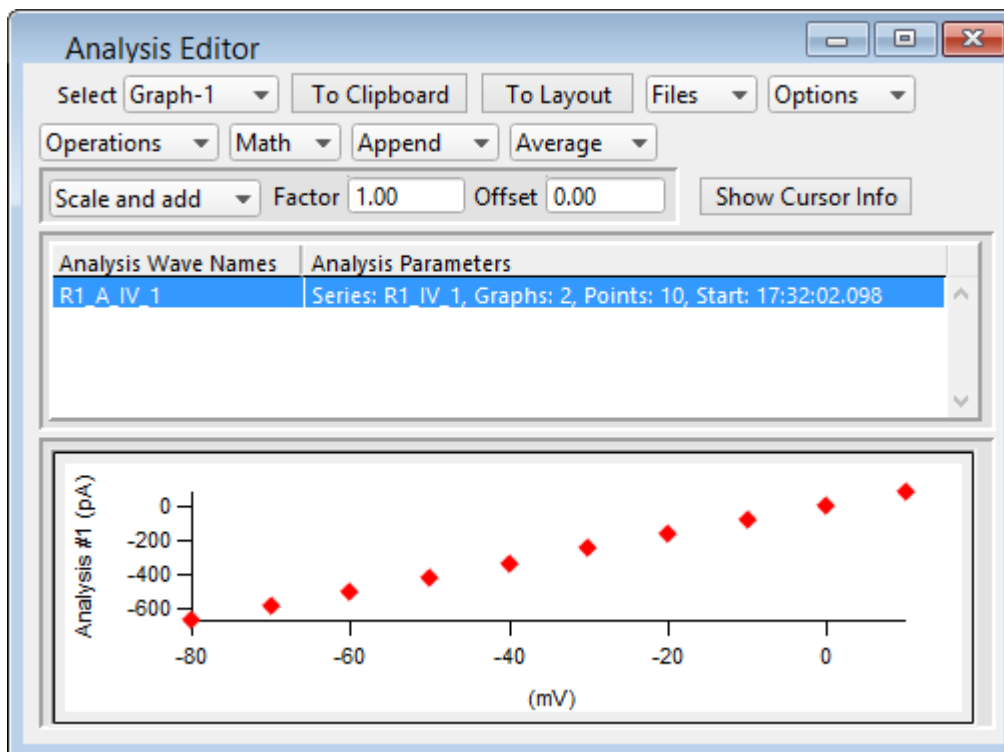


Figure 4-60. Analysis Editor

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.

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.



To Layout Copy/append the selected table or graph to a Layout window.



To Clipboard Copy the selected table or graph to the operating system (OS) clipboard.

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.

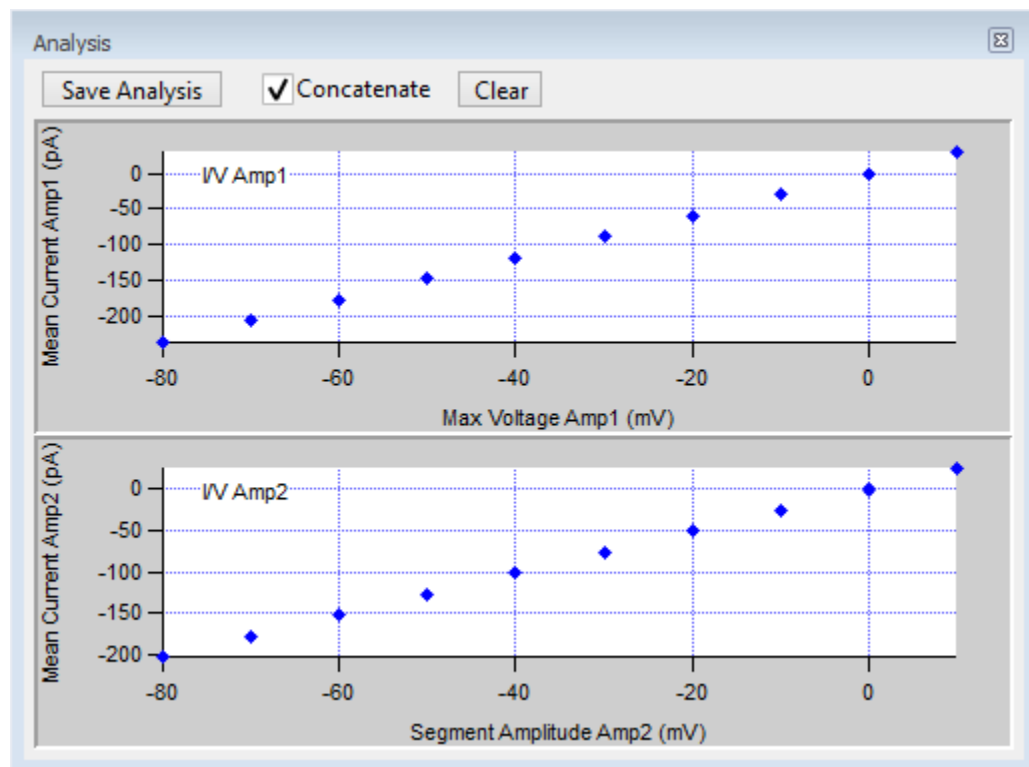


Figure 4-61. Analysis Window

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.

Concatenate Display new measurements along with the previous measurements.
 [only in the Acquisition Scope]

Clear Erase all measurements from the graph display.

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 access and display all of the Experiment's data objects, such as data waves, analysis graphs, layouts, images, metadata, Paradigms and Routines.

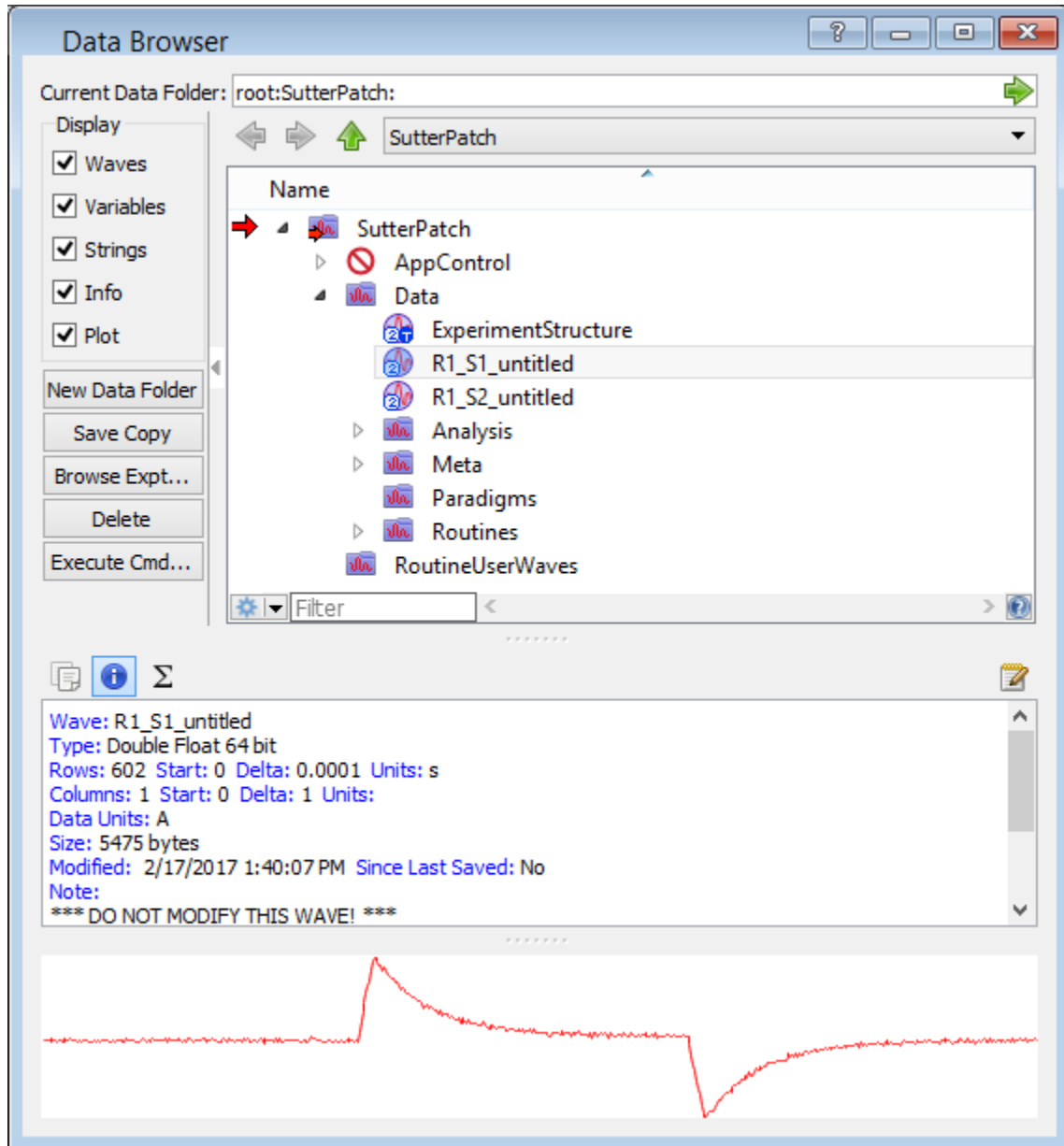


Figure 4-62. Data Browser

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

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

‘Analysis’ subfolder waves marked with “_En” contain tables of events; waves marked with

“_Zn” contain analysis results, such as from fits.

Warning! If this window is kept open during data acquisition, the Experiment can unexpectedly terminate.

Also, with the macOS, opening and closing windows can take a long time.

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 for the current Experiment.

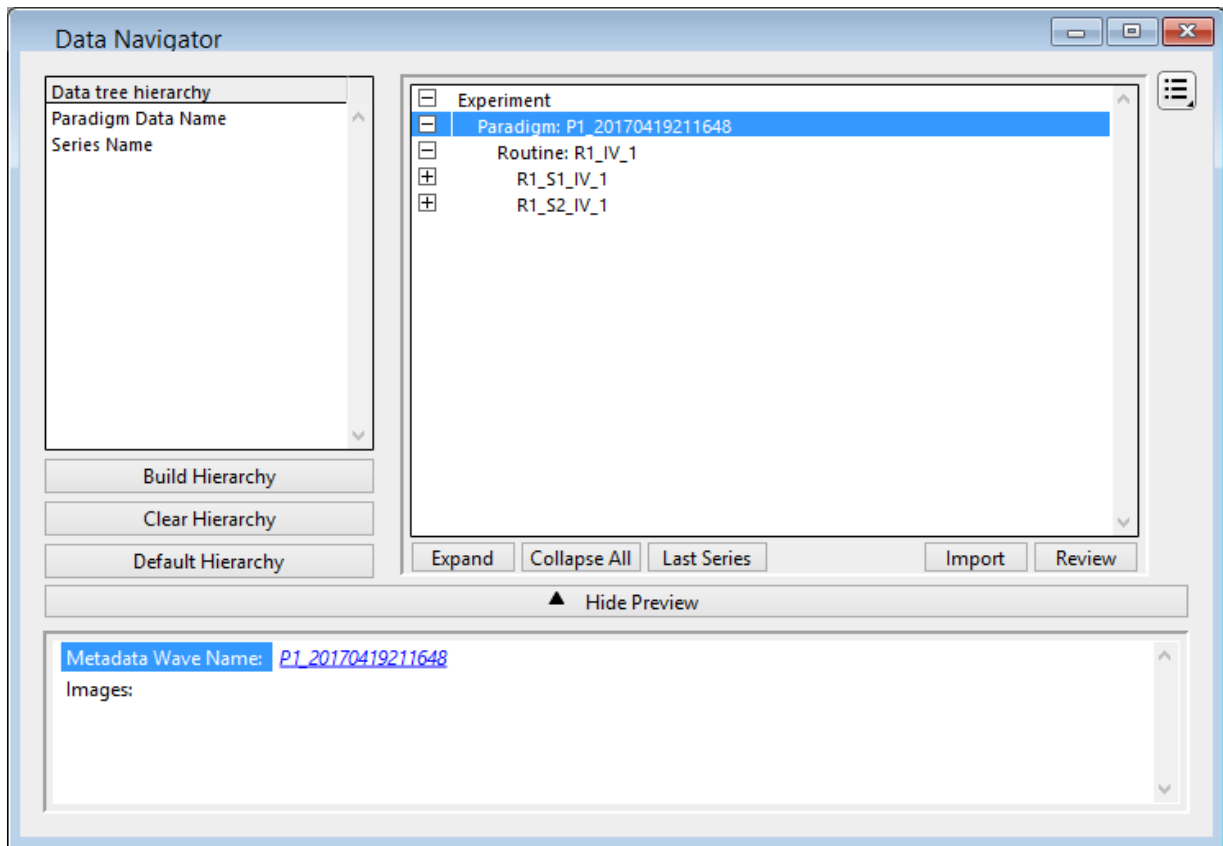


Figure 4-63. Data Navigator

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)

- All Categories
- Frequently Used
- Experiment Hierarchy
- Tag
- Operator
- Preparation - Animal
- Preparation - Tissue
- Preparation - Cell
- Experiment
- Amplifier
- Data Acquisition Settings
- Imaging
- Stimulus

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	The selected Routine or Series signals are opened in the Scope window for analysis.
Review	The selected Paradigm's signals and data are opened in the Scope window for review.

Note: Depending upon the data level, the same button displays the Analyze or Review commands.

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.
	[Preview sub-pane]	Displays the first signal of the selected Routine.
Signal:	[Preview]	Displays the selected Signal.
Sweep:	[Preview]	Displays the selected Sweep.



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.

Note: Use a mouse Shift-click to select a range of items, or CTRL-click to select separate items, to operate upon.

Paradigm:	Review	Display the Series data in the Paradigm Review window.
	Display	Display each signal in its own

		graph 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.
	Export Data (See Preferences)	Export the selected Paradigm data to file(s).
Routine:	Analyze	Display the Series data in a Scope (reanalysis) window.
	Display	Display each signal in its own graph 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

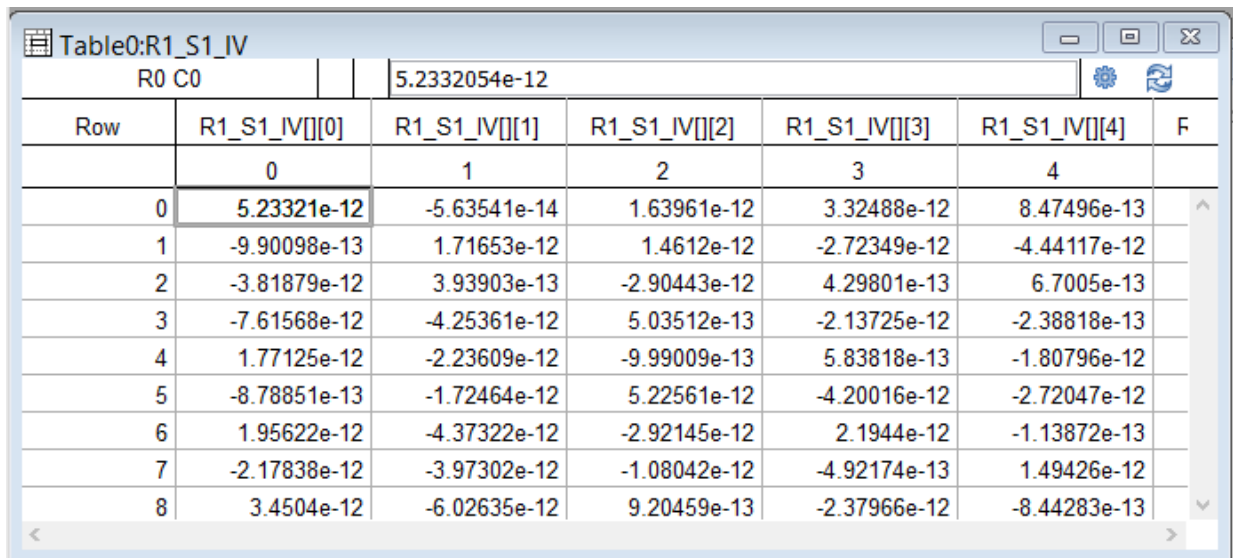
		closed and re-opened after the discard, then acquiring another Series will use the last discarded Series number.
	Export Data (See Preferences)	Export the selected Series to file(s).
Signal:	Analyze	Display the Series data in a Scope (reanalysis) window. All signals and sweeps display.
	Event Detection	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.
	Display	Display each signal in a graph window.
	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)
	Show Data in Data Browser	Open Igor's Data Browser window.
	Export Data (See Preferences)	Export the selected Signals to file(s).
Sweep:	Analyze	Display the Series data in a Scope (reanalysis) window.
	Edit	Display the Series data as numeric columns in a table.

Display	Display each signal in a graph window.
Extract Sweep	When a single sweep is selected, creates a graph of the sweep in the Analysis Editor.
Show Data in Data Browser	Open Igor's Data Browser window.
Export Data (See Preferences)	Export the selected sweeps to file(s).

Note: To export a portion of a sweep, use the Template Editor.

4.2.6 Data Table

The Data Table provides direct access to the sample points in a data Series, using a spreadsheet-style presentation.



The screenshot shows a window titled 'Table0:R1_S1_IV'. The table has a header row with 'R0 C0' and a value '5.2332054e-12'. Below this is a table with 7 columns: 'Row', 'R1_S1_IV[][0]', 'R1_S1_IV[][1]', 'R1_S1_IV[][2]', 'R1_S1_IV[][3]', 'R1_S1_IV[][4]', and 'F'. The data rows are numbered 0 to 8. The values in the columns are as follows:

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 4-64. 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.

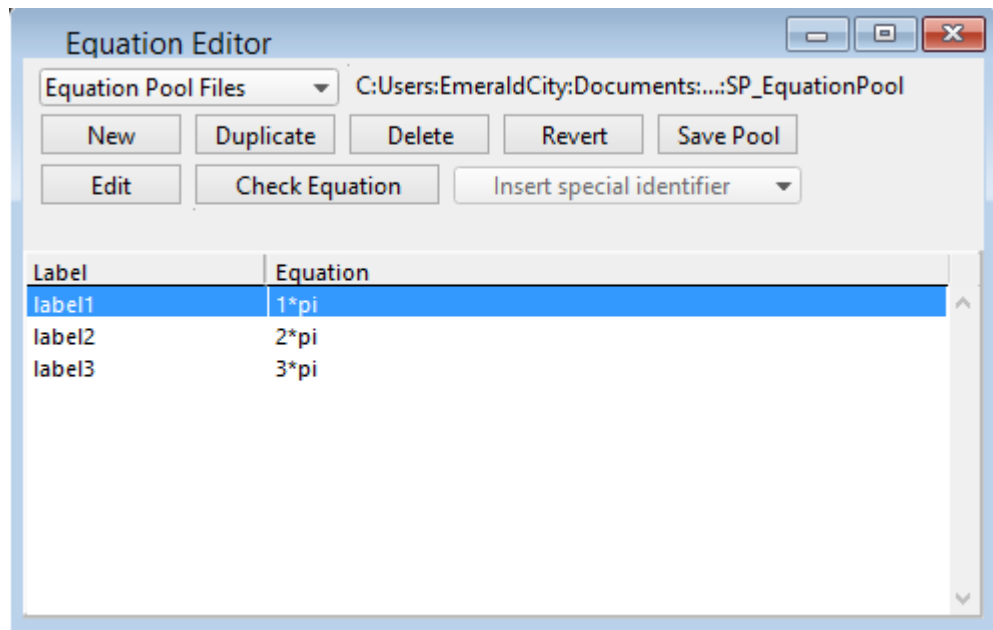


Figure 4-65. Equation Editor

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) 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'th analysis measurement value)
gx[1..8]	(n'th analysis graph x value)
gy[1..8]	(n'th analysis graph y value)
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..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)

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

IHold (amplifier holding current, A)

[± 0.000,000,020 (±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.

- 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).

- 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, except for comparison and bitwise operators, which associate from right to left.

Precedence	Operation Type	Operator
8	Comment	;
7	Exponentiation, Arithmetic operations: Left Shift, Right Shift	\wedge , \ll , \gg
6	Negation (logical) operations: Unary Negation, Logical Negation	-, !
5	Multiplication, Division, Remainder	*, /, %
4	Addition, Subtraction	+, -
3	Bitwise operations: And, Or, Nor, Xor	&, , nor, % \wedge
2	Comparison operations: Greater Than, Greater Than or Equal, Less Than, Less Than or Equal, Equal To, Not Equal To	>, >=, <, <=, !=
1	Logical operations: And, Or, Conditional	&&, , ?:
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

We recommend using parentheses to explicitly define the order of execution for expressions using Comparison and Logical operators.

There are also some differences in operator processing between the SutterPatch equation parser and the Igor Pro command parser:

Comment:	;	
SutterPatch		All characters to the right of a semi-colon are ignored
Igor Pro:		A semi-colon separates multiple commands on the same command line. An Igor comment uses a double slash: //

The Conditional operator “?:” is a shortcut for an if-else-endif expression. It evaluates as:

`<expression> ? <True> : <False>`

If the `<expression>` operand evaluates as non-zero, the `<True>` numeric operand is evaluated.

If the `<expression>` evaluates as zero, the `<False>` numeric operand is evaluated.

For complex expressions, only the real portion is evaluated.

Note: The “:” is a colon with 2 blank spaces around it.

An arithmetic left shift (`<<`) is the same as a bitwise left shift, whereby the least significant bit is padded with a zero. However, while an arithmetic right shift (`>>`) fills the most significant bit with its original value, thus preserving the sign, a bitwise right shift pads the most significant bit with a zero. A bitwise right shift can be constructed from existing operators.

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:

$$\text{equation} = \ln(r[1]) * 2.55 + 3$$

The natural log of the Routine Variable r[1] is multiplied by 2.55 and added to 3.

$$\text{label} = \text{power_to_volts}$$

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)

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 an innovative deconvolution algorithm. This technique finds events with high temporal fidelity, while also improving the signal-to-noise ratio (SNR).

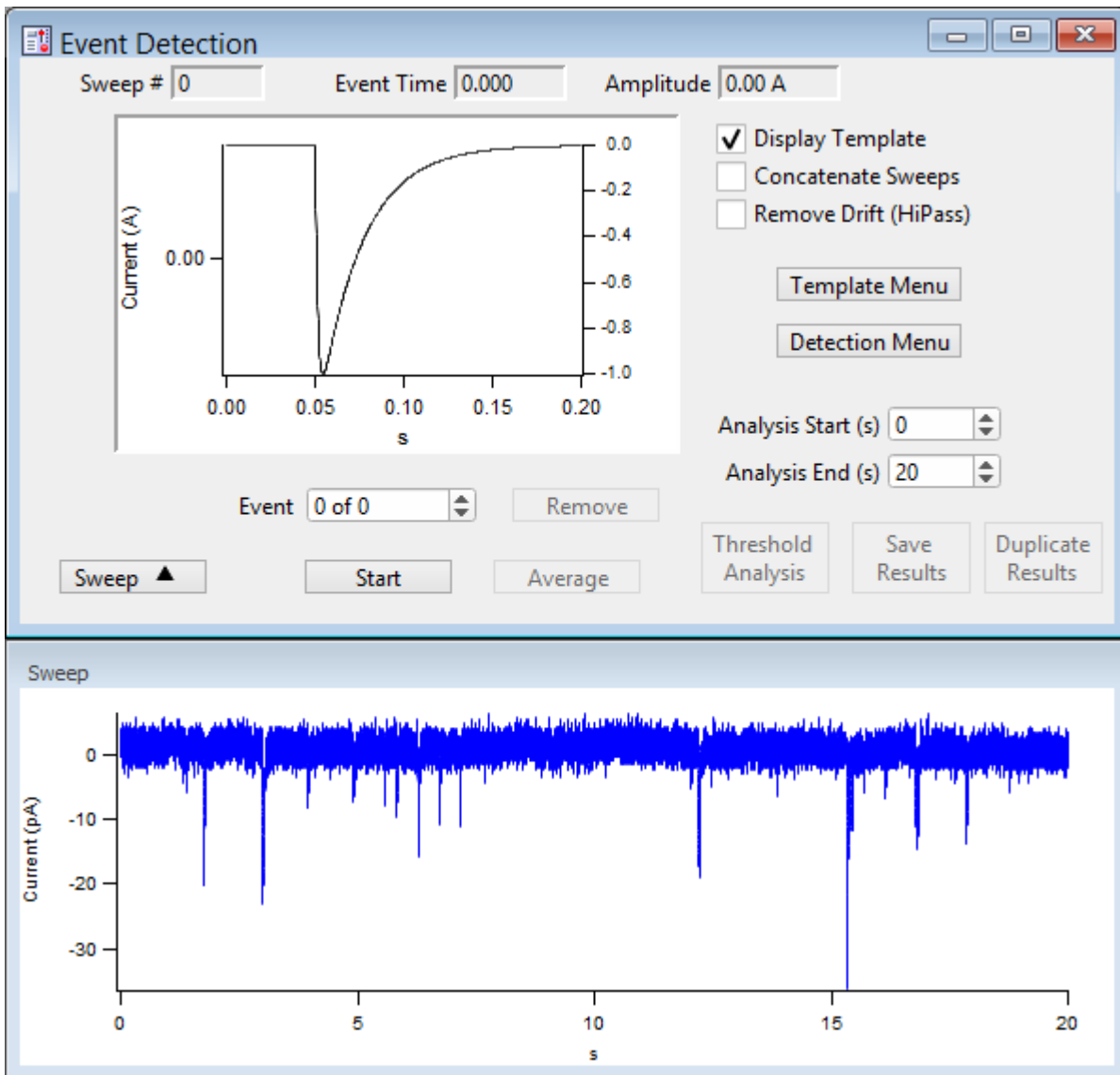


Figure 4-66. Event Detection

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.
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.
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.
Remove Drift (HiPass)	

Template Menu	Open the Template sub-panel to configure a 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)	[10 – 5,000] Time constant (τ) for the rising phase of the template event.
Decay Time (us)	[100 – 100,000] Time constant (τ) for the falling phase of the template event.
Create Template	Click this button to create the template.
Detection Menu	Open the Levels sub-panel to configure detection levels.
Threshold (xSD)	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. [0.1 – 10] Lower # = more noise-based events (false-positives) Higher # = more missed events (false negatives)
	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. [5e-13 – 1e-6]
Decay tau (us)	[<, >] [500 – 1,000,000] Set the decay tau as “less than” or “greater than” the tau value.
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.

Sweep	Show/Hide the sweep preview panel.												
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.												
	<table> <tr> <td>Total time analyzed =</td> <td>Includes the Start/End times for all sweeps (s).</td> </tr> <tr> <td>Number of events detected =</td> <td>Total number of events found.</td> </tr> <tr> <td>Event Frequency =</td> <td>(Hz)</td> </tr> <tr> <td>Average Event Amplitude =</td> <td>± 1 ms peak average (pA)</td> </tr> <tr> <td>Standard Deviation of Event Amplitude =</td> <td>(pA)</td> </tr> <tr> <td>Graphs:</td> <td> <ul style="list-style-type: none"> Cumulative probability vs. Amplitude Amplitude (Average) vs. Time Frequency vs. Sweep Number Amplitude vs. Sweep Number </td> </tr> </table>	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:	<ul style="list-style-type: none"> Cumulative probability vs. Amplitude Amplitude (Average) vs. Time Frequency vs. Sweep Number Amplitude vs. Sweep Number
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:	<ul style="list-style-type: none"> 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)													
	Note: For this measurement, events are measured relative to execution of the Routine's Start button, not to the sweep start.												
	Inter Event Interval (s)												

(See the SutterPatch Algorithms Appendix for a reference to the event detection algorithm.)

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

Ior

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/appended from windows with a 'Layout' button , such as the Scope window. Layout windows can also be automatically created by a Paradigm running an Export step.

Note: The Layout window is often created hidden behind other windows.



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, as does the Layout button in SutterPatch dialogs.

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.
<ul style="list-style-type: none"> By Event 	System settings, routine settings, and defined metadata events are displayed per Signal. Highlighted fields are editable.
<ul style="list-style-type: none"> 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 Firmware Version
 Amplifier Serial Number
 Amplifier Channel
 Number of Available Headstages
 Headstage Sequence Number
 Headstage Model

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
 Data Acquisition XOP Version

Paradigm

Paradigm Data Sequence Number
 Paradigm Data Base Name
 Paradigm Name
 Paradigm Description
 Paradigm Data Start Timestamp

Series (= Routine Data)

Series Sequence Number
 Series Base Name
 Routine Name
 Routine Acquisition Mode
 Routine Description
 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
 Input Offset Voltage [VC mode: '0' in Demo mode]
 Input Offset Lock On/Off [VC mode]
 Subtract Pip. Offset in Current Clamp [CC mode only]
 Input Signal Name

Input Signal Units	
Input Full-scale Minimum	
Input Full-scale Maximum	
Input Sampling Rate	
Virtual Signal Scaling Offset	[for Virtual Input channels]
Virtual Signal Math Type	[for Virtual Input channels]
Virtual Signal Equation	[for Virtual Input channels]
Virtual Signal Source Channel	[for Virtual Input channels]
Virtual Signal Source Signal Name	[for Virtual Input channels]
Virtual Signal Leak Display On/Off	[for Virtual Leak]
Virtual Signal Leak Zero Segment	[for Virtual Leak]
Virtual Signal Sweeps Processed	[for Virtual SweepAverage]
Virtual Signal Reference Sweep	[for Virtual SweepSubtract]
Virtual Signal Line Frequency Base	[for Virtual LineFreq]
Virtual Signal Filter Type	[for Virtual Filter/Smoothing]
Virtual Signal Filter Order	[for Virtual Filter/Smoothing]
Virtual Signal Filter Cutoff Frequency	[for Virtual Filter/Smoothing]
Virtual Signal LockIn Delay	[for Virtual LockIn]
Virtual Signal LockIn Attenuation	[for Virtual LockIn]
Electrode Fast Magnitude	[VC mode only]
Electrode Fast Time Constant	[VC mode only]
Whole-cell Compensation On/Off	[VC mode only]
WC Comp – Series Resistance	[only if WC Comp On]
WC Comp – Membrane Capacitance	[only if WC Comp On]
Series Resistance Correction On/Off	[VC mode only]
Series Resistance Prediction Value	[only if Series RCorr On]
Series Resistance Correction Value	[only if Series RCorr On]
Series Resistance Corr. Lag Time	[only if Series RCorr On]
Capacitance Neutralization On/Off	[CC mode only]
Capacitance Neutralization Mag.	[only if Cap Neut On]
Capacitance Neutralization Tau	[only if Cap Neut On]
Bridge Balance On/Off	[CC mode only]
Bridge Balance Resistance	[only if Bridge Bal On]
Current Clamp Dynamic Hold On/Off	[CC mode only]
Current Clamp Dyn. Hold Potential	[only if CC Dyn Hold On]
Current Clamp Dynamic Hold Speed	[undefined]
CC Dynamic Hold On for Acquisition	[only if CC Dyn Hold On]
Command Signal Name 1	
Command Signal Units 1	
Command Full-scale Minimum 1	
Command Full-scale Maximum 1	
Command Sampling Rate 1	
Command Holding Enabled 1	
Command Holding Value 1	[only if Cmd Hold 1 enabled]
Auxiliary Output Signal Units 1	
Auxiliary Output Holding Value 1	
Command Signal Name 2	[DIPA only]
Command Signal Units 2	[DIPA only]
Command Full-scale Minimum 2	[DIPA only]
Command Full-scale Maximum 2	[DIPA only]
Command Sampling Rate 2	[DIPA only]

Command Holding Enabled 2	[DIPA only]
Command Holding Value 2	[only if Cmd Holding 2 enabled]
Auxiliary Output Signal Units 2	
Auxiliary Output Holding Value 2	
Digital Holding Pattern (1 → N)	[1 – 8]

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.

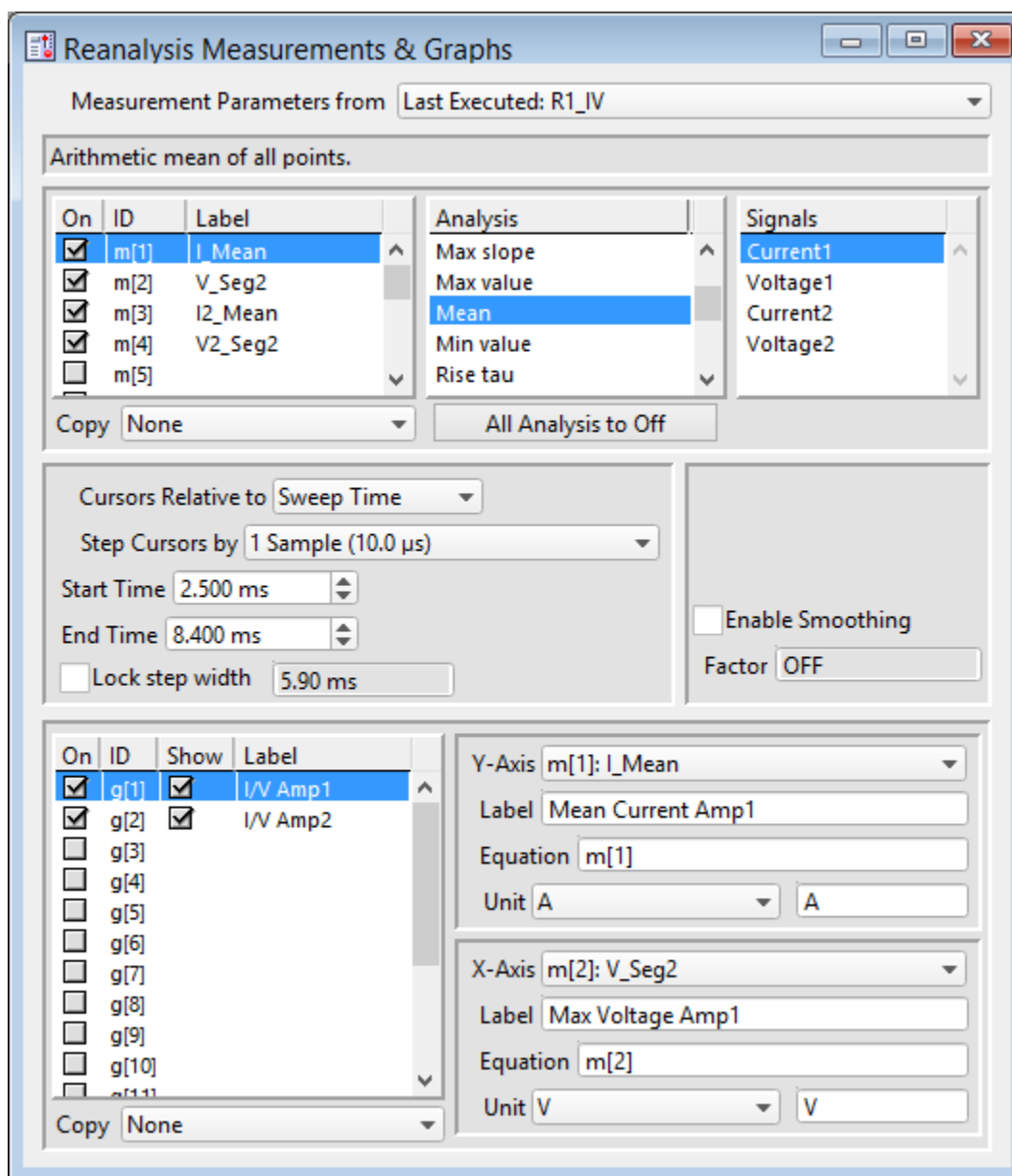


Figure 4-67. Reanalysis Measurements & Graphs

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.

- Current Settings (newly modified settings)
- Last Executed (last used version)
- Original Routine (original loaded version)

The Scope (Analysis) Measurements button also accesses virtual input signals.

Edit Virtual Signals

A virtual signal dialog allows modification of these “pseudo” input signals.

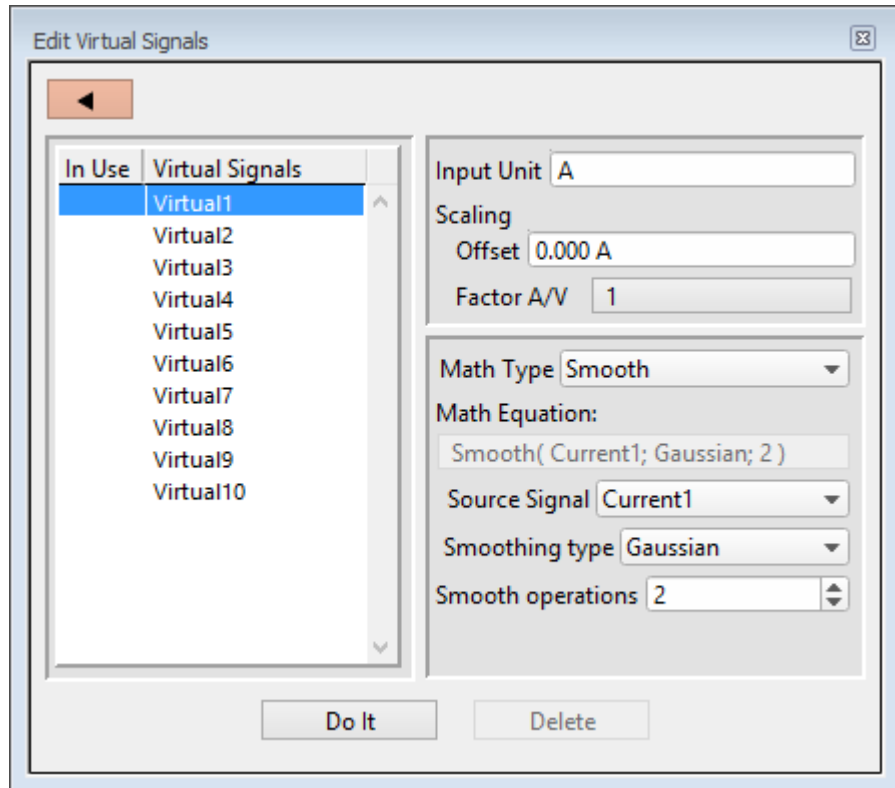


Figure 4-68. Edit Virtual Signals

Virtual input channels allow you to perform a variety of mathematical transformations on input signals. To enable a virtual signal, highlight a signal name. Changes to the highlighted signal are saved when you click the ‘Do It’ button, 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 fixed value from all data points in an input trace. This is useful for adjusting for an offset, or resetting a baseline.

Source Channel Select an input channel to process.

Baseline From Select how to calculate the subtraction value.

- Value Subtract a fixed value.
- Value Spinner adjusts in 1 pA or 1 mV increments.
- Trace Subtract the average of the entire input trace.
- Sweep Time Subtract the average of the data between the Start Time and End Time.

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 starting time of the Segment duration.

Start Time [derived value]

End Ratio Set the ending time of the data to be averaged, as a ratio relative to the ending time of the Segment duration.

End Time [derived value]

- **BesselFilter** A frequency-domain filter with excellent response characteristics for preserving the shape of a biological signal.

Source Channel Select an input channel to filter.

Filter Bandwidth Select a frequency range.

- **LowPass** Allow signal frequencies less than the cutoff frequency, and block all higher frequencies, such as high frequency noise.
- **HighPass** Allow signal frequencies greater than the cutoff frequency, and block all lower frequencies.

Filter Order [1, 2, 4, 8]

Number of “poles” in the filter

Cutoff Frequency (Hz) [100 to $< \frac{1}{2}$ the sampling rate]

Restrict frequencies from this boundary point onwards.

- **Differentiate** Apply differentiation to an input signal. The instantaneous rate of change in the signal is displayed.

Source Channel Select an input channel to differentiate.

- **Equation** Specify an equation to process an input signal.

Source Channel Select an input channel to process.

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 "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]

Access 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[#] Access the input trace (data

wave) in Scope position “n” for the last sweep of the current Series.

This numbering can differ from the Scope Position "n" if signals are re-arranged or hidden

Undo All changes in the equation editing session are discarded.

(See the Equation Editor for more details.)

- Integrate Display the integral of the data signal. This is equivalent to the signed area under a curve.

Source Channel Select an input channel to integrate.

- Leak Remove leakage current from the data signal. This is the small passive current when the cell is in a resting state.

This feature is only enabled when the Routine includes an output channel with P/N Leak Pulse enabled.

Source Channel Select an input channel to process.

Show Leak Display the accumulated leak currents after the subtracted data in a sweep. Display the average of the leak sub-pulses.

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 included in the current of the main signal.

- LineFreq Remove AC line frequency noise (hum) from the data signal.

Source Channel	Select an input channel for noise reduction.
Line Frequency	50 Hz Most of rest of world. 60 Hz Canada, (Caribbean), Central America, (Japan), Mexico, (South America), South Korea, Taiwan, USA.

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.

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

Calculations are made using 'conductance' (1 / resistance) instead of 'resistance'.

Current Channel Select a (source) input channel with a "current" signal.

Trace Kind Select the LockIn measurement to display.

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

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.

Cycles to Average [1 – 1000]

Cycles to Skip [1 – 1000]

V-reversal [± 1000 mV]

When using a calculated stimulus trace, enter the reversal potential for the ion under study, such as for (Na⁺) sodium spikes or (K⁺) potassium tail currents.

Note: See the SutterPatch Algorithms appendix for the math used in the LockIn computation.

- 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 sliding “window”.
- Note: For best performance, only odd values are used.
- 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 average.
----------------	-------------------------------------

Average Type	Cumulative Average all processed sweeps together.
--------------	--

RunAverage	Average the last “N” sweeps.
------------	------------------------------

Number of Sweeps	
------------------	--

- SweepSubtract Subtract a sweep from the input trace.

Source Channel	Select an input channel to process.
----------------	-------------------------------------

Reference Sweep	Select a sweep to be 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.

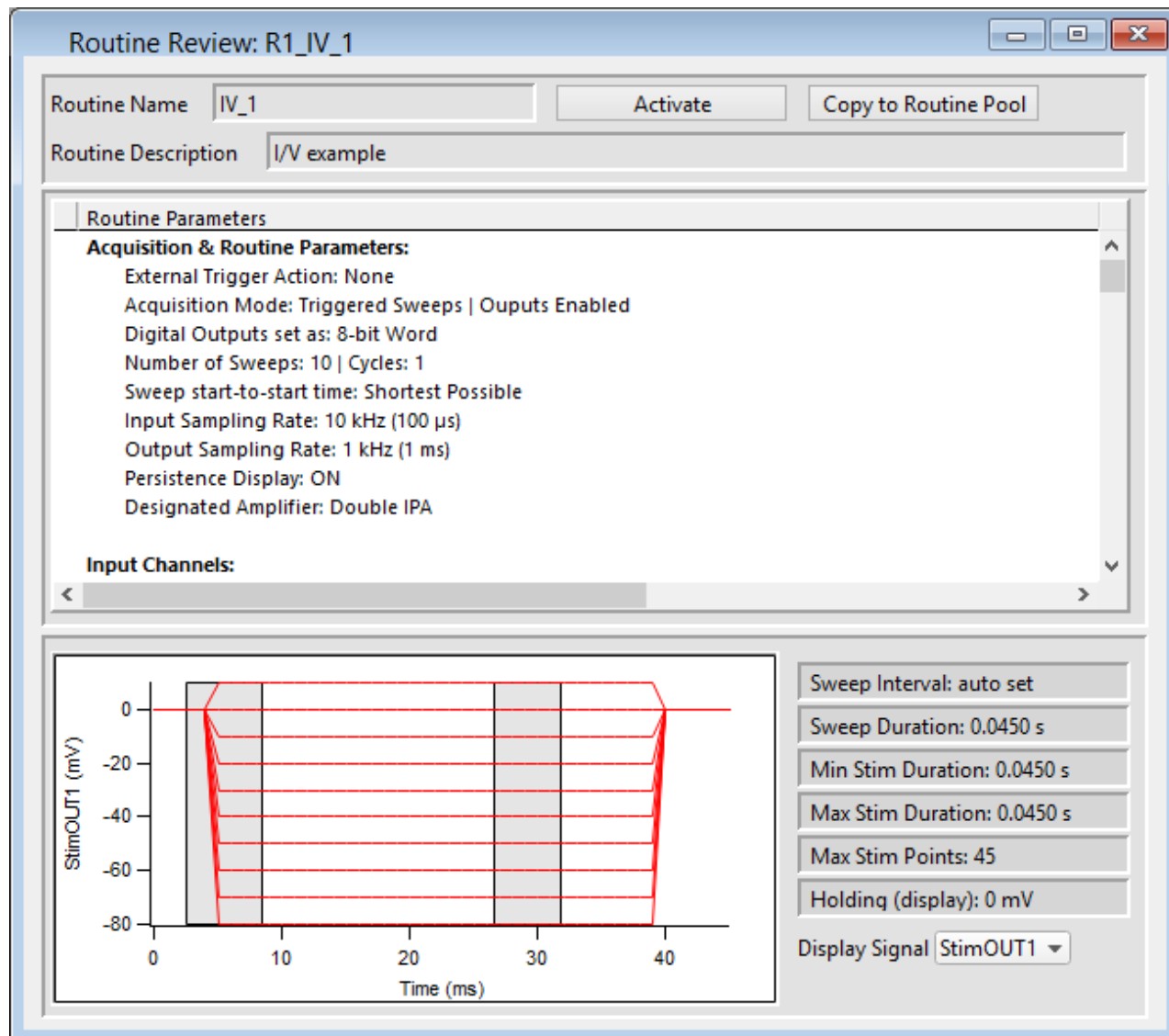


Figure 4-69. Routine Review

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)

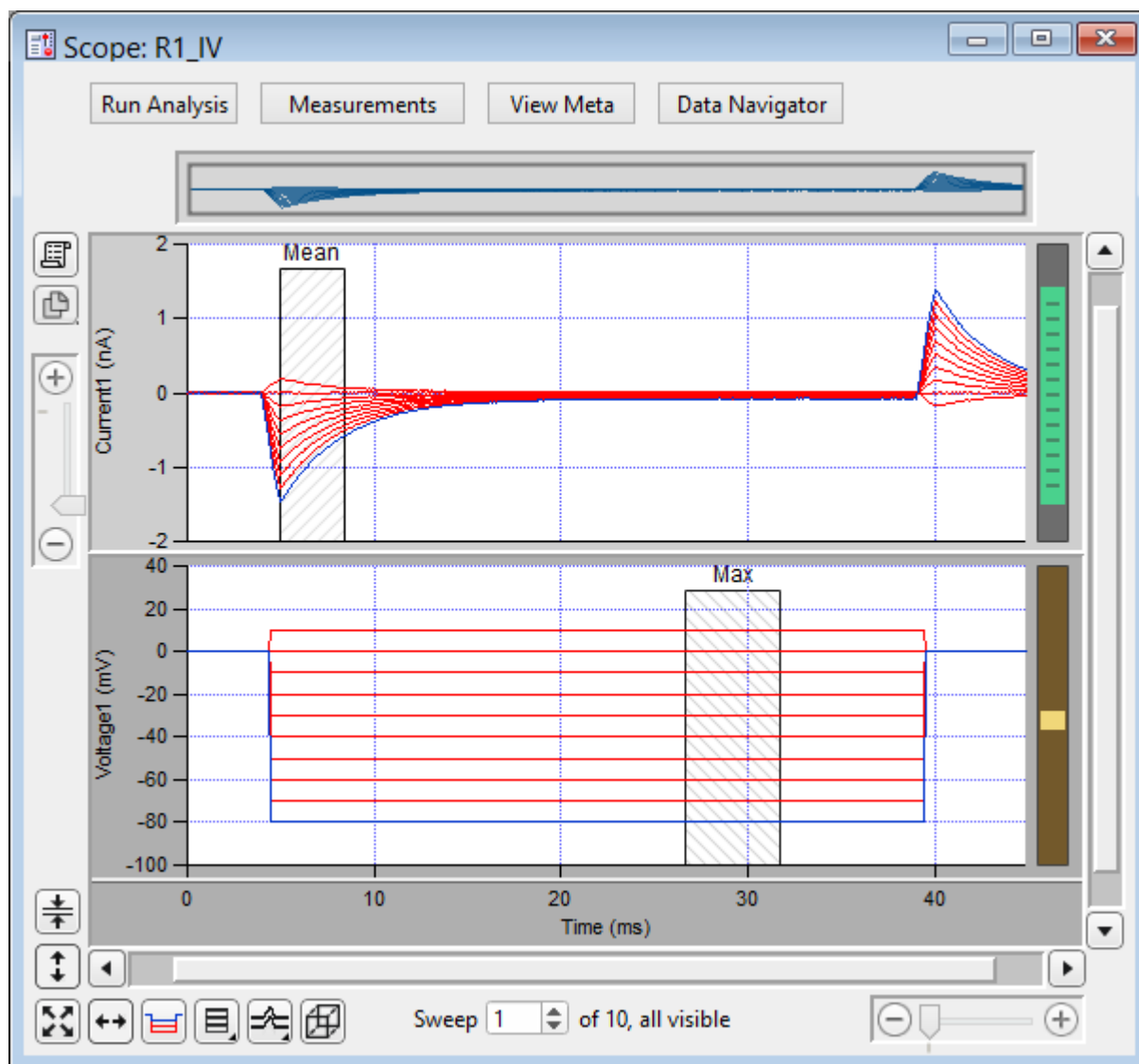


Figure 4-70. Scope (Reanalysis) Window

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:

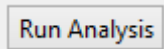
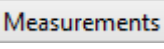
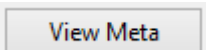
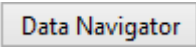

Navigation pane:











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 4-71. 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>Copy and paste into a new or append to an existing Layout window all Scope signals and Analysis graphs.</p>
<p>Copy to Clipboard</p>	<p>Copy the Scope window to the system clipboard.</p>

		
Center 	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.	
Autoscale 	Autoscale all visible signals. Rescale the Y-axes to their displayed sweeps data limits, and the X-axes to their full sweep duration.	
	Click to autoscale the Y-axis of the selected signal.	
	Click to full-scale all X-axes.	
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 with 'm' rows and 'n' columns.	
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.


	<p>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.</p>
<p>Sweep #:</p>	<p>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).</p>

Table 4-6, Scope Window Buttons

X- and Y-Axes	Same as the acquisition Scope
Main window	<p>Additional items:</p> <ul style="list-style-type: none"> • 3D View • Zero Baselines • Show All Sweeps (with triggered sweeps) • Select Sweeps (with triggered sweeps)
Data	<p>Additional items:</p> <ul style="list-style-type: none"> • Hide Sweep_# (with triggered sweeps) • Show Sweep_# Only (with triggered sweeps)

Note: IPA amplifiers record both stimulus and response signals via physical analog channels, therefore there are no timing delays between the recorded signals.

4.2.16 3D View Window

The Scope (Analysis) 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.

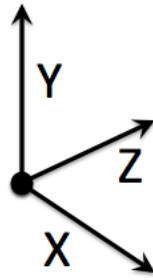


Figure 4-72. 3D Axes Definition

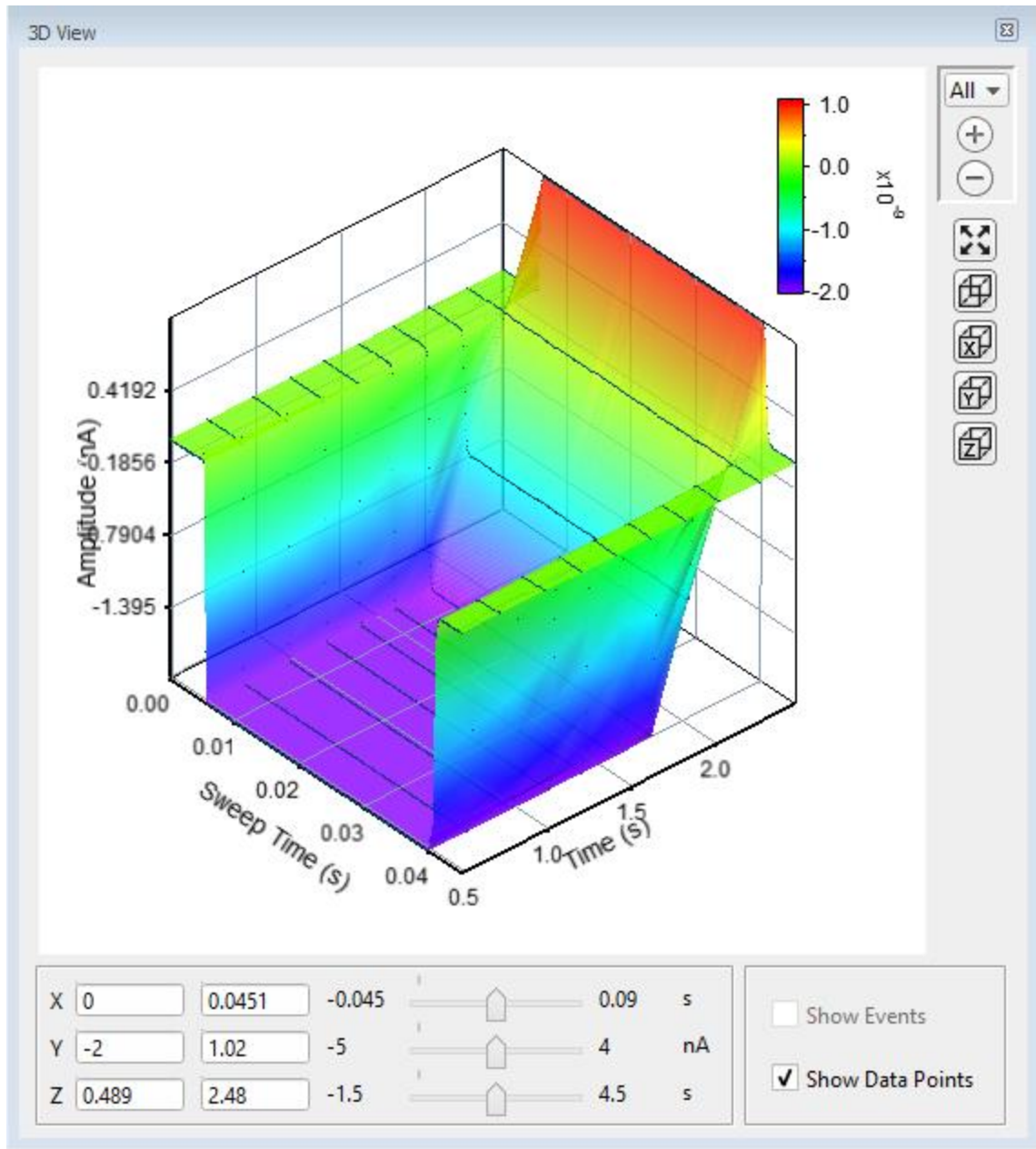





Figure 4-73. 3D View

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

A variety of optional experimental parameters (preparation, electrode, etc.) parameters can be associated with an Experiment, Paradigm, or Routine as user-configurable “metadata”.

Predefine the Metadata parameter values here.

Show Summary An overview of the user-defined metadata parameters.

Metadata Summary dialog

All parameters with defined values defined are displayed in the Summary, even from unselected ‘Metadata detail levels’. 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 metadata prompts before running:
▪ Expt	Experiment
▪ Pdgm	Paradigm
▪ Routn	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 The available metadata parameters are displayed by detail level, as set in SutterPatch > Set Preferences > Metadata.

[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 parameter used in the previous acquisition is written.
- Use a previous value Select from a drop-down list of the previous 20 metadata values used for acquisition in the experiment.
- 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.
Next 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

Delete

4.2.18 Single Channel Analysis

This analysis module provides single channel ion-channel analysis measurements, analyses and histograms of the active signal. Access via the Data Navigator / Analyze button followed by the Reanalysis Scope window 'Measure' button.

Single channel analysis is only available in the Sweeps display mode; Concatenated and Continuous display modes are not supported.

The Scope window data is overlaid with the first found event (based on the Transition Controls settings.)

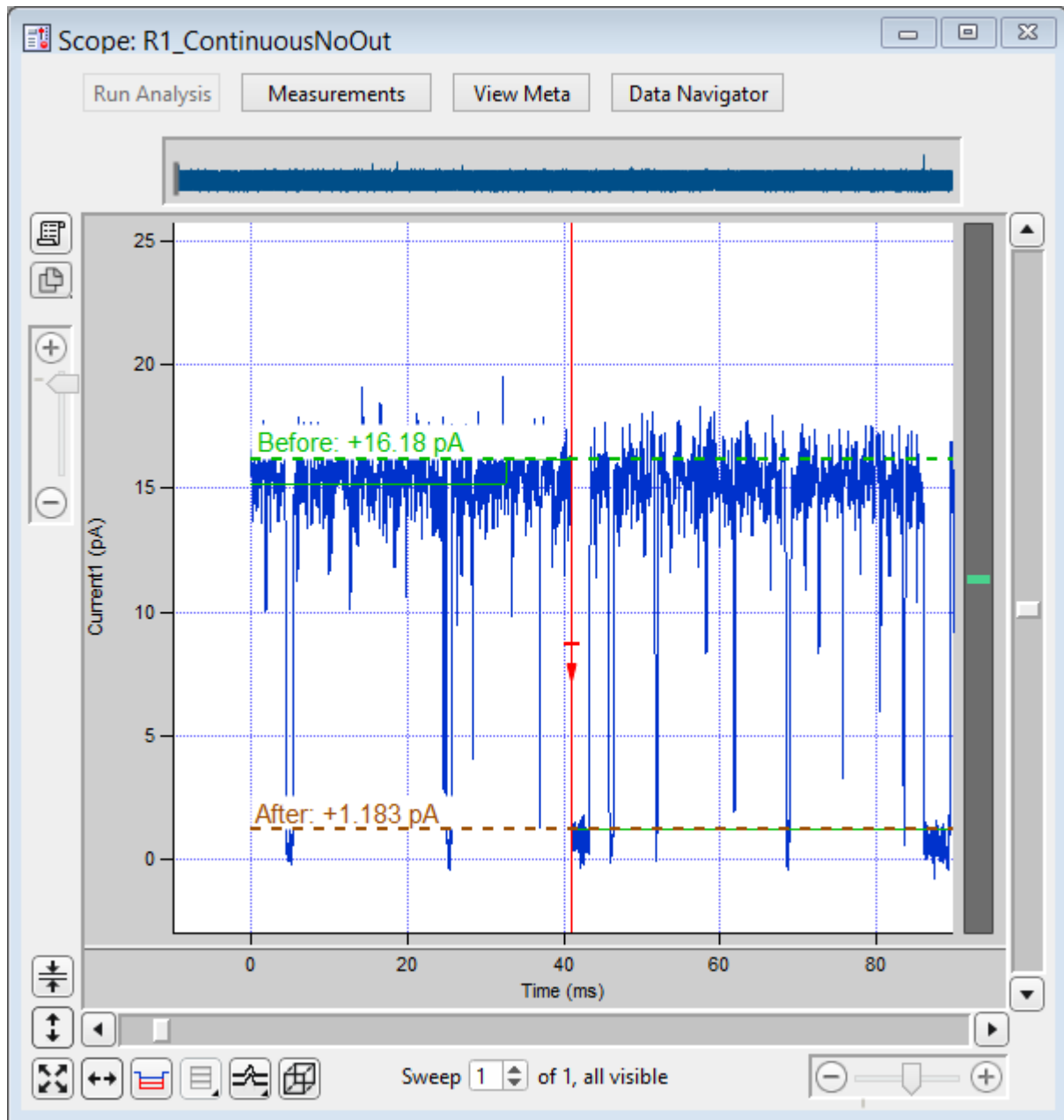


Figure 4-74. Single-Channel Scope

Whenever a level transition occurs, the “Before” and “After” levels, such as for open and closed channels, are displayed in the Scope window overlaid on the raw data.

- Use individual sweeps Perform analysis on a single sweep at a time.
- Use complete signal Perform analysis on the entire active signal.

[Descriptive information]

Single Channel Analysis Preferences

Dock to Scope

Baseline Average Duration = x.x ms

Mean Amplitude Duration = x.x ms

Cell Separator: Tab

Cell Separator: Comma

Invalid values: Use NaN

Invalid values: Use empty string

Digits in table entries:

Digits = 3, 4, 5, 7, 9, 11, 13, 15

Show All Signals

X-Width: [Sweep / 1 s / 500, 200, 100, 50, 20, 10, 5, 2, 1 ms]

The Scope window X-axis duration is set to this value for use with the target Transition Controls.

Show/Hide Current Amplitude Histogram Controls

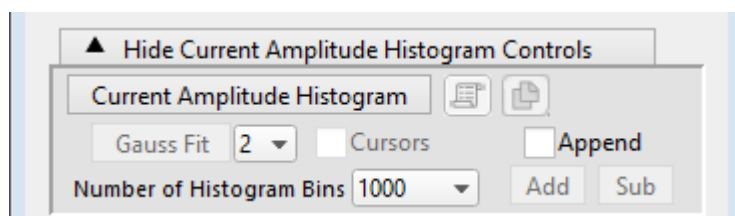




Figure 4-75. Current Amplitude Histogram Controls

The Current Amplitude Histogram is often the first analysis performed on an uncharacterized channel. It is used to determine:

- The quality of the recording.
- The number of levels in the open state.
- The first estimate of the open state amplitude(s).
- The first estimate of the baseline closed state.
- The frequency of openings.

Current Amplitude Histogram Click button to create a histogram plot.

 Copy to Layout Page Copy/append the current amplitude histogram to a Layout page.

 Copy to Clipboard Copy the current amplitude histogram to the clipboard.

Gauss Fit

Click button to perform a Gaussian fit on the histogram. SutterPatch will automatically find and fit up to the three largest peaks; adjust the fitting ranges as needed with manual cursors.

To fit additional smaller distributions, reposition the cursors and click the Append button.

Multi-peak amplitude (open level) differences and P(open) and P(closed) values are displayed in the graph. See the Table of Results for the fitting components.

[1, 2, 3]

Select the number of peaks (levels) to fit.

Cursors

Enable to display fitting cursors in the histogram plot. To reposition a cursor, drag its cursor symbol (labeled “A”, “B”, etc.) from the cursor bar onto the graph to the desired position.

Append

Modify the Current Amplitude Histogram.

Add

Add bins to the Current Amplitude Histogram.

Sub

Subtract bins from the Current Amplitude Histogram.

Number of Histogram Bins [4000, 2000, 1000, 500, 200, 100, 50]

Show/Hide Transition Controls

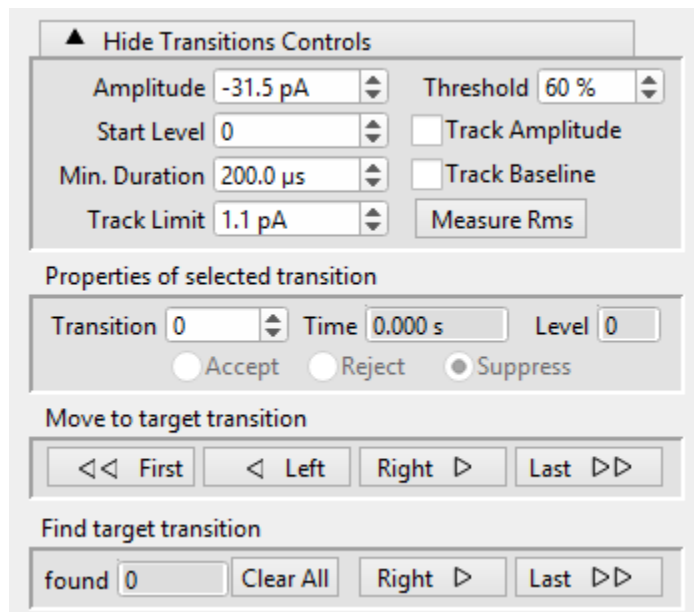


Figure 4-76. Transition Controls

Amplitude	The expected transition size for the ion-channel opening. For upwards-opening (positive) channels, use an unsigned value. For downwards-opening (negative) channels, use a negatively-signed value.
Threshold	[50 – 90%] The percentile of the Amplitude value that needs to be reached by the data to trigger finding a transition.
Start Level	The starting Closed or Open state of the ion-channel data under study. Closed = 0, Open = 1+. Note: All open states should have a positive level number. If transition levels are incorrectly reported as negatively signed, double-check the defined Amplitude value and its sign.
Track Amplitude	Automatically adjust the expected transition Amplitude based on the prior data.
Min. Duration	The minimum duration that the data needs to achieve at or above the Threshold level.
Track Baseline	Automatically adjust the baseline amplitude (Level 0) based on the prior data.
Track Limit	The maximum amount that the Amplitude or Baseline measurements can change while being automatically tracked.
Measure RMS	To measure the Root-Mean-Square noise in the signal, adjust the signal trace in the Scope window such that it shows a stretch of current without any event activity (i.e., all channels are closed), then click on the 'Measure RMS' button. The RMS value is displayed at the bottom of the dialog, and a 3 x RMS value populates the Track Limit field.

Properties of selected transition

Transition	Transition (event) number.
Time	Read-only field of time of transition start.
Level	Read-only field of open-channel level.
• Accept	

- Reject
- Suppress

Move to target transition

<< First	Move to the first found event.
< Left	Move to the prior found event.
Right >	Move to the next found event.
Last >>	Move to the last found event.

Find target transition

found	The number of found events.
Clear All	Reset the number of found events to zero and move to the first found event.
Right >	Find and move to the next event.
Last >>	Find all subsequent events and move to the last event.

For overlapping events, the first event duration is from the first Level 1 transition to the last Level 1 transition, for Level 1 openings; the second event duration is from the initial Level 2 transition to the last Level 2 transition, for Level 2 openings; etc.

Show/Hide Plots and Tables Controls

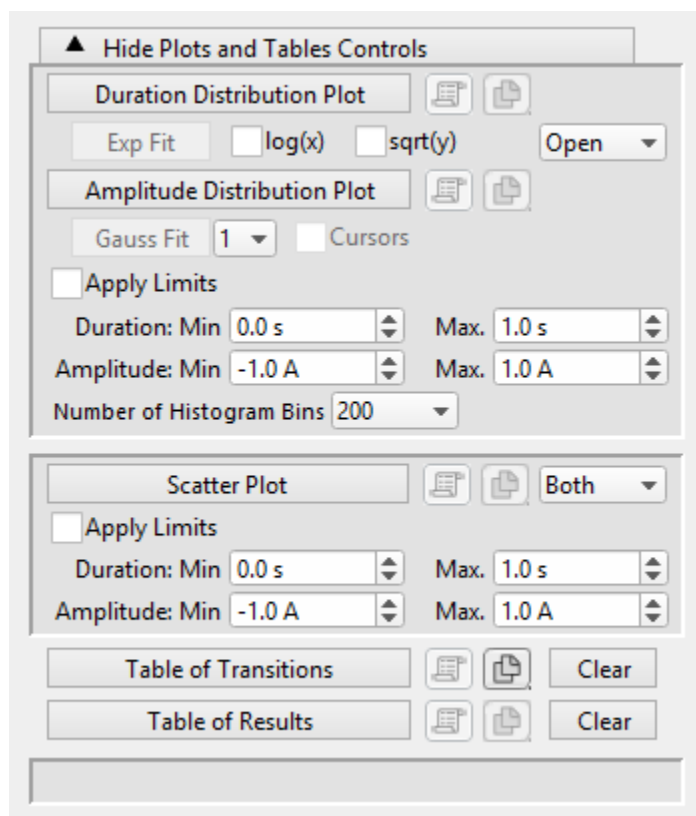


Figure 4-77. Plots and Tables Controls

Duration Distribution Plot

Create a dwell-time histogram plot.



Copy to Layout

Copy/append the Duration Distribution Plot to a Layout page.



Copy to Clipboard

Copy the Duration Distribution Plot to the Clipboard.

Exp Fit

log(x)

sqrt(y)

Open / Closed

Amplitude Distribution Plot:

Gauss Fit [1, 2, 3]

Cursors

Apply Limits

Duration: Min [0.0 – 1.0 s]

Max. [0.0 – 1.0 s]


Amplitude: Min [-1.0 – 1.0 A]

Max. [-1.0 – 1.0 A]

Number of Histogram Bins [1000, 500, 200, 100, 50, 20]


Scatter Plot

 Copy to Layout Copy/append the Scatter Plot to a Layout page.

 Copy to Clipboard Copy the Scatter Plot to the Clipboard.

Open / Closed / Both

Table of Transitions


 Copy to Layout Copy/append the Table of Transitions to a Layout page.

 Copy to Clipboard Copy the Table of Transitions to the Clipboard.

Clear

Table of Results

 Copy to Layout Copy/append the Table of Results to a Layout page.

 Copy to Clipboard Copy the Table of Results to the Clipboard.

Clear

Table Column Labels

Gaussian Fit

<u>Header</u>	<u>Label</u>
Source	Sweep_#
Analysis	Current Amplitude Histogram Fit / Amplitude Distribution Fit
Label1 / Value1	Amplitude
Label2 / Value2	p(closed)
Label3 / Value3	p(open)
Label4 / Value4	Gauss_y0_1
Label5 / Value5	Gauss_A_1
Label6 / Value6	Gauss_x0_1
Label7 / Value7	Gauss_width_1
Label8 / Value8	Success_1

Label9 / Value9	Gauss_y0_2
Label10 / Value10	Gauss_A_2
Label11 / Value11	Gauss_x0_2
Label12 / Value12	Gauss_width_2
Label13 / Value13	Success_2
Label14 / Value14	Gauss_y0_3
Label15 / Value15	Gauss_A_3
Label16 / Value16	Gauss_x0_3
Label17 / Value17	Gauss_width_3
Label18 / Value18	Success_3

Linear Exponential Fit

<u>Header</u>	<u>Label</u>
Source	Sweep_#
Analysis	Duration Distribution Fit
Label1 / Value1	Exp_y0 / #
Label2 / Value2	Exp_A / #
Label3 / Value3	Exp_Tau / #
Label4 / Value4	Exp_0 / #
Label5 / Value5	Success / #

Logarithmic Exponential Fit

<u>Header</u>	<u>Label</u>
Source	Sweep_#
Analysis	Duration Distribution Fit
Label1 / Value1	LogNormal_k0 / #
Label2 / Value2	LogNormal_k1 / #
Label3 / Value3	LogNormal_k2 / #
Label4 / Value4	LogNormal_k3 / #
Label5 / Value5	Success / #

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 displays in the upper portion of the window. Some warning messages also display here.

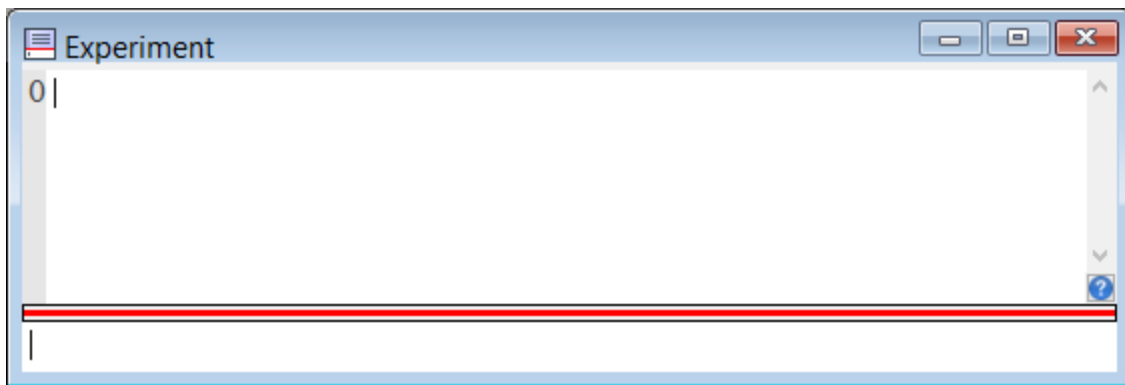


Figure 4-78. Command Window

This window is an Igor Pro code interpreter, providing programmatic interaction with SutterPatch. Commands can be entered into the command buffer in the lower portion of the window, or highlighted lines in the history section can be transferred into the command section for processing (to select all history text, use 'CTRL-A'), or the Paradigm step Execute can copy its command functions into this window for manual execution, when the Enter key is pressed.

A maximum of 400 characters can be entered into the command buffer, however they can be spread across multiple commands on multiple lines.

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.

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.

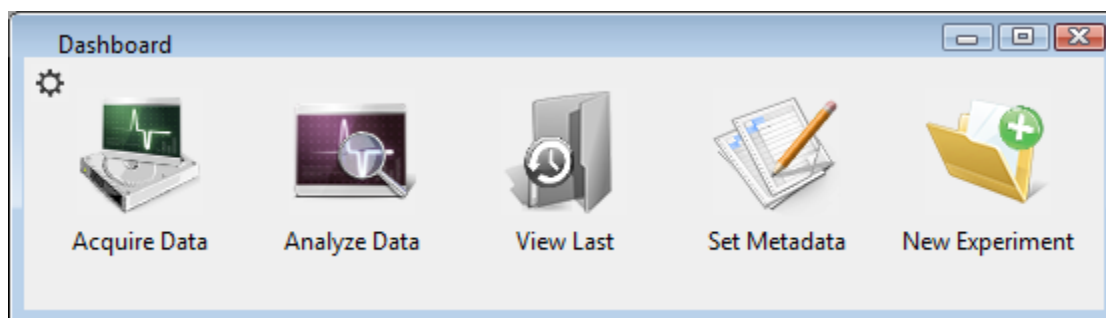



Figure 4-79. Dashboard

-  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 4-80. 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.

Axon Files

Data can be selected and exported to the Axon Instruments pCLAMP file formats via the Data Navigator ‘Available Actions’ button or a right-click menu. Set to the ABF or ATF file formats in the Set Preferences / Data Export section.

HDF5 Files

A modern efficient file format for saving and managing high volumes of data, available to Experiments in the Set Preferences / Files and Naming section.

HEKA Files

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.

Igor Files

Data can be selected and exported to the Igor Pro Binary Wave (*.ibw) file format via the Data Navigator 'Available Actions' button or a right-click menu. Select the Igor Binary (*.ibw) file format in the Set Preferences / Data Export section.

Igor binary waves (*.ibw) can be loaded into the SutterPatch Experiment via Data / Load Waves / Load Igor Binary. Find the files in the Data / Data Browser.

Template Files

Templates can be imported or exported via the Template Editor as Igor Binary Wave files (*.ibw).


Templates can also be used to export portions of data from a sweep.

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.

It is recommended that you create one Experiment per cell, to keep file sizes manageable.

Note: Even if immediately selected after saving an Experiment, you will still be re-asked to save the prior Experiment (due to internal processes.)

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.
--------------	---

Load Waves / Packages / Install HDF5 Package

Load Waves / New HDF5 Browser

Windows

The Windows menu provides access to graphs, tables and layouts.

Command Window	Process SutterPatch and Igor commands.
----------------	--

Control / Retrieve All Windows	Hidden unminimized windows can be brought into view with the menu command
--------------------------------	---

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.

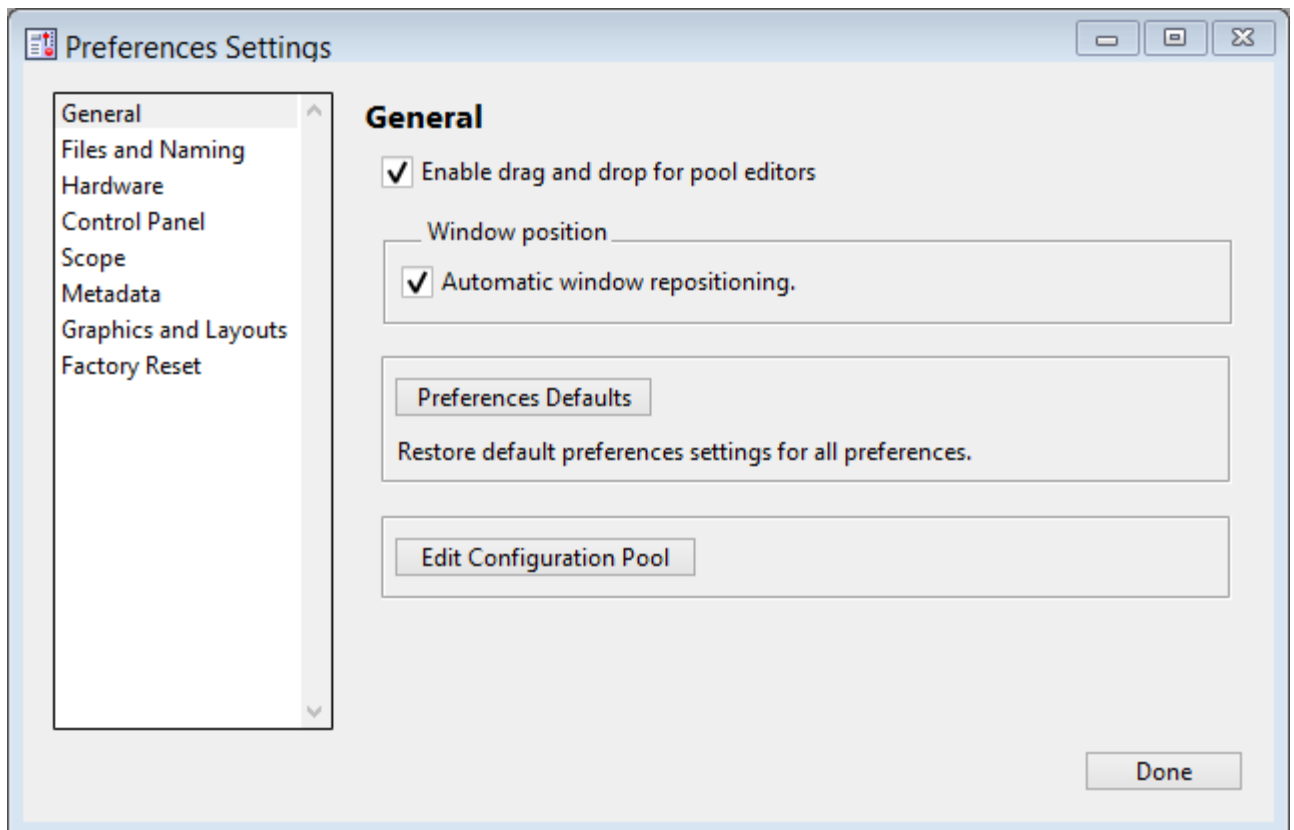


Figure 4-81. Preferences Settings

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)

One screen	Prevents a window spanning across monitors. If a “parent” window is moved to another monitor, it fully displays in the new monitor, while any child sub-window remains behind fully displayed in the original monitor.
------------	---

Note: You can also bring all unminimized windows into view with the Windows > Control > Retrieve All Windows menu command.

Preferences Defaults Restore default Preferences settings for all Preferences.

Edit Configuration Pool Edit and save user Preferences configurations.

Delete Configuration	Remove the named Configuration from the list. The last remaining name is not deletable.
----------------------	---

Add New Configuration	Create a new Configuration name for the current Preferences settings.
-----------------------	---

Configurations are selected for loading at the start of a new experiment.

Description	Enter the name for the Configuration (enclosed in double quotes).
-------------	---

Update Active Configuration (#)

The “active” Configuration is updated with the current Preferences settings.

This dialog opens with the active Configuration name listed. The number (#) indicates its position in the Configuration list.

Note: SutterPatch Configuration files use the *.spc file extension. However, this is reported by the OS as file type “PKCS #7 Certificates”.

ii. Files and Naming

Data files path: Browse to select a folder.

Enable automatic naming for experiment

Experiment file name example: (Maximum 34 characters)

[_____]

- pxp Standard “packed” experiment.

All experimental information is conveniently stored in one file.

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.

HDF5 Options

A modern efficient file format for managing high volumes of data.

The new settings become active after starting a New Experiment or restarting SutterPatch. It is strongly advised to enable the “automatic naming” option above, so that the matching H5 and PXP files are kept “in sync”.

Save to separate HDF5 file. Optional HDF5 file format for high volumes of data.

Data waves from the SutterPatch “data folder” (including raw data, metadata, Experiment structure, analysis results, images, etc.) are stored to a separate HDF5 file. Anything outside of the “data folder” remains part of the “.pxp” Experiment file.

The File / New HDF5 File command stores all existing data into the active HDF5 file, cleans-up the Experiment, and creates a new HDF5 file so that HDF5 acquisition can continue as if you had started a "New Experiment", but without restarting SutterPatch.

Note: This File menu item is only visible when the HDF5 file preference is enabled.

Keep only one Sweep in Memory [only displays when HDF5 is enabled]

Only hold the wave of one sweep in memory.

Data are stored to disk while acquisition runs, instead of storing all data at the end of an Experiment, which can be a time-consuming experience. This also minimizes memory allocation issues for big buffers.

The downside is that multi-sweep data cannot be processed online, e.g., subtracting the data of a "reference" sweep from other sweeps.

Save Options

(non-HDF5)

- 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. Data Export

- Igor Binary Save the signal formatted as an “Igor Binary Wave” file (*.ibw).
- ABF Format Save each signal of the routine formatted as an “Axon Binary File” v.1.8 (*.abf).
 - Export all selected sweeps to one file per signal
 - Ignore unselected sweeps
 - Replace unselected sweeps with NaN
 - Create individual files for each sweep
- ATF Format Save the signal formatted as an “Axon Text File” (*.atf).

iv. 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, oscillations can occur, and the patch-clamp seal can become unstable and 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.

If you are routinely losing cells when switching into Current Clamp mode, increase this setting from the default ‘0.5’ to ‘1’ or ‘2’.

Note: The electrode compensation reduction is done in the background, and does not affect the Control Panel current-clamp settings.

v. 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.

vi. 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.
- Use last y-scale The last setting of the Scope window Y-axis scaling is used.

Maximal sweeps displayed in persistence display

[2 – 100]

All

Improve data readability and system performance by restricting the number of sweeps displayed, which reduces the display processing load.

Display compression

Improve system performance by reducing the number of data points plotted, which reduces the display processing load.

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.
- Use last y-scale The last setting of the Scope window Y-axis scaling is used.

Signals Display

- Show all signals
- Use last selection
- Signals [] Enter signal numbers and/or ranges separated by commas.

Example: 1,2,4-5

Sweeps Display

- Show all sweeps
- Use last selection
- Sweeps [] Enter sweep numbers and/or ranges separated by commas.

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'.

vii. 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.

viii. Graphs 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

Note: Changes are only applied when creating a new layout page.

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

ix. Factory Reset

[Factory Reset] Click this button to reset SutterPatch to its 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.

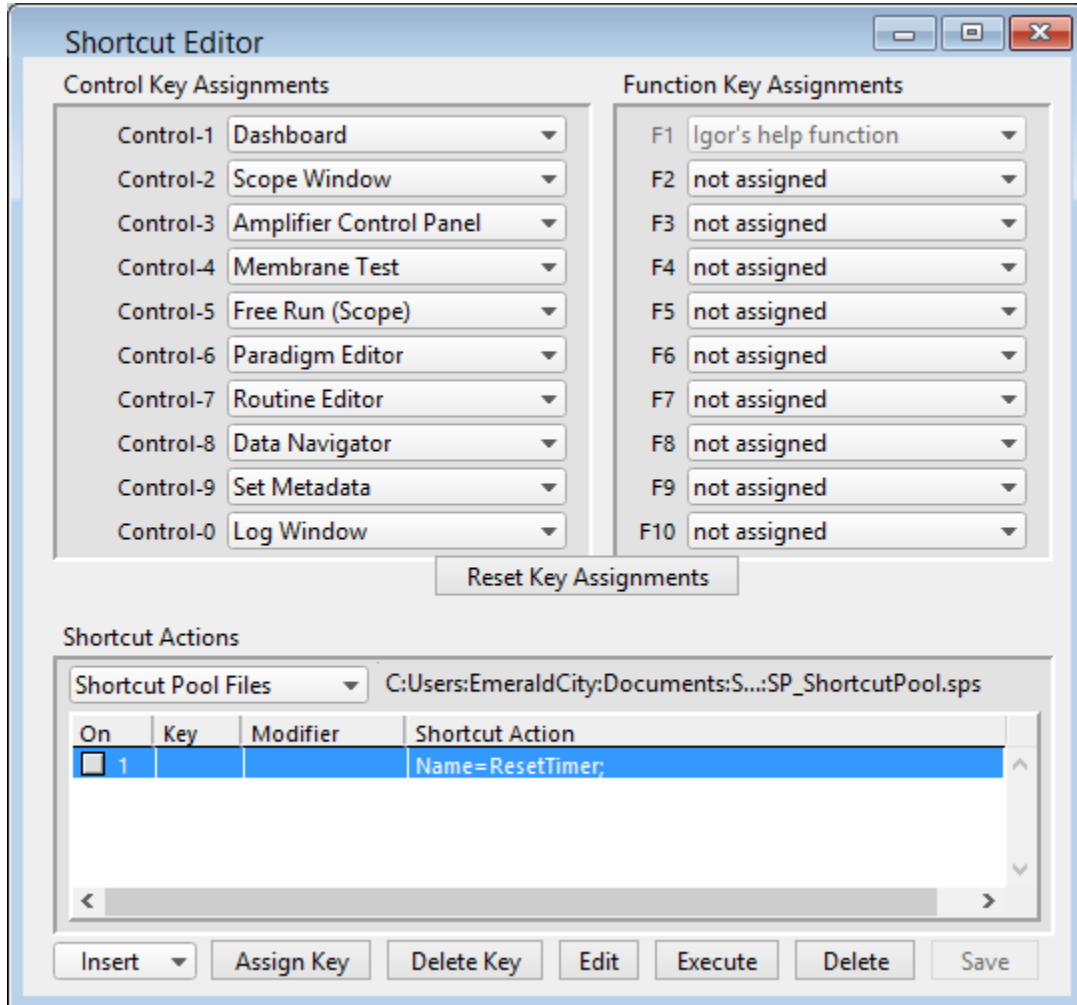


Figure 4-82. Shortcuts Editor

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 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 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	Dashboard
Control-2	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 Control key assignment:

Analysis Editor
 Camera Module
 Equation Editor
 Lock-In Adjustments
 Reset Acquisition
 Set Preferences
 Shortcut Editor
 Solution Editor
 Template Editor

Function Key Assignments

Computer keyboards usually include a set of Function keys for special actions. Configure a 'Function Key' assignment by clicking on its drop-down list and selecting a new menu or action item.

The list of available items for Function keys is the same as above for Control keys.

Function Keys:

F1	Igor Pro Help function	< reserved >
F2 – F10	not assigned	< available >

On some keyboards, you also need to press the 'Fn' key to use Function keys.

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 macOS Keyboard control panel. Even then, macOS will intercept some function keys.

Reset Key Assignments

Reset all Control and Function key assignments to their default settings.

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]

Shortcut Actions Table

Columns

On	Enable/disable the Shortcut Action.
Key	The assigned keyboard key.
Modifier	The keyboard "modifier key" used in a key combination - all keys are simultaneously pressed.

Windows

- CTRL Only use with keys '0 – 9'.

- ALT Keys '0 – 2' reserved by Igor for File / Recent Experiments.
- SHIFT Shift key.
- FUNC Function key.
- CAPS LOCK Ignored.

macOS

- Command Only use with keys '0 – 9'.
- Option Option key.
- Shift Shift key.
- 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.

These Actions operate similarly to Paradigm steps, with an additional Label field to name the Action in the Shortcuts menu.

Amplifier	Control an IPA amplifier's settings.
Analysis	Append, average, display and save analyses.
Camera	Take a photo or run live video.
Execute	Run an Igor or SutterPatch command.
Export	Send graphs to a Layout window.
Front Window	Set the specified window as the front window.
Hide Window	Hide the specified window.
Paradigm	Load & Run, Stop, Pause or Resume a Paradigm.
Reset Timer	Reset the Paradigm Editor Timer to zero.
Routine	Record data from a Routine.
Scope Operation	Control the display of the Scope window signals and sweeps.

Set Axis	Modify the axis scaling of a signal.
Set Checkbox	Set local and global checkboxes for conditional processing in Paradigm 'If' steps.
Set Variable	'Label' entry only displays in the Shortcut Action column.
Set Tag	Write a comment tag to the Paradigm metadata.
Start Acquisition	In open Scope acquisition window.
Stop Acquisition	In open Scope acquisition window.
View Last	Display the last recording in a Scope reanalysis window.
Write Log	Write a note to the Log window.

Assign Key

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.

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.)

Tips: If the CAPS LOCK button is on when assigning a key, the key is case insensitive.

Keyboards often have a Function (FN) button to allow special access to the Function keys.

Although the F1 function key is reserved in Igor Pro, it can be assigned if used with a modifier key.

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 Sample File

SP_EquationPool.txt	Equations for all amplifiers.
1. X3pi	3*pi
2. ElapsedTime	ParadigmTime - time
3. Temperature	aux[1]*1.23 - 273.15

Paradigm Pool Sample Files

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. Interactive_acquisition_1	Run an interactive acquisition stopping at a given analysis condition.
5. Start_two_Series	Start acquisition of two subsequent routines.
6. Start_ForEachSweep	Start acquisition of a routine, individually triggering each sweep.
7. Interactive_acquisition_2	Run an interactive acquisition loop that selects between 2 routines, and manually stop via a Checkbox.
8. Tuning_with_Input	Use the paradigm "Input" control to increment or decrement a Routine's stimulus output.
9. Toggle_Persistence	Use a Checkbox to toggle Scope trace persistence while acquiring a routine.
10. Switch_Headstages	Switch between multiple headstages.
11. Tuning_with_Keys	Use the keyboard to increment or decrement a Routine's stimulus output by 10 mV.

LockIn_IPA.spp	Paradigms for IPA lock-in tuning.
1. LockIn_Adjust_500Hz	
2. LockIn_Adjust_1kHz	
3. LockIn_DoAdjust	

Routine Pool Sample Files

SP_RoutinePool_DIPA.spr	Routines for two-headstage DIPA amplifiers.
SP_RoutinePool_IPA.spr	Routine 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	I/V 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 Sample File

SP_SolutionPool.spo Solutions for all amplifiers.

1. undefined

Template Pool Sample File

SP_TemplatePool.spt Templates 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:

Experiment Sample Files

ActionPotentials.pxp	Action potential data.
LargeAPs.pxp	Large action potential data.
MiniExample.pxp	Spontaneous miniature synaptic potential data.

4.3.9 Startup

The SutterPatch application startup sequence:

1. Click on the Igor Pro 8 icon to launch SutterPatch.
2. The application opens an Igor ‘Command’ window along with a “splash” screen displaying file opening information; both windows are then closed.
3. Next, a ‘Welcome to SutterPatch’ screen displays a few 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. Click ‘Start’, an Igor “Command” window opens, and the Welcome screen displays a progress bar while compiling the SutterPatch code; the Welcome screen is then closed.
5. A ‘Save experiment as’ dialog displays with standard file saving controls.
6. Next, if no Sutter amplifier is detected, the ‘No USB Connection’ pane allows you to try re-establishing the USB connections, or to select a hardware-emulation demonstration mode (IPA, Double IPA, dPatch, Dendrite).

In amplifier demonstration mode, the Amplifier Control Panel and acquisition Scope window are labeled with “Demo”, the input and output channels use simulated data, and most SutterPatch functions are available.

7. The SutterPatch files are initialized, the Dashboard panel displays, and additional SutterPatch windows display if they were open 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. TROUBLESHOOTING

6.1 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.

6.2 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.

6.3 Online 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.

6.3.1 Error Messages and Notifications

Some SutterPatch error messages or notifications will flash to get your attention, and automatically close after several seconds, and then write to the “History” window. To review such messages, see the Command window (Windows / Command Window).

6.4 Startup Issues

6.4.1 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.

6.4.2 Sample Parameter Files Not Installed

Problem: The SutterPatch installer fails to install the sample parameter files, as access is blocked to the Program Files or Users\..\Documents folders.

Solution: Disable any virus scanners or firewalls. If that does not help, then manually copy the sample parameter files into the Users\..\Documents\SutterPatch\Parameters folder.

6.4.3 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’.

6.4.4 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.

6.4.5 USB Communication Fails

Problem: When starting up SutterPatch in Windows, there is no USB communication with the computer.

Solution: The Windows “power plan” might have disabled the USB ports.

- 1) Go to the Windows Start screen, and enter “edit power plan” in the Windows Search box.
- 2) Click on “Change advanced power settings”.
- 3) Scroll down to “USB settings” and click on its [+] box.
- 4) Click on the “USB selective suspend setting” [+] box.
- 5) Change the “On battery” and “Plugged in” settings from ‘enabled’ to ‘disabled’, and click “OK”.

Also, sometimes a Windows 10 update can disable the computer’s USB ports. Either browse the web to find Device Manager and driver solutions to the problem, or restore the OS to an earlier version of Windows 10 until a Windows 10 update fix is released.

6.5 Acquisition Issues

6.5.1 Acquisition In Progress

- Problem:** Unable to start an acquisition because the system thinks it is already running.
- Solution:** Use the menu command SutterPatch / Reset Acquisition to clear the acquisition status.

6.5.2 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.

6.5.3 Acquisition Terminates

- Problem:** During acquisition, the recording terminates unexpectedly.
- Solution:** Close the Analysis / Data Browser window, if it is open.
- This window can consume a large amount of system resources, which can interfere with data acquisition.

6.5.4 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.

6.5.5 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.

6.5.6 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.

6.5.7 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.

6.5.8 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.

6.5.9 Post-Acquisition Delay

Problem: Every time acquisition completes, there is a delay with the program operations, as the entire *.pxp Experiment file is resaved when a recording stops.

Solution: Create new Experiments more often, so that file sizes are smaller and more manageable.

Or, change the file saving settings in Set Preferences / Files and Naming.

6.5.10 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.

6.5.11 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.

- 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.

6.5.12 IPA Amplifier Control Panel Issues

Problem: The IPA/DIPA Amplifier Control Panel is having odd problems.

Solution: You can reset the headstage controls by right-clicking almost anywhere in the Control Panel to select:

- **Reset Amplifier Controls** Reset all hardware settings of all attached Sutter IPA/DIPA amplifiers and their headstages to factory defaults.

6.6 Analysis Issues

6.6.1 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.

6.6.2 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'.

6.6.3 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.

6.7 General Issues

6.7.1 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.

6.7.2 Slow Display of Sweeps

Problem: When displaying a large number of sweeps, the display slows down.

Solution: Disable Persistence display in the Scope window.

6.7.3 Window Maximizing

Problem: Maximizing a window only maximizes the title bar.

Solution: Certain fixed-size windows and panels will not maximize (Action Potential Analysis, Amplifier Control Panel, Dashboard, Event Detection, Log, Paradigm Editor, Set Metadata, Set Preferences.) This is a reported Igor issue.

Also, if the active window is maximized, creating a new window might automatically "maximize" the new window.

6.7.5 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.

6.7.6 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.

6.7.7 Slow Window Opening/Closing

Problem: Window opening and closing is slow on the macOS.

Solution: Close the SutterPatch Data Browser. This function consumes a lot of system resources.

6.7.8 Wrong Preference 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.

6.7.9 Font Size Too Large

Problem: The font size is too large when using the display scaling in 'Scale and Layout'.

Solution: This can occur on high-resolution monitors running on older versions of Windows 10. Upgrade to the current version of Windows 10.

6.7.10 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.

6.7.11 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.

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- Warranty work will be performed only at the factory.
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- 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.

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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.

Section 10.3. Licensor may amend the terms of this Agreement and related company documents at any time with respect to any new releases, updates or versions of the Product, which if purchased by Licensee, will subject Licensee to the terms of the then current Licensor Software Licensing Agreement.

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 & DIPA 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 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 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-5. 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 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

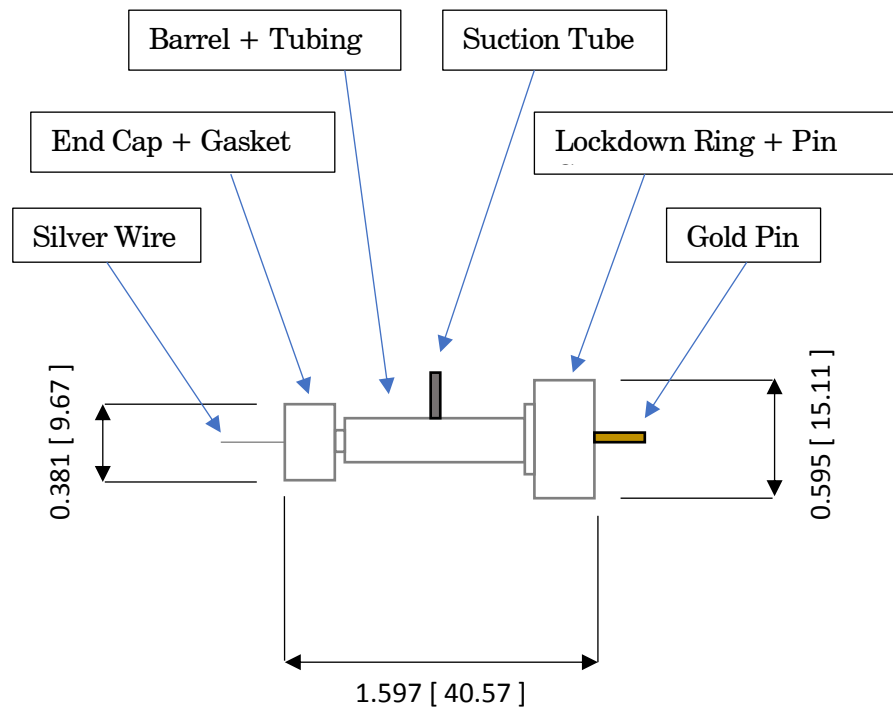


Figure E-0-1. Electrode Holder

Figure dimensions are in “inches [mm]”.

Model Cell Parts

- Model Cell
- Connector pins with crimp
- Ground wire

IPA Accessories

Patch Panel

IPA-PCH

This optional rack-mountable panel provides BNC ports for easy access to digital and auxiliary I/O connections.

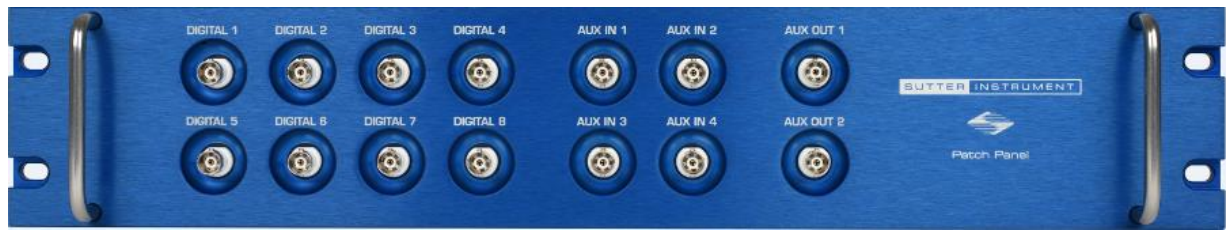


Figure E-0-2. Patch Panel

IPA Patch 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-6. IPA Patch Panel

Ground Point

GP-17

This optional machined brass tower provides reliable low-resistance connections for electrophysiology setups. The base plate mounts directly to air table tops (imperial and metric) with the included $\frac{1}{4}$ -20 and M6 screws. The plated connectors accept up to 9 banana plugs and 8 bare wires (up to 10 gauge). A “star” ground configuration is used to avoid ground loops.

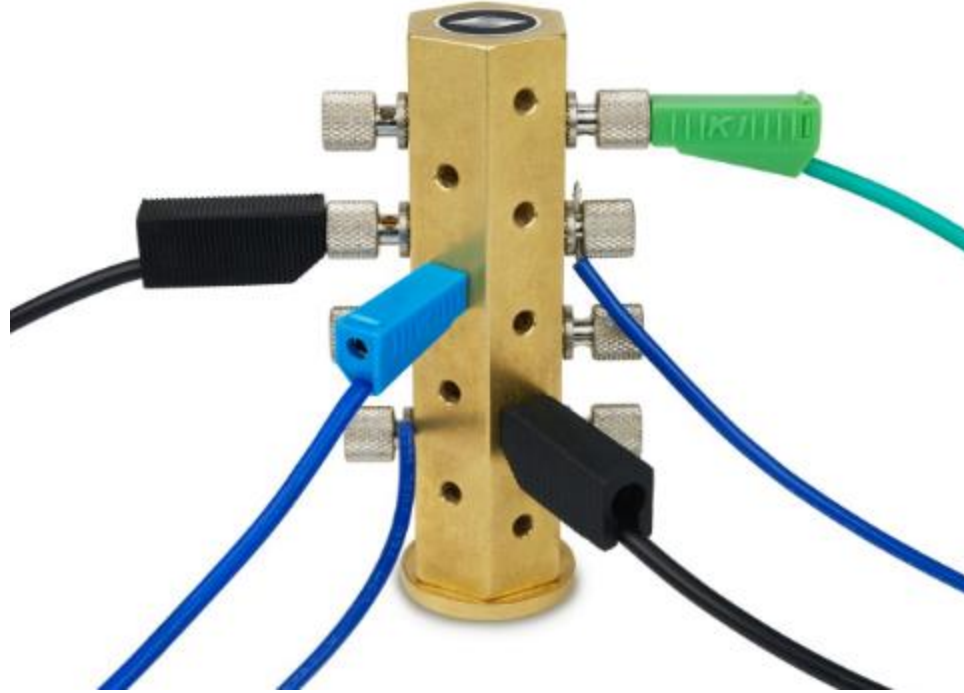


Figure E-0-3. Ground Point

The Ground Point 10-item kit includes cables with banana plugs and alligator clips. For very complex rigs, two sets of cables might be needed.

APPENDIX F. SUTTERPATCH ALGORITHMS

LockIn Computation

[for Routine Editor Virtual Input Channels]

Math used in the LockIn computation:

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

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

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

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

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

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

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

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

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

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

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

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

Event Detection Reference

[for Event Detection Analysis]

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.

Single Channel Fitting

[for Single Channel Analysis]

Math used in single channel fitting:

Gaussian Fit

$$y = y_0 + A * \exp(-((x - x_0) / \text{width})^2)$$

y_0 = offset

A = height of curve's peak

x_0 = position of center of peak

$\text{width} = \sqrt{2} * \sigma$

σ = standard deviation of the peak

Linear Exponential Fit

$$y = y_0 + A * \exp(-(x - x_0) / \text{tau})$$

Logarithmic Exponential Fit

$$y = k_0 + k_1 * \exp(-(\ln(x / k_2) / k_3)^2)$$